AN IMMUNOCOMPETENT INTESTINE-ON-CHIP MODEL AS PLATFORM FOR THE DISSECTION OF HOST-MICROBIOTA INTERACTION

Dissertation

To fulfill the requirements for the degree of

Doctor rerum naturalium (Dr. rer. nat.)

Submitted to the Council of the Medical Faculty of

the Friedrich Schiller Universität Jena

By M. Sc. Michelle Maurer

Born on 23 July 1990 in Grünstadt

1st Reviewer: PD Dr. Alexander S. Mosig, University Hospital Jena

2nd Reviewer: Prof. Dr. Bernhard Hube, University Hospital Jena

3rd Reviewer: Prof. Dr. Peter Loskill, Fraunhofer IGB Stuttgart

Date of the public disputation: 06 July 2021

“We'll never know what this is

'Til we take a look inside”

Lab Monkey, by Alice in Chains

CONTENT

Abbreviations

APC	antigen-presenting cell
C. albicans	*Candida albicans*
DC	dendritic cell
DSS	dextran sodium sulfate
GI tract	gastrointestinal tract
HIO	human intestinal organoid
hiPSCs	human induced pluripotent stem cells
IBD	inflammatory bowel diseases
L. rhamnosus	*Lactobacillus rhamnosus*
LPS	lipopolysaccharides
LTA	lipoteichoic acid
MAMPs	mircobe-associated molecular patterns
MPS	microphysiological systems
OoC	Organ-on-chip
PBMCs	peripheral blood mononuclear cells
PP	Peyer's patches
PRR	pattern recognition receptors
RA	retinoic acid
ROS	reactive oxygen species
S.typhimurium	*Salmonella enterica* subs. *enterica* ser. Typhimurium
TED	transepithelial dendrite
TGF-β	transforming growth factor beta
TLR	toll-like receptor
TSLP	thymic stromal lymphopoietin

Summary

Research investigating the human intestinal microbiota has recently gained momentum in the scientific literature. Mouse models have provided a number of indications on the involvement of the microbiota in health and disease. An alteration of the intestinal community called dysbiosis is associated with a range of conditions such as neurological diseases, diabetes, and inflammatory bowel diseases (IBD). However, the microbiota composition and the immune system substantially differ between mice and humans. To circumvent the inter-species differences and ethical issues related to animal experimentation, sophisticated *in vitro* models using human cells are at hand, which represent important cornerstones of microbiota research. Recently, organ-on-chip (OoC) platforms have been established to mimic organ-specific physiological microenvironments. By making use of microfluidic technologies and biocompatible materials, microphysiological systems (MPS) facilitate the co-culture of human host cells and microbes such as bacteria, fungi, archaea, protozoa, as well as viruses. Therefore, MPS provide the opportunity to study the interaction of host cells and microbiota and unravel underlying mechanisms.

In manuscript I, we describe the establishment of a microfluidic intestine-on-chip model comprising of epithelial cells in the luminal compartment and endothelial cells as well as macrophages in the vascular compartment. In this thesis, we characterized monocyte-derived phagocytes in the system using immunofluorescence staining, revealing the presence of two phenotypically distinct subsets. We found macrophage-like cells expressing high levels of CD68 and the fraktalkine receptor but also dendritic cell (DC)- like phagocytes with low CD68 and high CD103 expression. DCs formed protrusions that reached through the epithelial cells into the lumen, indicating luminal antigen sampling. We, therefore, assumed that supplementation of polarizing cytokines, combined with the environment of the intestinal tissue fostered the differentiation of monocytes into different myeloid lineages.

Another aspect of this thesis was to enable the colonization of the model with bacteria of the probiotic strain *Lactobacillus rhamnosus* and their interplay with the opportunistic fungus *Candida albicans*. First, we established a colonization protocol for *L. rhamnosus* and additionally perfused the endothelial layer with peripheral blood mononuclear cells (PBMCs) to increase resemblance to the *in vivo* situation. The intestine-on-chip model showed immune tolerance towards lactobacilli, in control

experiments and after luminal administration of lipopolysaccharides (LPS), which are normally present in the gastrointestinal tract. The amount of the cytokines Interleukin (IL-) 1β, IL-6, IL-8, IL-10, and tumor necrosis factor in the supernatants were measured using a cytometric bead array and revealed low-level cytokine secretion. Image analysis of junctional proteins E-cadherin and ZO-1 demonstrated enhanced expression of the proteins, and therefore an increased barrier function. However, when LPS was administered to the vascular compartment mimicking endotoxemia, the immune cells elicited an inflammatory response. In the presence of *L. rhamnosus*, secretion was even enhanced for certain cytokines in this condition. Even so, the increased cytokine release did not compromise barrier integrity. Instead, the presence of lactobacilli improved barrier function, when compared to experiments lacking the probiotics. This protective effect of *L. rhamnosus* has also been demonstrated by other research groups. Moreover, the bacteria also inhibited the growth of *C. albicans*, reduced tissue damage, and decreased translocation of the fungus from the luminal to the vascular compartment upon infection. Taken together, the intestinal model displayed organ-specific structures and included innate immune cells, which exhibited immune tolerance towards commensal bacteria and their products. Therefore, the platform allows detailed analysis of host-microbiota interactions and accompanying immune responses. In addition, the model facilitated studying the interplay of commensal bacteria and opportunistic pathogens under physiologically relevant conditions. Our intestine-on-chip model proved to be a valuable tool suitable for future investigation of diseases associated with intestinal inflammation.

In Manuscript II, established *in vitro* models and the potential of OoC models were discussed in reference to fungal infections. *Aspergillus fumigatus*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *C. albicans* are responsible for the largest share of fungal infections worldwide and require to be investigated under physiologically relevant conditions created using *in vitro* models. Although OoC models are reductionist and cannot yet be used for systemic analysis, they can make important contributions in broadening our understanding of microbiota-host interactions and fungal infections.

Zusammenfassung

In der wissenschaftlichen Literatur hat die Erforschung der humanen Darmflora zuletzt an Dynamik gewonnen. Mausmodelle haben eine Reihe von Hinweisen geliefert, dass unsere Darmbewohner in den Erhalt unserer Gesundheit und Krankheitsverläufe involviert sind. Veränderungen der Darmflora, Dysbiose genannt, stehen mit einigen Erkrankungen in Verbindung, zum Beispiel neurologischen Erkrankungen, Diabetes und entzündlichen Darmerkrankungen. Allerdings variiert die Zusammensetzung der Darmflora und das Immunsystem zwischen Maus und Mensch erheblich. Um die Artunterschiede und ethische Problematik von Tierversuchen zu umgehen, können stattdessen komplexe *in vitro*-Modelle mit humanen Zellen verwendet werden, die wichtige Säulen der Erforschung der Darmflora darstellen. Unlängst wurden Organ-on-Chip (OoC)- Plattformen etabliert, um eine organspezifische physiologische Mikroumgebung zu schaffen. Durch die Verwendung mikrofluidischer Technologien und biokompatiblen Materialien ermöglichen mikrophysiologische Systeme (MPS) die Ko-Kultivierung von humanen Wirtszellen und Mikroben, zum Beispiel Bakterien, Pilzen, Protozoen, aber auch Viren. Daher bieten MPS die Möglichkeit, die Interaktion von Wirtszellen und Darmflora zu untersuchen und zu Grunde liegende Mechanismen aufzuklären. In Manuskript I beschreiben wir die Etablierung eines mikrofluidischen Darm-on-Chip-Modells mit Epithelzellen in der luminalen Kammer und Endothelialzellen sowie Makrophagen in der vaskulären Kammer. In dieser Arbeit charakterisierten wir die aus Monozyten differenzierten Phagozyten im System mittels Immunfluoreszenzfärbung, was die Präsenz zweier unterschiedlicher Subtypen offenbarte. Wir fanden Makrophagen-ähnliche Zellen, die CD68 und den Fraktalkin-rezeptor auf hohem Niveau exprimierten, und darüber hinaus Phagozyten mit niedriger CD68- und hoher CD103-Expression, die dendritischen Zellen ähnelten. Dendritische Zellen formten Ausstülpungen, die zwischen epithelialen Zellen hindurch ins Lumen reichten und damit auf luminales Sammeln von Antigenen hinwiesen. Daher kann davon ausgegangen werden, dass die Differenzierung der Monozyten in verschiedene myeloide Zelltypen durch den Zusatz von polarisierenden Zytokinen zusammen mit der Umgebung des Darmgewebes begünstigt wurde. Ein weiterer Aspekt dieser Arbeit war es, die Kolonisierung des Modells mit dem probiotischen Stamm *Lactobacillus rhamnosus* und die Interaktion der Laktobazillen mit dem opportunistischen Pilz *Candida albicans* zu ermöglichen. Zunächst etablierten wir ein Kolonisierungsprotokoll

für *L. rhamnosus* und perfundierten die Endothelschicht zusätzlich mit mononukleären Zellen des peripheren Blutes, um die Ähnlichkeit mit der *in vivo*-Situation zu erhöhen. Das Darm-on-Chip-Modell zeigte Immuntoleranz gegenüber den Laktobazillen, in Kontrollversuchen, sowie nach der luminalen Zugabe von Lipopolysacchariden (LPS), welche normalerweise im Darmtrakt vorhanden sind. Die Menge der Zytokine Interleukin (IL)- 1β, IL-6, IL-8, IL-10 und des Tumornekrosefaktors in den Überständen wurde mittels Durchflusszytometrie gemessen und offenbarte eine niedrige Zytokinsekretion. Bildanalysen der zellkontaktvermittelnden Proteine E-cadherin und ZO-1 zeigten eine verstärkte Expression der Proteine, und daher eine Festigung der Barriere. Wenn LPS jedoch zur vaskulären Kammer hinzugegeben wurde um eine Endotoxämie nachzuahmen, riefen die Immunzellen eine inflammatorische Antwort hervor. In der Anwesenheit von *L. rhamnosus* war die Sekretion bestimmter Zytokine in dieser Kondition sogar erhöht. Trotzdem führte die erhöhte Zytokinsekretion nicht dazu, dass die Barriere-Integrität beschädigt wurde. Stattdessen verbesserte die Anwesenheit der Laktobazillen die Barrierefunktion im Vergleich mit Versuchen ohne den probiotischen Stamm. Dieser protektive Effekt von *L. rhamnosus* wurde auch von anderen Arbeitsgruppen gezeigt. Weiterhin inhibierten die Bakterien das Wachstum von *C. albicans*, reduzierten Gewebeschädigung und verminderten die Translokation des Pilzes von der luminalen in die vaskuläre Kammer nach der Infektion. Zusammengenommen erlaubt die Plattform eine detaillierte Analyse der Wirt-Darmflora-Interaktionen und damit einhergehende Immunreaktionen. Zusätzlich ermöglichte das Modell das Zusammenspiel kommensaler Bakterien und opportunistischer Pathogene unter physiologisch relevanten Bedingungen zu erforschen. Unser Darm-on-Chip-Modell erwies sich als nützliches Werkzeug, welches für die zukünftige Erforschung von entzündlichen Darmerkrankungen geeignet ist.

In Manuskript II wurden etablierte *in vitro*-Modelle und das Potenzial von OoC-Modellen in Bezug auf Pilzinfektionen diskutiert. *Aspergillus fumigatus*, *Histoplasma capsulatum*, *Cryptococcus neoformans* and *C. albicans* sind weltweit für den größten Teil an Pilzinfektionen verantwortlich und erfordern es, unter physiologisch relevanten, mittels *in vitro*-Modellen nachgestellten Bedingungen untersucht zu werden. Obwohl OoC-Modelle reduktionistisch sind und noch nicht für systemische Analysen verwendet werden können, können sie wichtige Beiträge leisten, um unser Verständnis von Interaktionen zwischen Wirtszellen und Darmflora, sowie Pilzinfektionen zu erweitern.

1. Introduction

1.1 The intestinal microbiota in human health and disease

The intestinal microbiota has received increasing attention within the scientific community over the last 15 years; the publication numbers have multiplied by 30 since 2000 (Li et al. 2020). However, it has been recognized for a long time that the intestinal microbiota has a major impact on the human body and mind. In 1986, it has been demonstrated, for the first time, that microbes can shape the brain chemistry of mice (Hegstrand and Hine 1986). In the literature, the terms microbiota and microbiome are often confused and used synonymously. In the present study, the term microbiota is used to refer to the collective of all bacteria, archaea, and eukaryotes inhabiting the gastrointestinal (GI) tract. The intestinal microbiota consists of commensal microorganisms, and commensals that are opportunistic pathogens, such as *Candida albicans*, given the appropriate conditions (Hube 2004). The microbiota ferments dietary fibers, which cannot be absorbed or digested in the small intestine, synthesizes vitamins, alters drug metabolism, and interacts with the immune system (Holscher 2017; Klaassen and Cui 2015; LeBlanc et al. 2013; Thaiss et al. 2016). In fact, the intestinal microbiota is often referred to as a distinct organ in the human body (Possemiers et al. 2011; Weiss and Hennet 2017). The impact of the microbiota on other organs has led to the use of the terms such as “gut-brain axis” and “gut-liver axis”, to describe the bidirectional relationship between the microbiota and the brain or the liver, respectively (Carabotti et al. 2015; Konturek et al. 2018). The microbiota composition varies greatly between individuals and is subjected to host genetics, age of the host, and environmental factors such as diet and lifestyle. Moreover, the configuration can change quickly in response to alterations of environmental factors, hampering the identification of a “healthy microbiota” and a pathological composition (Gilbert et al. 2018; Lloyd-Price et al. 2016). A common approach to determine the bacterial composition is to analyze fecal samples. The microbial DNA is extracted to sequence the 16S ribosomal RNA (rRNA) gene, but the evaluation can be error-prone (Poretsky et al. 2014). Storage conditions vary and can lead to unintentional bacterial growth and subsequently biased composition (Liang et al. 2020). Nevertheless, changes in the microbiota composition have been associated with neurological diseases such as Alzheimer’s disease, amyotrophic lateral sclerosis (ALS), and

depression but also obesity and diabetes (Mangiola et al. 2016; Patterson et al. 2016; Spielman et al. 2018). One of the key aspects in this regard is the balanced composition of the microbial community. 90% of the intestinal microbiota of human hosts consists of bacteria belonging to the phyla Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Verrucomicrobia, and Proteobacteria (Rinninella et al. 2019). Antibiotic treatment can cause alterations of the ratios of the individual phyla and overgrowth of individual species. This imbalance of the microbial community is called dysbiosis and is one of the hallmarks of inflammatory bowel disease (IBD) (Carding et al. 2015; Khan et al. 2019). Under homeostatic conditions, the interplay of the intestinal microbiota and host immune cells is finely tuned and depends on immune tolerance, a term describing the absence of proinflammatory responses to food and microbial antigens (Mowat 2003). Dysbiosis leads to a disturbance of intestinal immune homeostasis as cells of the innate and adaptive immune system induce inflammation when microorganisms translocate from the lumen into intestinal tissue. The release of proinflammatory cytokines under these conditions can result in damage to the epithelium and loss of barrier integrity (Weiss and Hennet 2017). It is still unclear, whether IBD is caused by dysbiosis or whether other factors of the condition such as strong proinflammatory responses lead to dysbiosis as a by-product. However, the deliberate modification of the intestinal microbial community for human well-being has also become a major field of investigation. The administration of probiotics, defined as microorganisms that convey a health benefit for the host when administered in appropriate amounts, represents a novel preventive and therapeutic strategy e.g. against microbial infections (Hill et al. 2014; Suez et al. 2019). The development of therapies that influence the microbiota or the introduction of probiotics requires studies of the interaction of host and microbiota. For such studies, the microbes need to be isolated and cultured under laboratory conditions. This appears to be challenging since the minimum requirements for bacterial growth are unknown for many species and many of them are anaerobic (Fodor et al. 2012). Due to the inherent complexity of microbiota research, adequate models are needed to dissect the interplay between the humans and their intestinal inhabitants, as well as interactions of individual members of the microbiota with each other.

1.2 The physiology of intestinal epithelium

Both the small and large intestine are lined by a single layer of columnar epithelium. In the small intestine, the digestion and absorption of nutrients take place. To increase the surface available for absorption, projections of intestinal tissue called villi intrude into the lumen, and the digestive area is further increased by microvilli on absorptive cells forming the brush border. In contrast, the large intestine, the colon, lacks villi, since its main task is the reabsorption of water and digestion of dietary fibers, of which the latter is performed by the microbiota. The crypts of Lieberkühn can be found in all parts of the small and large intestine. Here, stem cells are located, giving rise to all cell types in the mature epithelium (Mowat and Agace 2014). The largest part of epithelial cells consists of enterocytes that absorb nutrients, and play a vital role in establishing tolerance against food antigens and the microbiota (Miron and Cristea 2012). Approximately 25% of epithelial cells in the distal colon consist of goblet cells, which secrete a protective coat of mucus and immunoglobulin (Ig) A. There is only one loose mucus layer in the small intestine, while there are two layers in the colon: a dense inner layer, which is in direct contact with epithelial cells and devoid of microorganisms, and a loose outer layer to which microorganisms adhere. Impaired mucus production in the colon is associated with colon cancer and colitis (Hansson and Johansson 2010). Mucus consists of highly glycosylated glycoproteins called mucins that also serve as a nutrient source and attachment sites for the microbiota (Juge 2012; Sonnenburg and Backhed 2016). Paneth cells are only found in the small intestine and are located adjacent to the stem cells. They secrete antimicrobial peptides such as lysozyme and defensins to keep the stem cell niche sterile. In addition, normal stem cell function is maintained via the production of pro-epidermal growth factor (pro-EGF), WNT3, and Notch ligands (Clevers and Bevins 2013; Sato et al. 2011a). Enteroendocrine cells account for approximately 1% of the epithelial cells. They produce and release peptide hormones and have been demonstrated to sense microbial metabolites and respond by releasing cytokines (Worthington et al. 2018). Underneath the epithelium, the lamina propria provides a scaffold for the villi with its connective tissue, the vascular and lymphatic system. The lamina propria is part of the mucosa, together with the epithelial layer and a thin muscle layer beneath the lamina called muscularis mucosa. The intestinal epithelium does not only provide a physical barrier, there is also constant crosstalk with innate and adaptive immune cells residing in the mucosa. Via cytokine

signaling, the epithelial cells provide information about the luminal content including the microbiota (Wittkopf et al. 2014).

1.3 Friend or foe – the balancing act of the intestinal immune system

1.3.1 Homeostasis in the intestinal mucosa

The GI tract contains the largest number of immune cells in the body, which are organized in the gut-associated lymphoid tissue (GALT). The GALT comprises Peyer's patches (PPs), isolated lymphoid follicles, and mesenteric lymph nodes (Kelsall 2008). These lymphoid aggregates in the mucosa and submucosa are overlaid by follicle-associated epithelium comprising microfold cells (M cells), which are specialized in the uptake of luminal antigens. They are invaginated at the apical side and in close contact with immune cells (Mabbott et al. 2013). The intestinal mucosa comprises macrophages, DCs, B cells, T cells, eosinophils, and mast cells (Okumura and Takeda 2016). Due to the presence of the microbiota, immune responses are tightly regulated and exceptionally dynamic. On the one hand, immune cells need to elicit proinflammatory responses to adequately fight pathogens, on the other hand, the inflammatory response must be limited to avoid excessive inflammation and tissue damage. A dysregulated immune response towards the microbiota results in chronic inflammation and contributes to pathogenesis in IBD (Rubin et al. 2012). In order to trigger tolerogenic or proinflammatory immune responses, microbial antigens need to be recognized by antigen-presenting cells (APCs) that include macrophages, DCs, and B cells. APCs express pattern recognition receptors (PRRs) on their cell surface, enabling them to recognize microbial-associated molecular patterns (MAMPs). In addition to microbial patterns, these cells also respond to tissue damage by sensing and damage-associated molecular patterns (DAMPs). While MAMPs derive from microorganisms, DAMPs result from mutated or damaged host cells (Patten and Collett 2013; Tang et al. 2012). Toll-like receptors (TLRs) represent one major class of PPRs and bind MAMPs. TLRs are type I transmembrane glycoproteins expressed on the cell surface or within endosomes and bind MAMPs. Especially TLR4 plays an important role in the detection of microbial cell wall components. Lipopolysaccharides (LPS) are released from the outer membrane of Gram-negative bacteria and act as a potent stimulus of proinflammatory immune responses. First, LPS bind to LPB proteins, which

is followed by the binding of the LPS-LPB complex to CD14 on the surface of cells. This activates TLR4 and initiates a signaling cascade resulting in the production of proinflammatory cytokines such as interleukin (IL-) 1β, IL-6, and tumor necrosis factor (TNF) (Lavelle et al. 2010; Lu et al. 2008; Wright et al. 1990).

The small intestine under homeostatic conditions is shown in Fig. 1. As APCs, macrophages and DCs are essential for the maintenance of intestinal homeostasis. The distinction of macrophages from DCs regarding their specific roles and phenotypes has been challenging due to the lack of appropriate markers of each lineage (Bain and Mowat 2014). CD103 expressed by DCs alongside other cell surface markers has been demonstrated to be useful for the discrimination of the cell types (Gren and Grip 2016). Intestinal tissue-resident macrophages arise from circulating bone marrow-derived $CD14^{+}$ monocytes, displaying a proinflammatory phenotype in response to TLR stimulation. When they enter the intestinal mucosa, CD14 expression

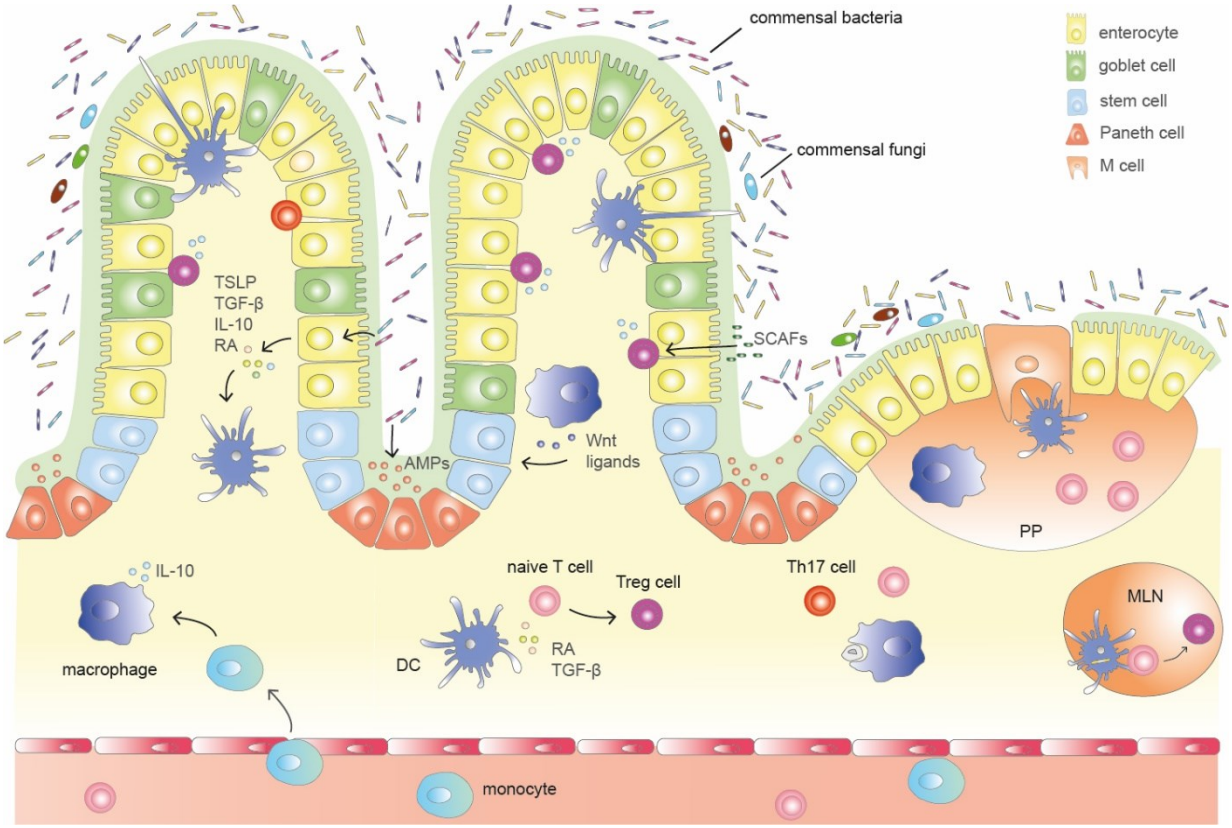


Fig. 1: Homeostasis in the small intestine. Under homeostatic conditions, the microbial community is highly diverse. Intestinal microbiota-derived metabolites such as short-chain fatty acids (SCAFs) stimulate Paneth cells to secret antimicrobial peptides (AMPs) and enterocytes to produce stimulants resulting in dendritic cells (DCs) adapting a tolerogenic phenotype. Tolerogenic DCs sample microbiota-derived antigens from the lumen, induce Treg cell conversion and prime naïve T cells in the MLN to become tolerogenic gut-homing T cells. Circulating monocytes replenish tolerogenic macrophages, which stimulate stem cell renewal and clear apoptotic cells. PP: Peyer's Patches; MLN: Mesenteric lymph node

decreases and they differentiate into tolerogenic resident macrophages. Adopting this phenotype is accompanied by an increase of phagocytic capacity and anti-inflammatory IL-10 secretion, while the production of proinflammatory cytokines and nitric oxide ceases. Although tissue-resident macrophages express TLRs, they do not react to TLR stimulation, a condition termed anergy. This mechanism is assumed to depend on transforming growth factor (TGF)-β signaling (Smith et al. 2001; Smythies et al. 2005). Anergy ensures that macrophages do not cause inflammation in response to commensal microbes. Tissue-resident macrophages represent a highly heterogeneous cell population with various subsets, which differ between different sections of the GI tract and are present in all tissue layers in varying numbers (Bujko et al. 2018; Ogino et al. 2013; Viola and Boeckxstaens 2020). The largest pool of macrophages is found in the colon (Nagashima et al. 1996). They phagocytose microbes that enter intestinal tissue, clear debris, and are involved in tissue remodeling. Moreover, they participate in stimulating the proliferation of intestinal stem cells in the crypts and wound healing (Pull et al. 2005). Macrophages have also been found to communicate with neurons in the muscularis externa, where they are involved in regulating peristaltic motility in mice (Muller et al. 2014).

In contrast to macrophages, DCs can potently induce adaptive immune responses by activation of T cells. Therefore, DCs form the bridge between the innate and the adaptive immune system (Banchereau and Steinman 1998). Different DC subpopulations exist that exhibit distinguished phenotypes and exert special functions within the mucosa. DCs derive either from monocytes (moDCs) or a common DC progenitor (CDP), giving rise to conventional DCs (cDCs) and plasmacytoid cells (pDCs). DCs effectively sample antigens from the lumen via two distinct routes: (i) M cells deliver the antigens to DCs residing in close proximity, or (ii) DCs acquire antigens from the lumen via endocytosis (Mann et al. 2013). During this process, they project protrusions between epithelial cells without disturbing barrier integrity by expressing tight junction proteins (Rescigno et al. 2001). If the sampled antigen is microbiota or food-derived, DCs convey tolerogenic signals, for example, by secretion of TFG-β and retinoic acid (RA) that converts naïve T cells into regulatory T cells (Treg cells) (Coombes et al. 2007). Treg cells are of major importance for the mediation of immune tolerance against the microbiota (Cebula et al. 2013; Cong et al. 2009).

1.3.2 Host-microbiota interactions – the probiotic strain *L. rhamnosus*

The microbiota and the host interact in manifold ways that we are just beginning to unravel. The administration of probiotic strains is used as a treatment strategy against infections, because of the known health benefits that it conveys via a series of mechanisms. For example, probiotics deprive pathogens of nutrients and space and block epithelial receptors thereby reducing available attachment sites (Monteagudo-Mera et al. 2019). Like DCs and macrophages epithelial cells recognize microbes by expression of PRRs. This leads to an activation of tissue-protective measures and upregulation of junction proteins resulting in enhanced barrier integrity (Artis 2008). Paneth cells secrete antimicrobial peptides in response to PRR stimulation, however, it has been shown that e.g. α-Defensin in turn influences microbial composition (Salzman et al. 2010; Vaishnava et al. 2008). A number of microbial metabolites exert effects on epithelial cells such as tryptophan catabolites, which bind to the aryl hydrocarbon receptor (AhR) and thereby induce stem cell differentiation (Metidji et al. 2018). Short-chain fatty acids (SCAFs) produced by the microbiota represent an important energy source for epithelial cells, however, they are also involved in promoting epithelial cell turnover and tight junction expression (Park et al. 2016; Zheng et al. 2017). Epithelial cells also secrete molecules that unfold their effects in the mucosa where immune cells reside. Important drivers of tolerogenic immune responses are IL-10, thymic stromal lymphopoietin (TSLP), RA, and TFG-β produced by epithelial cells. Moreover, it was demonstrated in an *in vitro* model that the presence of probiotics led to an upregulation of TSLP and TFG-β secretion by epithelial cells (Zeuthen et al. 2008). The impact of the immune cells on epithelial cells manifests, for example, in the secretion of fibroblast growth factor 2 by Treg cells interacting with IL-17, which results in epithelial cell damage repair (Song et al. 2015). The microbiota also directly acts on immune cells and *vice versa*: Treg cell activation can be induced by microbiota-derived butyrate, while Treg cells govern IgA secretion in PPs leading to enhanced microbial variability (Kawamoto et al. 2014; Smith et al. 2013).

Lactobacillus rhamnosus is one of the best-studied probiotic strains (Segers and Lebeer 2014). It is a Gram-positive rod-shaped bacterium with a size of 2 to 4 µm in length and 0.8 to 1 µm in width. It is facultative anaerobic, non-motile, and does not form spores. Like other Lactobacilli, it exploits a range of carbohydrates such as rhamnose, giving it its name, and metabolizes these through fermentation to yield lactic acid (Valík et al. 2008). A study involving human volunteers demonstrated that orally

administered *L. rhamnosus* shape the microbial communities in the GI tract by increasing the occurrence of beneficial bacteria and reducing potentially damaging bacteria (Toscano et al. 2017). *L. rhamnosus* acts on host cells in various ways, including upregulation of mucus production and mediation of immune responses (Martin et al. 2019; Segers and Lebeer 2014). The immunomodulatory properties of *L. rhamnosus* are well documented (Fong et al. 2016; Mileti et al. 2009; Rocha-Ramirez et al. 2017). Factors secreted by *L. rhamnosus* induce expression of several TLRs on mononuclear phagocytes and induce secretion of IL-8 and other chemokines in macrophages (Fong et al. 2016; Rocha-Ramirez et al. 2017; Veckman et al. 2003). One of these factors might be lipoteichoic acid (LTA), the Gram-positive equivalent of LPS, which has been shown to induce *IL-8* mRNA production in Caco-2 cells and is involved in modulating anti-viral immune responses via TLR3 signaling (Claes et al. 2012; Mizuno et al. 2020). Attachment of *L. rhamnosus* to epithelial cells and mucus is facilitated by SpaCBA pili, thin bulges of the bacterial cell surface, which might also be involved in shaping immune responses (Kankainen et al. 2009; Segers and Lebeer 2014). Among these factors, lactic acid is a controversially discussed metabolite, which is not exclusively produced by *L. rhamnosus*. Lactic acid has been shown to have antimicrobial activity, which might be due to its capacity to permeabilize the outer membrane of Gram-negative bacteria (Alakomi et al. 2000; De Keersmaecker et al. 2006; Makras et al. 2006).

1.3.3 The inflamed mucosa

Disturbing the finely coordinated interplay of microbiota and host can result in inflammation of the intestinal mucosa summarized in Fig. 2. When the intestinal barrier is breached and microbes translocate from the lumen into the tissue, an inflammatory response is initiated. $CD14^+$ monocytes are recruited from the circulation into the tissue, where they differentiate into macrophages and secrete proinflammatory cytokines such as TNF, IL-1β and IL-6 (Hine and Loke 2019). At the same time, recognition of MAMPs, e.g. via TLR-dependent signaling, leads to a decline in endocytic capacity in DCs (Hemmi and Akira 2005). Subsequently, they migrate to secondary lymphoid organs and present antigens to T cells. Naïve T cells differentiate into effector T cells, either into memory T cells or effector cells, which can further be subdivided into cytotoxic (Tc) cells, with the ability to kill infected cells directly, or T helper (Th) cells. Different kinds of infections result in different Th responses:

Intracellular microbes and viruses induce a Th1 response, while parasitic infections are engaged by Th2 cells. In the intestinal tract, Th17 cells, named after their capacity to produce IL-17, are the crucial drivers of proinflammatory responses towards microbial pathogens. In order to induce the differentiation of naïve T cells into effector T cells, APCs present antigens via the MHCII, while cytokine signals drive the T cell polarization to specific lineages. The antigen/MHC complex is recognized by the T cell receptor (TCR). The interactions are further stabilized by the co-receptor CD4. Only in the presence of co-stimulatory molecules CD80, CD68, or CD40 expressed on APCs and their recognition by CD28 and CD40L, respectively, T cells differentiate into effector cells (Gaudino and Kumar 2019; Ni and O'Neill 1997). Primed T cells acquire gut-homing properties, which ensures a tissue-specific response. The tendency for a T cell to become proinflammatory depends on three DC attributes: an upregulated expression of co-stimulatory molecules and processed antigens, and an enhanced ability to secrete proinflammatory cytokines (Bernardo et al. 2018a). T cell-mediated

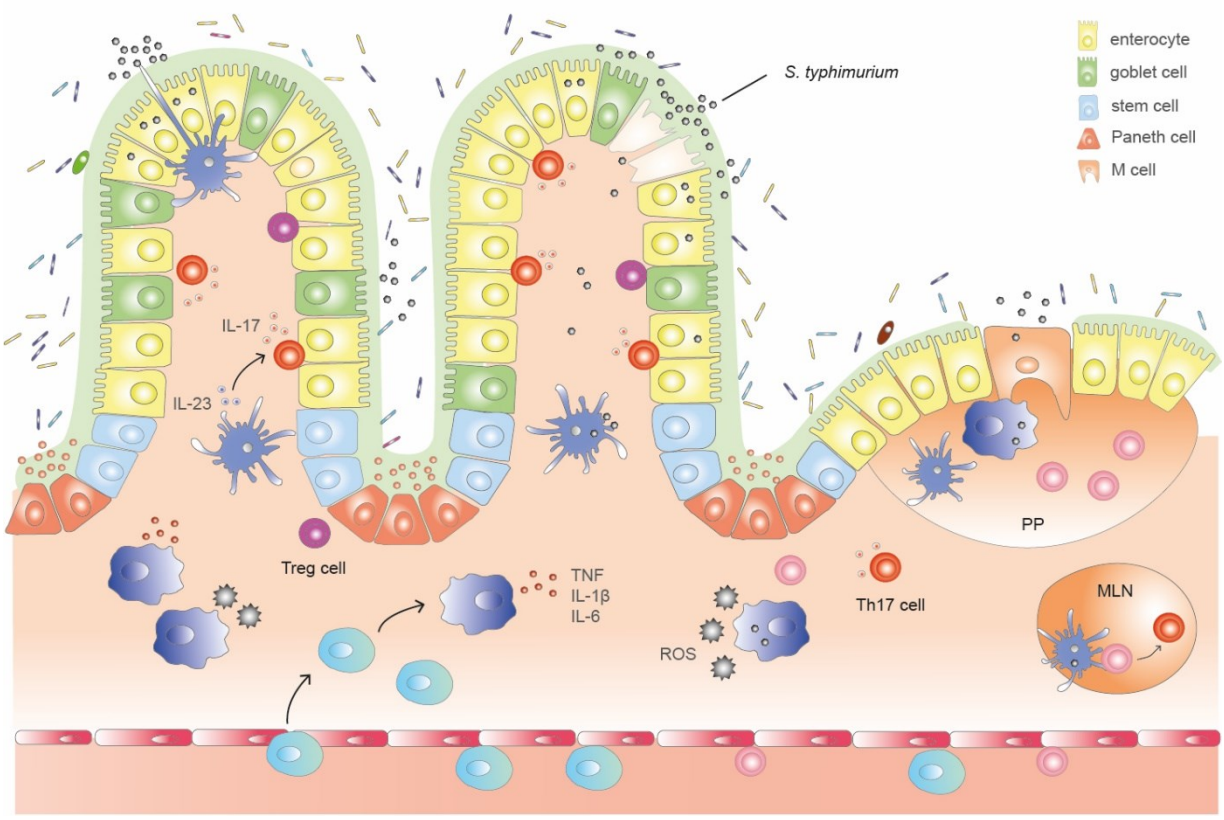


Fig. 2: Dysbiosis in the small intestine caused by *S. typhimurium*. Food-borne *S. typhimurium* induces alterations of the intestinal community by competing with commensals. The pathogens translocate into intestinal tissue, for example, in areas of damaged epithelium, via M cells or uptake by epithelial and dendritic cells. Infection leads to enhanced production of antimicrobial molecules by Paneth cells, Th17 cell expansion, the release of reactive oxygen species (ROS), and proinflammatory cytokines. Circulating monocytes are attracted to the site of inflammation and differentiate into proinflammatory macrophages. PP: Peyer's Patches; MLN: Mesenteric lymph node

immune responses result in the recruitment of other immune cells such as neutrophils, which are attracted by IL-17 (Kalyan and Kabelitz 2014). If the inflammation is not resolved properly, it can become a chronic condition as seen in IBD (Onali et al. 2019). A bacterial pathogen that actively induces inflammation in the mucosa is *Salmonella enterica* serovar Typhimurium (*S. typhimurium*); a rod-shaped, Gram-negative, facultative anaerobic bacterium that is one of the major causes of enteric diseases in humans. Following the ingestion of contaminated food or water, *S. typhimurium* uses flagella to move towards the epithelial cells and establishes contact via fimbriae (Gart et al. 2016). Three different mechanisms are employed to gain access to the intestinal tissue: transcytosis via M cells, induced uptake by epithelial cells, and presumably also by DCs (Broz et al. 2012). After being phagocytosed by macrophages and DCs, the pathogens persist in *Salmonella*-containing vacuoles (SCVs), replicate and exit the host cells to infect other cells (Steele-Mortimer 2008). During inflammation, macrophages produce reactive oxygen species (ROS), which react with luminal thiosulphate to form tetrathionate. Unlike commensals, the pathogens can use tetrathionate as an electron acceptor and therefore gain a growth advantage (Winter et al. 2010). Examining the pathogenesis of *S. typhimurium* contributed significantly to developing a general understanding of pathogen-detection and host immune responses.

1.3.4 Fungal infections and the opportunistic nature of *C. albicans*

Fungal infections are on the rise worldwide. Contributing factors are, for example, procedures that compromise the immune system like stem cell and organ transplantation and the administration of immunosuppressive drugs such as TNF-antagonists (Lockhart and Guarner 2019). Antibiotic treatment reduces the frequency of beneficial bacteria and therefore enables overgrowth of fungal species (Guinan et al. 2019). Immunocompromised individuals are more susceptible to become infected with fungal pathogens, of which *Aspergillus fumigatus*, *Histoplasma capsulatum*, *Cryptococcus neoformans* and *Candida albicans* are the most clinically relevant ones (Brown et al. 2012). Fungi do not only interact with humans as pathogens but also as commensals. The bacterial microbiota has received greater attention than our fungal inhabitants, which seem to be less stable than bacterial communities (Hallen-Adams and Suhr 2017). Nevertheless, fungi-bacteria-host interactions play an important role to maintain intestinal homeostasis (Enaud et al. 2018; Tso et al. 2018). A fungal

species that can switch between a commensal and pathogenic state is *C. albicans*, which inhabits the human GI tract of approximately 50% of the western population (Bougnoux et al. 2006). It can cause life-threatening systemic infections with the GI tract representing the major source (Gouba and Drancourt 2015). *C. albicans* can become pathogenic, e.g. after antibiotic treatment, and transitions from yeast to hyphal growth, which can result in the invasion of the epithelial layer and subsequent dissemination into the bloodstream (Filler and Sheppard 2006; Kornitzer 2019). In the pathogenic state, *C. albicans* uses a range of mechanisms to enter intestinal tissue such as transcellular translocation, which induces necrosis in epithelial cells, and induction of endocytosis by M cells (Albac et al. 2016; Allert et al. 2018). Epithelial cells release chemokines and proinflammatory cytokines upon recognition of *C. albicans*; however, the immune system seems to be only activated when a certain threshold of fungal burden is reached (Tong and Tang 2017).

1.4 Models to study host-microbiota interactions

The increasing interest in the intestinal microbiota is accompanied by advances in -omics technologies, such as metabolomics and metatranscriptomics, which help to identify microbial molecules exerting effects on the host and affected genes of both the host and intestinal habitant (Heintz-Buschart and Wilmes 2018). Mouse models have played a crucial role in microbiota research and are commonly used to investigate the complex interactions of the microbiota and the host on a whole-body scale (Faith et al. 2010; Franklin and Ericsson 2017). Many physiological and immunological aspects are conserved between mice and humans, however, there are also substantial differences between the species. For example, the diet differs since mice are exclusively herbivores unlike most of the human population; the human mucosal surface in the small intestine has folds called *plicae circularis* that are inhabited by mucus-associated bacteria and are absent in mice. Generally, mice display a thicker mucus layer, lower intestinal pH values and oxygen tension in the mucus than humans. Moreover, the microbiota composition of mice heavily depends on the provider, strain, and housing conditions, which influences the reproducibility of the studies (Friswell et al. 2010; Hugenholtz and de Vos 2018). In addition, there are many immunological differences concerning innate and adaptive immune responses, especially DC and T cell biology (Gibbons and Spencer 2011; Mann et al. 2013; Mestas and Hughes 2004). These

differences can impede the translation of findings from mouse models to the human situation.

In vitro models represent an emerging tool for microbiota research (Arnold et al. 2016). Standard two-dimensional (2D) cell culture using human primary cells or cell lines is widely used but has major limitations regarding microbiota studies and tissue physiology. Static cell culture leads to an overgrowth of bacteria and an accumulation of metabolic waste products. The cells are deprived of shear stress, which is pivotal for normal tissue function of many cell types. In addition, these models lack 3D tissue architecture (Basson 2003; Mosig 2016; White and Frangos 2007). Recently, advanced *in vitro* models have been developed to create organ-specific tissue in a more physiological environment. In these platforms, called organ-on-chip (OoC) or microphysiological systems (MPS), cells are usually cultured on permeable membranes, which are embedded in fabricated biochips. Shear stress and flow can be imitated using microfluidic technologies. MPS allow the simultaneous culture of multiple cell types in organ-specific tissue architecture and multiple MPS have already been established mimicking organs such as heart, liver, and lung (Groger et al. 2016; Huh 2015; Mathur et al. 2015). The use of intestinal OoC platforms allows dissection of the microbiota-host interplay in a tightly controlled, reproducible environment. Modeling the complex and unique structures of the GI tract is challenging.

Several intestine-in-chip models have recently been developed sharing some common features (Ashammakhi et al. 2020). This includes optically transparent and gas permeable biochip material, enabling the monitoring of the growth of cells by light microscopy, and gas exchange. Initially, cells are kept statically to allow them to adhere to the membrane. Furthermore, standard readouts comprise confocal fluorescence microscopy, assessment of barrier integrity via transepithelial electrical resistance (TEER) measurement or fluorescent molecules like FITC-dextran, and analysis of signaling molecules and metabolites in the supernatants. In contrast to conventional 2D cell culture, the overgrowth of bacteria can be prevented by continuous perfusion. The pioneering study was conducted by Kim and colleagues in 2012, showing that perfused adenocarcinoma-derived epithelial cells Caco-2 can grow out and form villi and crypt-like structures within five days (Kim et al. 2012). It is known that villus morphogenesis is mediated by Wnt signaling. Caco-2 cells basolaterally secreted the Wnt-antagonist Dickkopf-1 (DKK-1), which prevented villus morphogenesis in static cell culture. It was demonstrated that removal of DKK-1 by basolateral perfusion and

flowrate-dependent upregulation of the Wnt receptor FZD9 induced villus morphogenesis (Shin et al. 2019). The generated structures did not only resemble the 3D architecture of the small intestine but also allowed the colonization with *L. rhamnosus* for over one week (Kim et al. 2012). The human-microbial crosstalk (HuMIX) model created by Shah and co-workers in 2016 followed another approach and integrated an oxygen gradient (Shah et al. 2016). The model consisted of three compartments, with the lowest being perfused with oxygenated cell culture medium, supplying Caco-2 cells cultured in the overlaying compartment. The third and uppermost compartment harbored *L. rhamnosus* grown on a mucus layer under hypoxic conditions to mimic the *in vivo* oxygen content. By co-culturing another commensal *Bacteroides caccae*, the researchers were able to show that the transcriptomic response of the host cells differed from the one observed when only *L. rhamnosus* was present. A combination of *L. rhamnosus* and prebiotics, non-digestible dietary fibers promoting the growth of beneficial microbes, were used in a follow-up study (Greenhalgh et al. 2019). It was demonstrated that the metabolites produced during microbial fermentation of dietary fibers induced downregulation of genes associated with drug resistance and procarcinogenic pathways in Caco-2 cells. The development of intestine-on-chip models that recreate a physiological environment and enable the colonization with commensal bacteria have great potential to broaden our understanding of microbiota-host interactions and make important contributions to biomedical research.

2. Aim of the thesis

The intestinal microbiota has attracted considerable attention over the last 15 years. Sophisticated *in vitro* models based on human cells are needed to elucidate host-microbiota interactions and the interplay of commensals and pathogens in a physiological microenvironment. Many intestine-on-chip platforms have been developed but lack cells of the innate immune system. The presence of phagocytes in the system is indispensable to study microbiota-host interactions under conditions resembling the *in vivo* situation. Macrophages and DCs contribute to homeostasis in the GI tract yet seem to have different functional properties. For the sake of simplicity, “we” is used in the following, irrespective of whether the experiments were carried out by me alone or by colleagues and collaborators. The individual contributions are listed under “Author contribution statement”. We developed a microfluidic intestine-on-chip model comprising of two individually perfused compartments, which were separated by a porous membrane. The vascular compartment harbored human umbilical vein endothelial cells (HUVECs) and phagocytes, while Caco-2 cells in the luminal compartment formed 3D villi-like structures that resembled the physiological architecture of intestinal tissue. In this study, we aimed to characterize phagocytes in the endothelial as well as in the epithelial layer in respect of macrophage and DC markers and phagocytic capacity. To enhance the physiological relevance of the model, the endothelial layer was perfused with PBMCs. In the next step, we established a protocol to colonize the model with the probiotic strain *L. rhamnosus*. We studied how the presence of these commensal bacteria affected immune signaling and barrier integrity under two distinct conditions. In the first set up, LPS was administered to the luminal compartment representing homeostatic conditions. In a second set up, LPS was added to vascular compartment to mimic endotoxemia. As proof of concept, we performed microbial interaction studies by co-culturing *L. rhamnosus* with the opportunistic fungus *C. albicans*. We investigated whether the presence of the probiotics affected fungal growth, tissue damage and fungal translocation into the vascular compartment. We also discussed *in vitro* models used for the investigation of fungal infections. *Aspergillus fumigatus*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *C. albicans* are responsible for the largest share of fungal infections worldwide. We aimed at highlighting recent progress in organ-on-chip technologies that can alleviate the development of treatment strategies.

3. Manuscripts

3.1 Manuscript I

A three-dimensional immunocompetent intestine-on-chip model as *in vitro* platform for functional and microbial interaction studies

---

Michelle Maurer, Mark S. Gresnigt, Antonia Last, Tony Wollny, Florian Berlinghof, Rebecca Pospich, Zoltan Cseresnyes, Anna Medyukhina, Katja Graf, Marko Gröger, Martin Raasch, Fatina Siwczak, Sandor Nietzsche, Ilse D. Jacobsen, Marc Thilo Figge, Bernhard Hube, Otmar Huber, Alexander S. Mosig

Published in:

Biomaterials, 2019, 220:119396

The human intestinal tract is a unique ecosystem that has attracted increasing attention within the last 15 years or so. It is appreciated that the composition of microorganisms, comprising bacteria, fungi, and protozoa, has a far-reaching impact on human health and disease. It is generally accepted that the administration of probiotic bacteria conveys a health benefit, given the appropriate amount. However, we are just beginning to understand the crosstalk between host cells, commensal microbiota, and potentially pathogenic microbes. Recently developed microphysiological systems (MPS) hold the potential to study the crosstalk in a physiological microenvironment. We have developed a microfluidic intestine-on-chip model that mimics the three-dimensional structure of intestinal tissue, includes an endothelial layer, macrophages, and dendritic cells, and can be colonized with the probiotic strain *L. rhamnosus*. Immune cells tolerate these probiotics but elicit an inflammatory response when endotoxemia is mimicked by the administration of lipopolysaccharide into the endothelial compartment. Moreover, the intestinal model serves as a platform for functional microbial studies. *L. rhamnosus* significantly reduces the growth of *C. albicans* and limits its translocation from to luminal into the endothelial layer. The intestine-on-chip model serves as a useful tool to investigate intestinal crosstalk and the effect of alterations in the community.

Biomaterials 220 (2019) 119396

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

A three-dimensional immunocompetent intestine-on-chip model as *in vitro* platform for functional and microbial interaction studies

Michelle Maurer[a,c], Mark S. Gresnigt[b], Antonia Last[b], Tony Wollny[c], Florian Berlinghof[c], Rebecca Pospich[d,e], Zoltan Cseresnyes[f], Anna Medyukhina[f], Katja Graf[b], Marko Gröger[a,g], Martin Raasch[a,c], Fatina Siwczak[a], Sandor Nietzsche[i], Ilse D. Jacobsen[a,d,h], Marc Thilo Figge[f,h], Bernhard Hube[b,h], Otmar Huber[a,c], Alexander S. Mosig[a,c,*]

[a] Center for Sepsis Control and Care, Jena University Hospital, Friedrich-Schiller-University of Jena, Jena, Germany
[b] Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knoell-Institute, Jena, Germany
[c] Institute of Biochemistry II, Jena University Hospital, Jena, Germany
[d] Research Group Microbial Immunology, Leibniz Institute for Natural Product Research and Infection Biology-Hans Knoell Institute, Jena, Germany
[e] Division of Immunodermatology and Allergy Research, Hannover Medical School, Hannover, Germany
[f] Research Group Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany
[g] University of California, UCSF Broad Center of Regeneration Medicine and Stem Cell Research, San Francisco, USA
[h] Institute of Microbiology, Faculty of Biological Sciences, Friedrich Schiller University, Jena, Germany
[i] Centre for Electron Microscopy, Jena University Hospital, Friedrich-Schiller-University of Jena, Jena, Germany

ARTICLE INFO

Keywords:
Microphysiological system
Gut-on-chip
Microbiota
Mucosal immunity
Candida albicans
Lactobacilli

ABSTRACT

Alterations of the microbial composition in the gut and the concomitant dysregulation of the mucosal immune response are associated with the pathogenesis of opportunistic infections, chronic inflammation, and inflammatory bowel disease. To create a platform for the investigation of the underlying mechanisms, we established a three-dimensional microphysiological model of the human intestine. This model resembles organotypic microanatomical structures and includes tissue resident innate immune cells exhibiting features of mucosal macrophages and dendritic cells. The model displays the physiological immune tolerance of the intestinal lumen to microbial-associated molecular patterns and can, therefore, be colonised with living microorganisms. Functional studies on microbial interaction between probiotic *Lactobacillus rhamnosus* and the opportunistic pathogen *Candida albicans* show that pre-colonization of the intestinal lumen of the model by *L. rhamnosus* reduces *C. albicans*-induced tissue damage, lowers its translocation, and limits fungal burden. We demonstrate that microbial interactions can be efficiently investigated using the *in vitro* model creating a more physiological and immunocompetent microenvironment. The intestinal model allows a detailed characterisation of the immune response, microbial pathogenicity mechanisms, and quantification of cellular dysfunction attributed to alterations in the microbial composition.

1. Introduction

Commensal microorganisms of the intestinal microbiota support the digestion and absorption of nutrients by the gut. Microbial colonization is supported by the host *via* a mucus layer secreted by epithelial cells organized in a complex tissue comprising villi and crypts that form a tight and protective barrier between the microbiota and the circulation. A physiological communication between the members of the intestinal microbiota and their host is crucial for the maintenance of homeostasis in the human body. Thus, dysregulation and imbalance of these interactions known as dysbiosis are directly associated with the development of human diseases, including diabetes [1], obesity [2], inflammatory bowel disease (IBD) [3], cancer [3], depression [4] and non-infectious inflammatory diseases caused by opportunistic pathogenic fungi [5].

However, current knowledge on the impact of the microbiota on health is based solely on descriptive and correlative studies. So far, the lack of suitable experimental models prevented mechanistic studies on the complex cellular and molecular signalling processes within the microbiota under physiological conditions. Current *in vitro* models lack

* Corresponding author. ASM Center for Sepsis Control and Care, Jena University Hospital, Friedrich-Schiller-University of Jena, Jena, Germany.
E-mail address: alexander.mosig@med.uni-jena.de (A.S. Mosig).

https://doi.org/10.1016/j.biomaterials.2019.119396
Received 14 May 2019; Received in revised form 8 July 2019; Accepted 28 July 2019
Available online 02 August 2019
0142-9612/

the required tissue complexity with major limitations for the co-culture of living bacteria with human intestinal cells. On the other hand, animal models have limitations in the transferability to the human situation, since the composition of the microbiota, as well as immune system considerably varies between men and mice and even between individual mouse strains [6]. Established cell lines, such as Caco-2 cells, cultured as monolayers on extracellular matrix-coated standard plastic dishes or transwell inserts are frequently used to study human intestinal epithelial cells *in vitro*. Although these two-dimensional culture techniques have elucidated numerous aspects of intestinal epithelial cell biology, it should be noted that they do not recreate typical microanatomical structures of the human intestine.

The intestine is the primary site for interaction of the gut microbiota consisting of commensal microorganisms and opportunistic pathogens [7]. The continuous exposure to microbial-associated molecular patterns (MAMPs) in the intestinal lumen requires the immune system to weigh tolerogenic and protective responses continuously. It is becoming increasingly clear that dysregulation of this tolerant immune response and alterations in the gut microbiota contribute to chronic inflammatory diseases and breakdown of the gut barrier [3,8]. Although three-dimensional organoid cultures allow the *in vitro* differentiation of microanatomical structures in high detail [9], these models have limitations in their ability to reflect a tolerant immune response to the colonising microbiota. Recently a microfluidically perfused model of the human intestine has been described that allows circulation of peripheral blood mononuclear cells (PBMCs) and effectively recapitulates structural features of intestinal microanatomy and physiology, including a polarised intestinal tissue differentiation [10,11]. Although this system represents a major advancement in emulating the human intestine, models are still required that are capable of stably cultivating commensal microbiota in an immune responsive environment and consider the balanced response to the microbiota and its metabolites.

The Toll-like receptor 4 agonist lipopolysaccharide (LPS) derived from gram-negative bacteria is a ubiquitously present MAMP in the human intestinal lumen [12]. However, upon dysfunction of the intestinal barrier, LPS can translocate into the circulation and in more substantial amounts eventually triggers a systemic inflammatory response syndrome associated with multiple organ failure [13]. Phagocytes play a critical role in gatekeeping MAMPs between the intestinal lumen and the circulation. In mice, it has been shown that tissue-resident phagocytes such as mucosal macrophages (mMphs) and dendritic cells (DCs) are central players in the release of inflammatory cytokines [14]. Both cell types populate the normal human intestinal mucosa but play distinct complementary roles in the selective local immune response and tissue homeostasis with mMphs as the most abundant mononuclear phagocytes in the intestinal lamina propria [15]. They account for most of the uptake of microbes that cross the epithelial barrier and are required to maintain an anti-inflammatory milieu in the mucosa by scavenging of apoptotic and damaged cells [16,17]. The expression of G protein-coupled chemokine receptor CX3CR1 on mMphs has been demonstrated as a central receptor in regulating intestinal barrier integrity [18,19]. The homeostatic function of $CX3CR1^+$ mMphs is complemented by $CD103^+$ DCs that represent another primary antigen-presenting cell type of the intestine [20]. These cells continually sample their environment for antigens derived from food, microbiota, and self-antigens [21] by extension of transepithelial dendrites into the intestinal lumen [22]. During homeostasis, intestinal DCs are considered to be tolerogenic, whereas mMphs are actively involved in tissue remodelling.

To resemble these conditions *in vitro*, we developed a three-dimensional model of the human intestine composed of endothelial and epithelial cell layers forming organotypic microanatomical villus- and crypt-like structures. For the first time, we demonstrate physiological interactions between epithelial and endothelial cells in an immunocompetent environment. This is created by tissue resident mMphs and DCs in a microphysiological model resembling essential conditions within the human intestine. We studied the biological intestinal homeostasis, barrier functionality and immunotolerance to stimulation with LPS at the intestinal luminal side of the model. To emulate dysregulation, imbalance and inflammation, the endothelial side was stimulated with LPS in a model of endotoxemia. Furthermore, we colonized the intestinal model with non-damaging living bacteria (*Lactobacillus rhamnosus*). Similar to *in vivo,* where the intestinal microbiota antagonizes microbial associated pathogenicity by limiting overgrowth and translocation through the intestinal epithelial barrier, we validated *L. rhamnosus'* antagonistic interactions on the opportunistic pathogenic fungus *Candida albicans* in our intestinal model.

2. Material and methods

2.1. Cell isolation and culture

Endothelial cells: Human umbilical cord vein endothelial cells (HUVECs) were isolated and seeded at a density of 2.5×10^4 cells/cm^2 in Endothelial Cell Medium (ECM) (Promocell, Heidelberg, Germany) up to passage 4 as described previously [23]. The study was approved by the ethics committee of the Friedrich Schiller University Jena (2018–1052), and all donors were informed about the aim of the study and gave written consent.

Caco-2 cells: The human epithelial colorectal cell line Caco-2 was kindly provided by the Institute for Laboratory Medicine, Clinical Chemistry and Pathobiochemistry, Charité - University Medicine Berlin. Cells were cultured in DMEM (GIBCO Darmstadt, Germany) supplemented with 10% foetal calf serum (FCS, Life Technologies), 20 ng/ml gentamicin (Invitrogen), 1% MEM non-essential amino acids (GIBCO). 5×10^5 Caco-2 cells are seeded in a 25 cm^2 flask and cultured in DMEM medium (Life Technologies) containing 10% FCS. The medium was changed every three to four days and cells used from passage 30 to 50.

Peripheral blood mononuclear cells and primary macrophages: PBMCs of three different healthy donors were isolated by Ficoll density gradient centrifugation as described previously [66] and seeded in 6- well plates with a density of 1.0×10^6 cells/cm^2 in X–VIVO 15 medium (Lonza, Cologne, Germany) supplemented with 10% (v/v) autologous human serum, 10 ng/ml human granulocyte macrophage colony-stimulating factor (GM-CSF) (PeproTech, Hamburg, Germany), 10 ng/ml M-CSF (PeproTech), 100 U/ml penicillin and 100 µg/ml streptomycin (Pen/Strep) (Thermo Fisher, Darmstadt, Germany). After 1 h incubation in a humidified cell incubator at 5% CO_2 and 37 °C the cells were washed twice with X–VIVO 15 medium and cultivated for 24 h before subcultured with endothelial cells in the biochip were terminal macrophage differentiation was performed in the supplemented X–VIVO 15 medium.

2.2. Biochip cell culture

MOTiF biochips made from polystyrol (PS) were obtained from microfluidic ChipShop GmbH (Jena, Germany). Biochips were manufactured by injection moulding as described elsewhere [23]. The chamber above the membrane has a height of 700 µm; the chamber under the membrane has a height of 400 µm. The width of the afferent and efferent channels is 0.8 mm and 2 mm, respectively. The height of these channels is 0.6 mm and 0.4 mm, respectively. Upper and the lower chamber including channel systems have a volume of 220 µl and 120 µl, respectively. A 12 µm thin polyethylene terephthalate (PET) membrane with a pore diameter of 8 µm and a pore density of 1×10^5 pores/cm^2 (TRAKETCH Sabeu, Radeberg, Germany) was integrated. An area of 1,1 cm^2 is available for cell culture. Chips and channels structures were sealed on top and bottom side with an extruded 140 µm thin PS foil using a low-temperature proprietary bonding method. Gas permeable silicon tubing was used for perfusion allowing oxygen equilibration during experiments. Additionally, PS bulk material and 140 µm thin PS bonding foil allowed re-diffusion of oxygen. Ramping

structures have been introduced into the chip bulk for prevention of unfavourable flow conditions and trapping of stationary air bubbles. For hydrophilization of the whole surface, the biochips were treated with oxygen plasma.

Intestine-on-chip models were assembled by staggered seeding of endothelial and epithelial cell layers. HUVECs were seeded with a density of $1.5 \times 10^5\,cm^{-2}$ in the upper chamber of a sterilised biochip in EC medium. Following 48 h of culture, macrophages were seeded on top of the confluent HUVEC cell layer with a density of $5 \times 10^4\,cm^{-2}$ in M199 medium (Life Technologies) supplemented with 10% FCS), 10% (v/v) autologous serum, 68×10^{-7} M L-glutamine (Sigma-Aldrich), 25 μg/mL heparin (Sigma- Aldrich), 7.5 μg/ml endothelial mitogen (Thermo Fisher), 5 μg/ml ascorbic acid (Sigma-Aldrich), 10 ng/ml GM-CSF, 10 ng/ml M-CSF and Pen/Strep. After 72 h of static culture and daily medium exchange, Caco-2 cells were seeded in the bottom chamber of the biochip with a density of $4{,}5 \times 10^5\,cm^{-2}$ and cultured in DMEM. The inlets of each biochip were sealed and the whole intestinal model cultured upside down for 24 h under static conditions. Subsequently, the fully assembled organ-on-chip model was used in experimental series as described. The endothelial side of the model was perfused with M199 medium with a flow rate of 50 μl/min (shear stress: 0.07 Pa). The luminal side of the model was perfused with 50μl/min (shear stress: 0.01 Pa). For LPS stimulation cells were treated with 100 ng/ml LPS (Sigma-Aldrich, Taufkirchen, Germany). Supernatants were collected and replaced with fresh medium containing 100 ng/ml LPS after 24 h. Supernatants were collected again after an additional 24 h. Cells were cultured in a humidified cell incubator at 5% CO2 and 37 °C.

2.2.1. Microbial strains and culture conditions

L. rhamnosus ATCC 7469 was grown on Man, Rogosa, and Sharpe (MRS) agar plates (Carl Roth, Karlsruhe, Germany) at 37 °C and 1% O_2. For use in experiments, bacterial cells were grown at 37 °C without agitation in MRS broth (Carl Roth, Karlsruhe, Germany). Prior to experiments, lactobacilli were collected by centrifugation, washed twice in PBS and diluted to an optical density OD_{600nm} of 0,5 in DMEM.

The *C. albicans* wild-type strain SC5314 was grown on YPD plates (2% peptone, 1% yeast extract, 2% glucose, 2% agar) at 30 °C. For use in experiments, *C. albicans* cells were grown overnight (o/n) in YPD medium (2% peptone, 1% yeast extract, 2% glucose) at 30 °C and 180 rpm. Prior to infection, yeast cells from an o/n culture were collected by centrifugation, washed two times with phosphate-buffered saline (PBS), the cell number was determined using a Neubauer chamber system, and adjusted to 5×10^3 cells/120 μl in DMEM.

Host cells were cultured in antibiotic-free medium for 72 h. After 24 h of LPS treatment, the bacterial suspension was inoculated onto the epithelial layer and incubated for 30 min to allow attachment to the epithelium. Subsequently, unattached cells were removed and PBMCs inoculated. The endothelial layer was cyclically perfused with a flow-rate of 50μl/min (shear stress: 0.07Pa) while the luminal chamber was linearly perfused with 25μl/min (shear stress: 0.03Pa). After 24 h of culture in the chip, supernatants were plated on MRS agar (Carl Roth). The membrane was lysed in 0.2% Triton-X 100 for 15 min at RT and removed before the suspension was pelleted, washed with PBS and plated.

2.3. Immunofluorescence staining

Cells were fixed with either 4% paraformaldehyde for 10 min at room temperature or methanol for 20 min at −20 °C. For permeabilization and blocking of unspecific binding sites, cells were incubated in PBS including 0.1% saponin (Sigma Aldrich) and 3% goat serum (Dianova, Hamburg). Staining was performed with antibodies against CD68, CD103, E-cadherin, VE-cadherin, β-Catenin (BD Biosciences, Heidelberg, Germany), occludin, ZO-1 (Invitrogen, Karlsruhe, Germany), CEACAM-1, CX3CR1, CYP3A4 (Merck-Millipore, Schwalbach, Germany), α-defensin (abcam, Cambridge, UK), mucin 2 (Acris, Herford, Germany), villin (Santa Cruz Biotechnology, Heidelberg, Germany), von Willebrand factor (Dako, Hamburg, Germany) and secondary antibodies goat-anti-mouse-Cy3, goat-anti-rabbit-AF488, goat-anti-rabbit-AF647, DAPI (Invitrogen) and Phalloidin (ThermoFisher). Samples were embedded in fluorescent mounting medium (Dako). Imaging was performed with an AxioObserver Z1 fluorescence microscope equipped with an ApoTome-2 (Carl Zeiss AG, Jena, Germany). Images were analysed with ImageJ2 software (Fiji).

2.4. Scanning electron microscopy

Cells were fixed with 2.5% (v/v) glutaraldehyde in cacodylate buffer for 120 min. Afterwards, the samples were washed twice with cacodylate buffer for 10 min and dehydrated in ascending ethanol concentrations (30, 50, 70, 90 and 100%) for 10 min each. Subsequently, the samples were critical-point dried using liquid CO_2 and sputter coated with gold (thickness approx. 4 nm) using a SCD005 sputter coater (BAL-TEC, Liechtenstein) to avoid surface charging. Finally, the specimens were investigated with a field emission (FE) SEM LEO-1530 Gemini (Carl Zeiss NTS GmbH, Oberkochen, Germany).

2.5. Image analysis and quantification

Images were acquired as Z-stacks utilising a Zeiss Apotome microscope and were saved in the Zeiss native image format „CZI". The Apotome images were first processed by Zeiss' ZEN software to provide optical sectioning. Further preprocessing included deconvolution using Huygens Professional (SVI, Hilversum, Holland), applying the „Spinning disk" deconvolution module by setting the pinhole spacing at 4 μm, according to SVI guidelines. The deconvolved images were analysed using Imaris 9.2.1 (Bitplane, Zürich, Switzerland). Automated quantification of confocal fluorescence microscopy data was performed by customised software, which was implemented in the programming language "python" (https://www.python.org/) and is available upon request.

Cell-based analysis: The three-dimensional image data were used to identify individual cells applying nuclear staining as guidance. Here the „Cells" module of Imaris was applied, where the DAPI channel was selected to create the cell nuclei, whereas the E−(VE-) cadherin or the ZO-1 labelling channel provided information about the membrane location. The segmented nuclei served as seeds to assist the search for the cell membranes. After successful segmentation, the area between the nuclei and the cell membrane was identified as cytoplasm. Particle analysis was applied when necessary, where the intensity and morphometry of additional components (for example the von Willebrand factor) was characterised in the cytoplasmic area.

Membrane-based analysis: The 3D image stacks were preprocessed in Zen and Huygens Professional as described above. In analysing the (V) E-cadherin and ZO-1 distribution, cell membranes were segmented using the Surfaces module of Imaris 9.2.1. The deconvolved Z-stacks were locally thresholded using the Otsu algorithm with a 2-μm neighbourhood diameter preceded by Gaussian blurring with a spatial filter size of 0.4 μm. The segmented membrane objects were limited to items with sphericity in the range of 0.0–0.9 and volume above 100 voxels in order to exclude artefacts. Macrophages were segmented as surfaces objects in Imaris. Here the Gaussian blurring was executed with a filter size of 0.645 μm, and the local Otsu thresholding was applied at a 2.4-μm neighbourhood diameter. The macrophage volume was limited to the 60–1200 μm [3] range in order to avoid detecting fragments or large clusters.

Quantification: Statistical information was extracted from the segmented images in Imaris 9.2.1. Here we measured the mean and the standard deviation of the fluorescence intensity for all channels within all regions of the segmented surfaces and cells; the area, the volume and

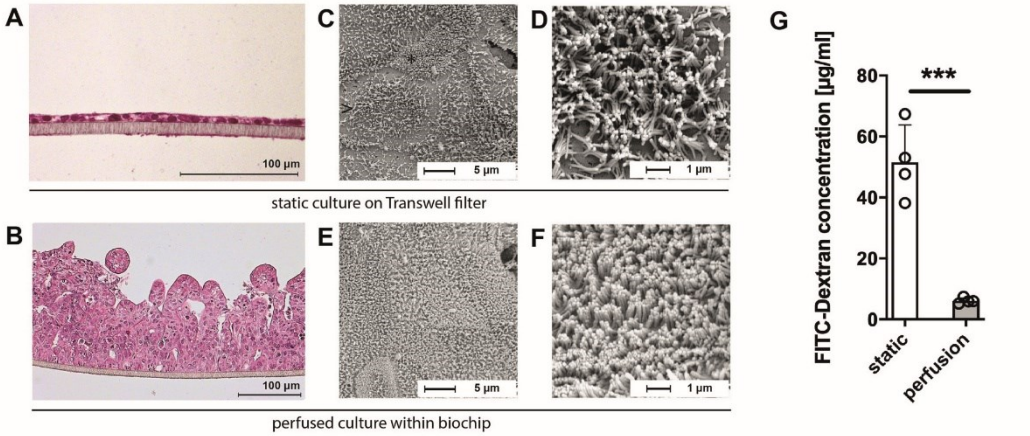


Fig. 1. Caco-2 cells form crypt and villus-like structures under perfusion conditions in the biochip. A, B) Histological H&E staining of Caco-2 cell layers cultured A) statically in the transwell, and B) under perfused conditions in the biochip. C-F) Scanning electron microscopy of Caco-2 cell layers cultured for 7 days under C, D) static conditions on transwell filters, and E, F) under perfusion conditions in the biochip. G) Permeability of epithelial cell layers for FITC-labelled dextran beads (3–5 kDa) under static and perfused conditions in the biochip. A-F) microscopy images are representative of three independent experiments, G) data from four independent experiments, data presentation shows the mean ± standard deviation., Statistical test was made using the Student's t-test, ****p < 0.0001.

the sphericity of the entire cells, the cell compartments and the cell membranes; the number of cells per Z-stack, etc. These analysis results were saved in Excel spreadsheets and used for statistical analysis and plotting.

Ellipticity index: The reconstructed surfaces were assigned a best-fitting ellipsoid in Imaris. According to the relationship amongst the main axis of the ellipsoid (a, b, c), we characterized the surfaces as prolate (a = b < c; cigar-shaped) and oblate (a < b = c; disk-shaped), by using the following indices:

$$e_{prolate} = \frac{2a^2}{a^2 + b^2} * \left(1 - \frac{a^2 + b^2}{2c^2}\right),$$

$$e_{oblate} = \frac{2b^2}{b^2 + c^2} * \left(1 - \frac{2a^2}{b^2 + c^2}\right).$$

2D analysis: Automated quantification of VE-cadherin staining in 2D was performed by customized software, which was implemented in the programming language Python. Prior to the analysis, images were preprocessed with a median filter (size 3 pixels) to remove spike noise. Then each image was thresholded at 100 intensity levels (out of 255, 8-bit) to obtain a binary mask of the cellular junctions. After that, connected regions were identified and regions with an area less than 20 µm [2] were assigned to a separate class of small particles. To evaluate the integrity of the cellular junctions, we computed the total area of cellular junctions, as well as the mean and maximum intensity of the cellular junctions. For computing the mean intensity, only those pixels were used that belonged to the foreground after the thresholding step. Moreover, we computed the total area and area fraction of the small particles. In the latter case, the area of the small particles was normalized by the total area of the cellular junctions.

2.6. Cytokine profiles

Supernatants were collected after indicated time periods and immediately frozen at −80 °C. Cytokines were detected using CBA assay (BD Biosciences) according to the manufacturer's protocol. Enhanced sensitivity flex set was used for the measurement of TNF and IL-1β release. Secretion of IL-6, IL-8 and IL-10 was analysed using standard CBA flex sets. The analysis was performed on a BD FACS-Canto II cytometer with FACSDiva software. Data analysis was performed using FCAP Array V3 software (Softflow, Pecs, Hungary).

2.7. Permeability assay

Fluorescein isothiocyanate (FITC)-dextran (Sigma-Aldrich) with an atomic mass of 3–5 kDa was used. The medium in both chambers is replaced by preheated PBS. Subsequently, 150 µl FITC-dextran solution is added in the lower chamber containing Caco-2 cells and is incubated for 30 min at 37 °C. The biochip is turned upside down during incubation allowing FITC-dextran to permeate through the cell layer under static conditions. After washing of the lower chamber with PBS, the solution was collected from the upper chamber. All steps were performed protected from light.

2.8. Statistics

For each experiment replicates have been performed as indicated in the figure legend. Statistical analysis has been performed with GraphPad Prism 6.05 (GraphPad Software, La Jolla, CA, USA). For analysis of statistical significance, the tests indicated in the figure legend have been performed. A p-value < 0.05 was considered statistically significant.

3. Results

The epithelial cell layer of the intestinal model is formed by Caco-2 cells cultured on a 10 µm thin porous polyethylene terephthalate (PET) membrane suspended in the biochip and continuously perfused with cell culture medium. During cell culture, microfluidic perfusion ensures a constant removal of metabolic waste products and a continuous resupply with nutrients [23]. In contrast to conventional cell culture approaches such as transwell filters (Fig. 1 A), Caco-2 cells cultured in microfluidically perfused biochips form a self-organised three-dimensional cell layer with columnar epithelium (Fig. 2 B) and increased microvilli formation at the apical cell surface within 7 days of culture (Fig. 1C - F). Permeability measurements confirmed an increased barrier function of the three-dimensional cell layer compared to conventional cell culture under static conditions where only two-

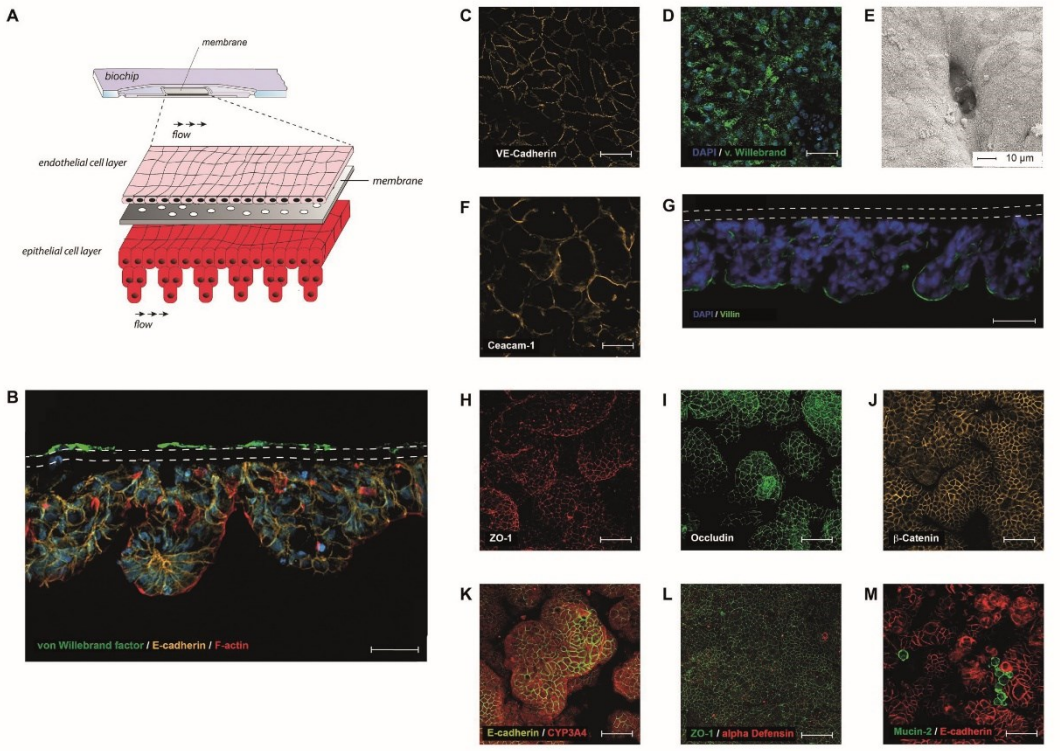


Fig. 2. Design of the intestine-on-chip model featuring organotypic microanatomy and expression of endothelial and intestinal epithelial cell type markers. A) A porous membrane suspended in the biochip serves as a scaffold for the multi-layered intestinal model composed of endothelial and epithelial cells. Microchannels integrated into the biochip separately perfuse both epithelial and endothelial layers at 50 µl/min. B) Cross-section of the three-dimensional intestinal model: endothelial cells express von Willebrand factor (green), epithelial cells express E-cadherin (orange) and F-actin (red). Both cell layers are separated by a porous membrane (dashed line). Actin filaments are stained with phalloidin (red). Scale bar 100 µm. Nuclei were stained with DAPI (blue). C-D) Endothelial cells form a confluent monolayer and express C) VE-cadherin (orange) and D) von Willebrand factor (green). E-N) Epithelial cell layer: E) Scanning electron microscopic image of the crypt-like structures. Expression of F) CEACAM-1 (orange); G) villin (green) (DAP blue, dashed lines marks membrane); H) ZO-1 (red); I) occludin (green); J) β-catenin (orange); K) E-cadherin (orange); CYP3A4 (red); L) α-defensin (red); ZO-1 (green); M) mucin-2 (green); E-cadherin (red); C-D, F-J) Scale bar 50 µm. Nuclei were stained with DAPI (blue). C-M) Representative images of four independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

dimensional Caco-2 monolayers are formed (Fig. 1G).

The multi-layered tissue model was separately perfused by two microchannels at the endothelial and the luminal side of the model (Fig. 2A). The vasculature of the intestine was mimicked by co-culture of HUVECs on the membrane opposite to the epithelial layer. Endothelial cells formed a confluent lining in the biochip that express endothelial cell marker proteins VE-cadherin (Supplementary Video S1) and von Willebrand factor (vWF) (Fig. 2B–D). In the epithelial cell layer, we observed the cellular self-organisation into three-dimensional structures with morphological features of crypts and villi that fully cover the membrane serving as cell substrate (Fig. 2 E-J, Supplementary Video S2). The formation of a three-dimensional Caco-2 cell layer upon microfluidic perfusion has also been reported by Ingber and colleagues, demonstrating the differentiation of these cells into a polarized columnar epithelium that contains cells with markers of absorptive, mucus-secretory, enteroendocrine, and Paneth cell populations [11]. Also in our model, villus-like structures appear polarised and express at the outer cell borders the carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1, Fig. 2 F), a protein expressed by intestinal epithelial cells (IEC) *in vivo* at the apical surface of intestinal villi [24]. Further, villin, a protein controlling the shape and motility of IEC by interconnecting the brush borders to the cytoskeleton [25] was found expressed at the tip of villus-like structures (Fig. 2 G). These structures present themselves in a well-defined organisation expressing tight junction proteins ZO-1 and occludin (Fig. 2H, I), both critically involved in the maintenance of barrier function [26]. Further, β-catenin (Fig. 2 J), an essential regulator of epithelial cell proliferation and differentiation [27], and cytochrome P450 3A4 (CYP3A4, Fig. 2 K), a major CYP enzyme responsible for metabolizing over fifty percent of prescribed drugs [28] are stably expressed by the epithelial cell layer. In contrast to static culture, Caco-2 cells differentiate under perfusion conditions into cell-subtypes expressing specific cell type markers such as α-defensin, a marker of Paneth cells (Fig. 2 L) and Mucin-2, a marker protein of Goblet cells (Fig. 2M).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.biomaterials.2019.119396

The lack of tissue-resident mMphs and DCs is a limitation of most currently available *in vitro* gut models. These two cell types are required to initiate immune responses causing inflammation and to prevent pathogen dissemination, yet also for mediation of immunotolerance to

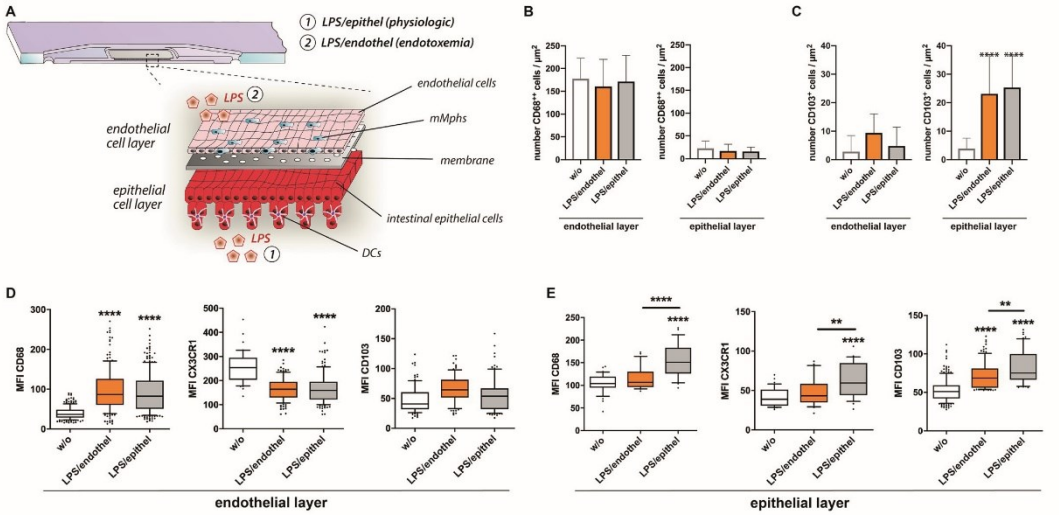


Fig. 3. Mobility and phenotype marker expression of mPCs upon LPS stimulation. A) The distribution pattern of mPCs with the majority of mMphs populating the endothelial cell layer and DCs residing in the epithelial cell layer. Two different LPS stimulation conditions were tested for recreating 1) physiological contact of epithelial cells with LPS; and 2) conditions of endotoxemia, with LPS exposed to the endothelial cell layer. B-C) Total number of B) $CD68^{++}$ cells or C) $CD103^{+}$ cells per µm [2] within the endothelial or epithelial cell layer of the non-stimulated model (w/o), and the model stimulated at the endothelial side (LPS/endothel) or the epithelial side (LPS/epithel). D-E) Mean fluorescence intensity (MFI) of CD68, CX3CR1 and CD103 expression by mPCs in the D) endothelial and E) epithelial cell layer. Whisker plots: 10th – 90th percentile, the box represents the 25th and 75th percentile with the line in the box marking the median. Data points outside whiskers mark outliers. A-D) Statistical testing with one-way ANOVA and Tukey's correction, $^{**}p < 0.01$, $^{****}p < 0.0001$ vs. non-stimulated condition (w/o); Data of five independent experiments is shown.

commensal bacteria and derived MAMPs in the gut lumen. To simulate these functions *in vitro*, human primary monocytes were seeded on the endothelial cell layer and differentiated in the presence of macrophage colony stimulating factor (M-CSF), and granulocyte-monocyte colony stimulated factor (GM-CSF). After 7 days of culture, monocytes differentiated into two phenotypically distinct monocyte-derived phagocyte subsets (mPCs) resembling features of mMphs and DCs, respectively. The functionality of these mPCs was studied in the presence of LPS, both at the endothelial and epithelial cell layer (Fig. 3 A). Expression of CX3CR1 with a high level of CD68 ($CD68++$) defined mMph surrogates, whereas DCs were defined by expression of lower CD68 levels ($CD68^{+}$) and the presence of the DC marker integrin alpha E (CD103). It has been reported that mMphs depend on CX3CR1 to be maintained within the lamina propria and to prevent translocation of commensal bacteria to mesenteric lymph nodes [29]. In accordance with these reports, we found that LPS exposure to the intestinal luminal side of the model triggered mMphs and DCs to self-organise within the tissue. LPS stimulation at the intestinal luminal side supported the formation of $CD68^{+}/CD103^{+}$ DCs and triggered DC invasion into the epithelial cell layer (Fig. 3C, D, E) with some of these DCs forming dendrites through the epithelial cell layer contacting the luminal space (Supplementary Figure 1). In mice, lamina propria-resident $CD103^{+}$ DCs were shown to migrate into the epithelium during homeostasis to facilitate capture of bacterial antigens [21]. In the intestinal model, a significantly increased expression of CD103 by DCs was observed at the luminal side upon epithelial cell stimulation (Fig. 3 D, E, Supplementary Figures 2 and 3). Thus, a physiological contact of epithelial cells with LPS contributed to improved DC maturation within the epithelial cell layer. LPS stimulation also upregulated CD68 and CX3CR1 in mMphs that predominately populate the endothelial cell layer (Fig. 3 B, D, E).

Endotoxemia represents a disease condition in which LPS leaks into the bloodstream. This condition was simulated by exposing the endothelial lining to LPS. Although we observed an upregulation of CD68 in mPCs, we were unable to find increased upregulation of CX3CR1 in mMphs within the epithelial cell layer as observed upon intestinal luminal LPS stimulation. Further, CD103 expression by DCs was significantly decreased in the epithelial barrier compared to LPS stimulation at the luminal side (Fig. 3 B - E). These results demonstrate guided mobility and adapted activation pattern of mPCs depending on the microenvironment created by the endothelial and epithelial cell layers with their individual responsiveness to LPS exposure.

Subsequently, we studied the responsiveness of mPCs depending on their differentiation and distribution pattern induced by the different LPS stimulation sites. In general, no adverse effects were observed on barrier functionality despite the presence of mPCs in the model, irrespective of the side of LPS exposure (Fig. 4 A). Under simulated physiological conditions with LPS present at the luminal side of the model, we observed immunotolerance reflected by the absence of a release of pro-inflammatory cytokines. However, emulated endotoxemia with LPS exposure to the endothelial layer induced a robust inflammatory response characterized by release of TNF, IL-1β, IL-6, and IL-8 (Fig. 4B–E).

The proinflammatory cytokines TNF, IL-1β, IL-6, and IL-8, are essential mediators of human intestinal inflammation and LPS-induced cell damage [30]. Moreover, these cytokines are critically involved in the loss of intestinal barrier function frequently observed in IBD [31,32]. A decrease in the expression of endothelial VE-cadherin, and E-cadherin/ZO-1 expressed by IECs is directly associated with intestinal permeability in IBD [33]. Similar observations were made in our intestinal model, were direct exposure of the endothelial lining to LPS significantly reduced expression of VE-cadherin with a diffuse and disrupted expression pattern along the cell borders. In addition, an analysis of endothelial cells by quantification of the cellular ellipsoid index (0 = line, 1 = circle) revealed significant morphological

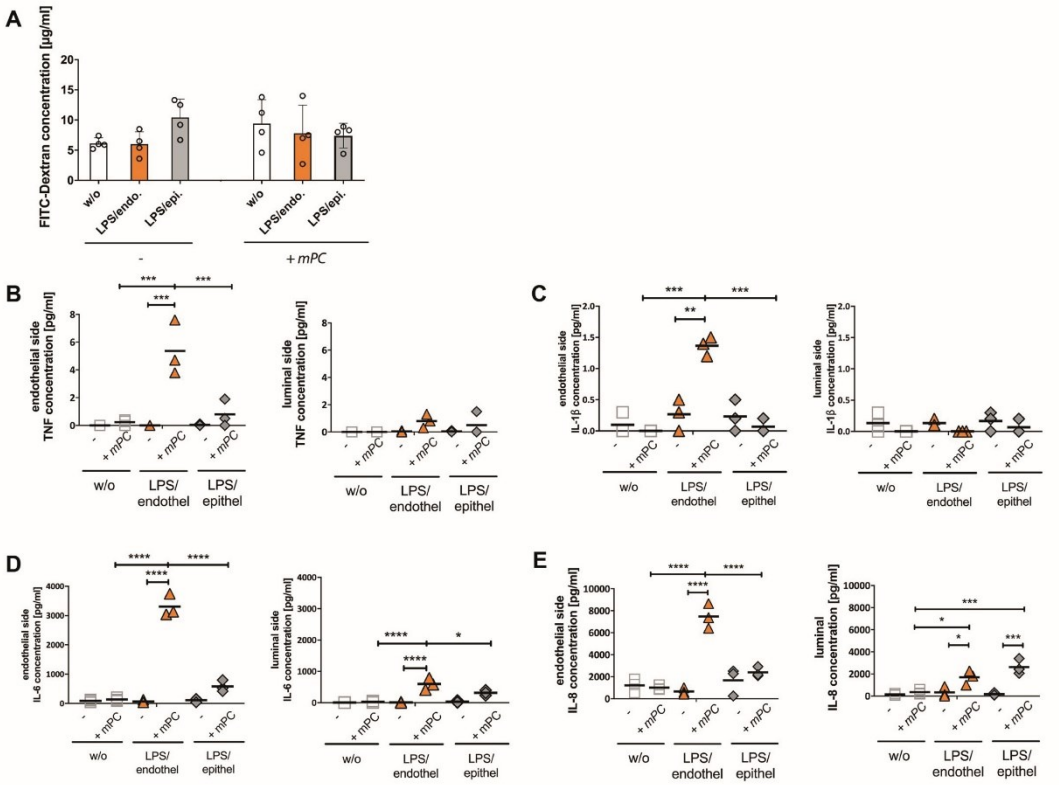


Fig. 4. Permeability and cytokine release upon LPS stimulation. A) Permeability of the intestinal model for FITC-dextran, and B-E) release of proinflammatory cytokines B) TNF, C) IL-1β, D) IL-6, and E) IL-8 at the endothelial or luminal side of the intestinal model without LPS stimulation (w/o), and upon LPS stimulation at the endothelial cell layer (LPS/endothel) or the epithelial cell layer (LPS/epithel) in absence (−) or presence (+) of mPCs. B-E) data is shown as scatter plots with mean values ± standard deviation, statistical significance was calculated by two-way ANOVA with Sidak's multiple comparisons test (****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). Data of three independent experiments are shown.

alterations of endothelial cells directly exposed to LPS. Endothelial cells exposed to LPS appeared elongated with a diffuse VE-cadherin expression, indicating endothelial dysfunction [34]. In contrast, we observed an even increased VE-cadherin expression and no alterations of endothelial morphology when LPS was perfused at the intestinal lumen of the model (Fig. 5A and B; Supplementary Figure 4). Similar observations were made for E-cadherin and ZO-1 expression in the epithelial cell layer. For both proteins, we found an increased expression level when the epithelial cell layer was stimulated with LPS. However, the expression of E-cadherin and ZO-1 was significantly reduced when the endothelial cell layer was exposed to LPS (Fig. 5C, D; Supplementary Figs. 4B and C).

The observed immunotolerance at the luminal side represents a crucial physiological feature required for stable colonisation with living bacteria in the presence of functional mPCs. Therefore, we next colonised the luminal side with the probiotic bacterium *L. rhamnosus* in the presence of LPS at the luminal side resembling physiological conditions. Alternatively, LPS was added to the endothelial layer mimicking endotoxemia. We observed that independent from the side of LPS stimulation, *L. rhamnosus* stably colonised the epithelial cell layer without translocating through the tissue barrier (Fig. 6 A). Further, a trend towards an improved barrier function was observed upon luminal colonisation with *L. rhamnosus* (Fig. 6 B). Infection of the intestinal tract is often accompanied by infiltration of the intestine by leukocytes [35]. We therefore introduced circulating PBMCs into the vascular perfusion circuit of the endothelial compartment to investigate the potential immunomodulatory effects of *L. rhamnosus* in the intestinal model. Measurement of the cytokine release profiles for IL-1β, IL-6, IL-8, IL-10, and TNF in intestinal models colonised with *L. rhamnosus* showed a significant increase of cytokine release and PBMC adhesion upon LPS stimulation of the endothelial cell layer compared to LPS stimulation at the luminal side (Fig. 6C, D). Though, we observed only slightly increased IL-1β and IL-6 levels and no alterations of IL-8, IL-10 and TNF release associated with the luminal colonisation with *L. rhamnosus*.

L. rhamnosus was described among other probiotic bacteria to reduce enteric colonisation and infection with opportunistic pathogens. Multiple mechanisms, including nutritional competition, reduction of barrier permeability, and the modulation of the immune response have been discussed in this context [36,37]. We found that *L. rhamnosus* colonisation significantly increased the expression of E-cadherin and ZO-1 even in the presence of PBMCs (Fig. 6 E, F) and improved cell viability at the epithelial cell layer indicated by lower LDH release (Fig. 6 G). Lactobacilli have been shown to counteract infections of the gastrointestinal tract (GI) by opportunistic pathogens [38,39]. The

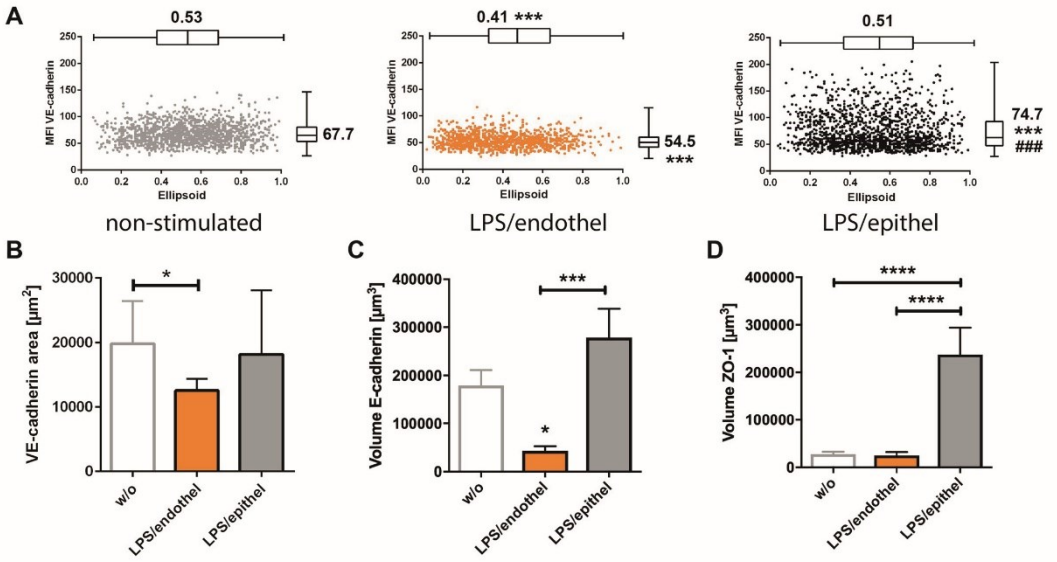


Fig. 5. Expression of VE-cadherin, E-cadherin and ZO-1 upon LPS stimulation. A) Expression pattern of VE-cadherin and endothelial cell morphology under non-stimulated conditions, and upon exposure of the endothelial layer to LPS (LPS/endothel) or the epithelial cell layer (LPS/epithel). Mean fluorescence intensity (MFI) of VE-cadherin expression is plotted for each individual cell analysed against its ellipsoid index (0 = line, 1 = circle). B) Quantification of VE-cadherin expression area in endothelial cells. C) Expression of E-cadherin and D) ZO-1 in the epithelial cell layers by z-stack image analysis and quantification as the volume of expressed protein levels. Statistical testing performed by one-way ANOVA with Tukey's correction; *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$ vs. non-stimulated condition (w/o) or between indicated conditions. Data of four independent experiments are shown.

yeast *C. albicans* usually exists as a harmless commensal in healthy individuals [40]. However, it can also become a pathogen causing severe systemic infections as part of ultra-low diversity microbial communities in the gut, i.e. after antibiotic treatment of critically ill patients with suppressed immune function [41]. *L. rhamnosus* has been shown to interfere with colonization of *C. albicans* in the gut by limiting fungal growth [38,42]. However, most of these studies were performed in absence of epithelial cells [43,44] or in monolayered epithelial cell cultures [45–47]. We therefore studied the interaction of *C. albicans* with *L. rhamnosus* a more complex and immunocompetent microenvironment reflected by the intestine-on-chip model. The luminal side of the intestinal model was infected with *C. albicans*, and the impact of *L. rhamnosus* pre-colonization was analysed for the resulting fungal burden after 24 h of co-culture. We found that *L. rhamnosus* colonisation not only limit the growth of *C. albicans* at the luminal side of the model (Fig. 6H), but also reduces the translocation of the fungus over the intestinal barrier into the endothelial compartment emulating the vasculature (Fig. 6 I, J, K).

4. Discussion

The intestinal epithelium is one of the most dynamic and rapidly renewing tissues in the body, which requires continuous remodelling as well as disposal of effete cells. In order to simulate the human intestine as a stable and immunocompetent ecosystem, we developed a microfluidic organ-on-chip model forming a three-dimensional epithelial cell layer that can be colonised by living bacteria. We could demonstrate that the model, in contrast to monolayer cultures, exhibits near physiological 3D tissue architecture. Similar to normal intestinal epithelial our model demonstrates immunotolerance of resident mMphs and DCs to MAMPs (LPS) present in the intestinal lumen, whereas the presence of LPS in the vascular compartment elicited strong inflammatory responses. Further, we were able to include an artificial microbiota consisting of living lactobacilli that was shown to protect against invasion of the opportunistic pathogen *C. albicans* by lowering fungal burden overgrowth in the intestinal lumen and limiting fungal translocation through the gut barrier. Our observations are in agreement with reports from *in vivo* studies [38] and demonstrate the usefulness of the intestine-on-chip model as a platform for functional studies on microbial communication and host-microbe interaction *in vitro*.

A potential limitation of our model is the use of the intestinal epithelial Caco-2 cell line, isolated from a colorectal tumour [48]. However, the cell line was shown to exhibit features more similar to human small intestine [10,11] and to retain stem cell-like capabilities able to recreate microanatomical structures of the human intestine *in vitro* [11]. Caco-2 cells cultured under microfluidically perfused conditions can self-organise and differentiate into a polarised columnar epithelium containing cells with markers of absorptive, mucus-secretory, enteroendocrine, and Paneth cell populations [11], which is not observed in Caco-2 cells cultured under static conditions [49]. The use of perfused conditions is thus imperative to evolve a more physiological microenvironment facilitating proper cell differentiation and increased mucus secretion to provide a suitable substrate layer that could be efficiently populated by living bacteria. Moreover, microfluidic perfusion and related shear forces on cell layers have been recently shown to prevent detrimental bacterial overgrowth *in vitro* [50].

Recently the HuMiX system, a multichannel intestine chip consisting of Caco-2 cells has been described [51]. This system allowed substantial advances in modelling host-microbe interactions *in vitro* but lacks endothelial cells and an immunocompetent environment created by mPCs. Further, microorganisms cultured in the chip are separated by a nanoporous membrane from the epithelial cells preventing their direct interaction. To fully mimic host-microbe interaction direct interactions of epithelial cells, immune cells, and the microbes are required.

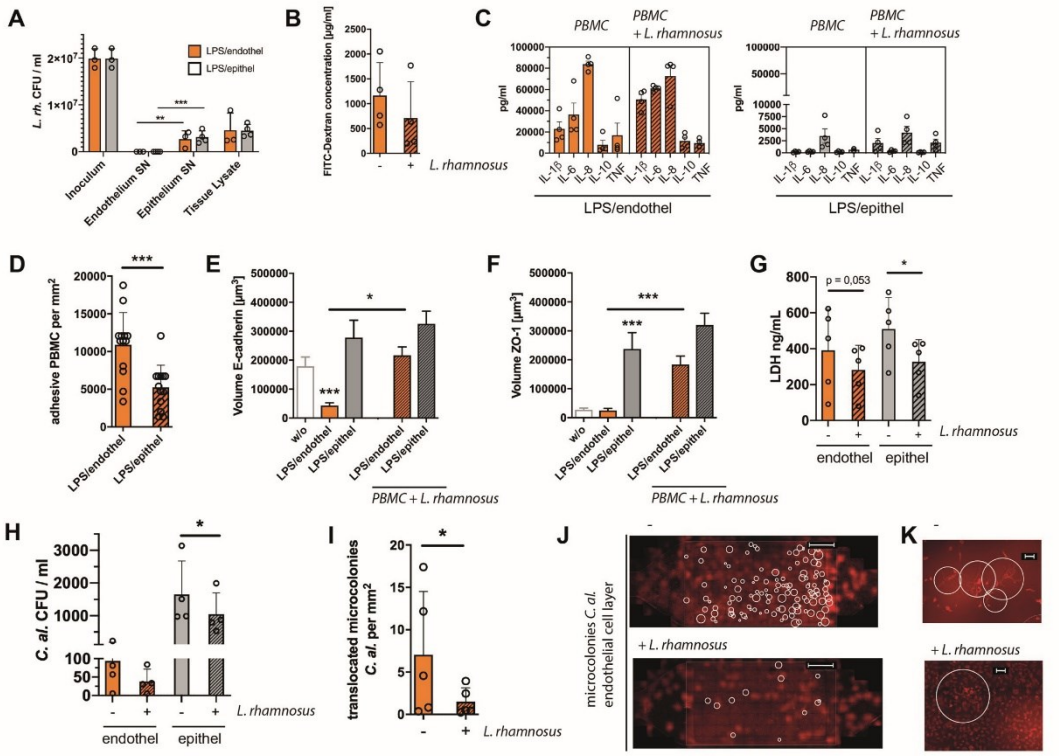


Fig. 6. Colonisation of the intestinal model with *L. rhamnosus* and *C. albicans*. A) Colony forming units (CFU) of *L. rhamnosus* from inoculated medium (Inoculum), in the supernatant at the endothelial side (Endothelium SN) or the luminal side (Epithelium SN), and in both cell layers after tissue lysis (Tissue Lysate). Colonisation was performed with LPS exposed to the endothelial side (LPS/endothel, orange bars) or LPS exposed to the epithelial cell layer (LPS/epithel, grey bars). B) Permeability assays with FITC-dextran in the model without *L. rhamnosus* (−) or *L. rhamnosus* colonised at the luminal side (+). C) Cytokine release in the supernatant at the endothelial side with endothelial cells stimulated with LPS (LPS/endothel) or epithelial cells stimulated with LPS (LPS/epithel). PBMCs were circulated at the endothelial side without *L. rhamnosus* (PBMC), and with *L. rhamnosus* colonising the luminal side of the model (PBMC + *L. rhamnosus*). D) Adhesive PBMCs in the presence of *L. rhamnosus* at the luminal side upon stimulation with LPS at the endothelial (LPS/endothel) or epithelial cell layer (LPS/epithel). E-F) Expression of E) E-cadherin and F) ZO-1 quantified as the volume in the epithelial cell layer. G) Release of LDH in the supernatant of the intestinal luminal side and vascular side with and without *L. rhamnosus* colonisation. H) Colony forming units (CFU) of *C. albicans* (*C. a.*) co-cultured without and with *L. rhamnosus* at the epithelial and endothelial side. I-K) C. Microcolonies formed by *C. albicans* upon translocation from the luminal side in absence or presence of *L. rhamnosus*. I) Quantified results of microcolony formation. J) low (scale bar 2 mm) and K) higher magnification (scale bar 100 µm) of formed microcolonies. J, K) Representative images of four independent experiments. Circles indicate identified microcolonies, fuzzy red background is caused by endothelial cells. E-F) A-C) Statistical testing with one-way ANOVA and Tukey's correction, $^{*}p < 0.05$, $^{***}p < 0.001$ vs. non-stimulated condition (w/o) or between indicated conditions. D, G, H, I) Two-tailed ratio paired *t*-Test, $^{*}p < 0.05$, $^{***}p < 0.001$ between indicated conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The self-formation of the three-dimensional epithelial cell layer in our model allows the colonisation of living microorganisms directly at the luminal side of the intestinal model. Further, the organotypic microenvironment supports the self-organization of mMphs and DCs guided by physiological LPS gradients ranging from the luminal to the vascular side. Already present at birth, mMphs share many characteristics with their monocyte-derived counterparts in the adult intestine including high-phagocytic activity [52]. These findings support the emerging view that instead of their origin, the location and the surrounding microenvironment determines the functions of tissue-resident macrophages [53,54]. After their arrival in the mucosa, invading monocytes undergo a process of local cell differentiation and acquire the expression of CX3CR1 and MHCII [55]. During homeostasis, resident intestinal mMphs are replenished continuously by circulating monocytes with up to 50% of mature mMphs [56]. Similar to the *in vivo* situation these cells were found to express high levels of CX3CR1 upon LPS stimulation of the intestinal lumen. These mMphs primarily populated the endothelial side of the model, whereas CD103 expressing DCs were mainly recruited to the luminal side of the epithelial cell layer [19,21]. *In vivo* CX3CR1^{+} mMphs initiate innate and adaptive immune responses to fungi in the intestine via expression of c-type lectin receptors [57]. Furthermore, it has been recently shown that CX3CR1^{+} mMphs are critically involved in limiting microbiota-induced intestinal inflammation. They promote tissue homeostasis by limiting expansion of microbe-specific T helper 1 (Th1) cells and support the generation of microbiota specific regulatory T-cells. Interestingly, this study also demonstrated that colonization with microbes that adhere to the epithelium can compensate for intestinal microbiota loss and activate homeostatic immunoregulatory mechanisms [19]. Under physiological conditions *in vivo* mMphs do not respond to inflammatory triggers such

as LPS allowing them to act as efficient scavengers without inducing inflammation that usually ensues upon encounter of MAMPs and would compromise intestinal homeostasis [58]. In the intestinal model, we could demonstrate that LPS is well tolerated at the luminal side of the model with physiological homeostasis in the presence of commensal *L. rhamnosus*.

The maintenance of epithelial integrity has been shown to rely on the function of mMphs and DCs that reduce epithelial susceptibility to inflammatory insults and drive intestinal epithelial cell renewal and differentiation [59,60]. Further, mMphs stimulate the differentiation of regulatory T-cells and thereby contribute to intestinal homeostasis [61]. Recruited monocytes can differentiate into mature mMphs under inflammatory conditions exerting repair functions as has been described for monocytes infiltrating the liver [62,63]. We were able to demonstrate that by integration of mMphs and DCs tissue homeostasis can by mediated and LPS is tolerated at the luminal model side. Further, primary leucocytes (PBMCs) can be perfused in the model in the presence of LPS without detrimental effects on immune cell activation.

Colonisation of the intestine with *L. rhamnosus* did not induce inflammatory responses by release of proinflammatory cytokines. Instead, colonization improved cell viability of the intestinal model and was associated with increased E-cadherin and ZO-1 expression in the epithelial layer under simulated conditions of endotoxemia. These observations are in agreement with recent *in vivo* studies in mice, were an improved E-cadherin expression was also induced by lactobacilli [36]. Although the mechanism of the barrier improvement remains speculative, a potential explanation might be cytoprotective induction of heat shock proteins that were reported to contribute to cell protection by modulation of tight junction protein expression [64]. Stable colonisation of an intestinal model with *L. rhamnosus* was also shown by Kim *at al* [10]. However, PBMCs circulated in the model caused injury of villi and the intestinal barrier through the release of IL-8 induced by LPS present at the luminal side [10]. Here we demonstrate that in presence of mMphs and DCs physiological immunotolerance to LPS can be established, without detrimental effects of immune cell activation. This allows endothelial PBMC perfusion and a stable co-culture of living bacteria and LPS at the luminal side of the model. A pro-inflammatory immune response associated with tissue damage was only observed in the endotoxemia model, with *L. rhamnosus* colonisation ameliorating inflammation-associated tissue damage.

In vivo, continuous checks and balances between immune tolerance and the induction of inflammatory responses upon translocation of microorganisms are required. We observed increased leukocyte recruitment to the endothelial cell layer when LPS was present at the vascular side of the model creating an inflammatory environment that resembles conditions of IBD. The number of adhesive PBMCs under these simulated disease conditions was significantly higher compared to the more physiological conditions when LPS was present at the luminal side of the model. These observations are in agreement with clinical reports from IBD patients where blockade of recruitment of leucocytes into the intestine represents a promising treatment option for the patient [65]. The model has proven a suitable platform to elucidate mechanisms of host-microbe interactions in an immunocompetent environment in studies on leucocyte recruitment, immune cell differentiation, and the establishment of an adapted immune response to commensal and pathogenic microorganisms. Nevertheless, further improvements will be performed in follow up studies. These improvements should consider the contribution of other immune cells on immune surveillance of microorganisms in the gut, i.e. B- and T-cells organised in lymphoid follicles of the Peyer's patches and within gut-associated lymphoid tissue.

We could further demonstrate the feasibility of our model to investigate microbial interactions between commensal and opportunistic microorganisms with functional consequences for the intestinal barrier under near-physiological and well-controlled conditions *in vitro*. We here show for the first time that *L. rhamnosus* colonisation can diminish invasion of *C. albicans* into the "bloodstream" in a microphysiological environment created by our intestine-on-chip system. Similar to the *in vivo* situation where the bacterial microbiota protects against *C. albicans* overgrowth [38,39], we could demonstrate a protective effect of probiotic *L. rhamnosus* limiting the overgrowth and translocation of *C. albicans* to the endothelial cell compartment. It is tempting to speculate that also luminal exposure to LPS contributes to a reduced translocation of pathogenic microbes into the vascular compartment by improving the intestinal barrier.

Our intestinal model has proven to be a valuable tool to systematically explore the underlying mechanisms of microbial communication, host-microbe interactions, microbial pathogenicity mechanisms, and immune cell activation under physiologically relevant conditions *in vitro*. Thus, it represents a powerful platform for the investigation of disease mechanisms, driven by (opportunistic) pathogens, i.e. under well-defined conditions of dysbiosis created in synthetic microbiomes that are composed of a limited number of microbial community members. Further, it allows the screening and development of novel treatment strategies for IBD by supporting and maintaining physiological conditions of the human microbiota that keep opportunistic pathogens in their commensal state and prevent the onset of related inflammatory diseases.

Author contributions

M.M., T.W., F.B., M.G., M.R., F.S. contributed experiments for organ-on-chip development. M.M., M.S.G., A.L., R.P., K.G. contributed microbiological experimentation and functional interaction studies. Z.C., A.M., M.T.F. contributed image analysis and quantification strategies. S.N. contributed electron microscopy. I.D.J., B.H., O.H., A.S.M. contributed design and supervision of experiments. B.H., A.S.M. designed the study. A.S.M. wrote the manuscript. I.D.J., B.H., O.H., A.S.M. contributed funding acquisition. All authors read and approved the manuscript.

Conflicts of interest

M.R. and A.S.M. hold equity in Dynamic42 GmbH. M.R. is CEO of Dynamic42 GmbH. A.S.M. consults to the company.

Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical or time limitations.

Acknowledgement

We thank Melanie Ulrich, Nora Mosig and Tobias Voigt for excellent technical support. We thank André Scherag for advice on statistical tests. This work was financially supported by the Deutsche Forschungsgemeinschaft (DFG) through the CRC/TR124 FungiNet to I.D.J. (project C5), M.T.F. (project B4) and B.H. (project C1), through the CRC 1278 PolyTarget to M.T.F. (project Z01) and through Cluster of Excellence "Balance of the Microverse", funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC 2051 – Project-ID 390713860 to I.D.J, M.T.F., B.H. and A.S.M. The work was further financially supported by the German Ministry for Education and Research (BMBF) (grant no. 01EO1002) to I.D.J, O.H., B.H. and A.S.M., an Alexander von Humboldt postdoctoral research fellowship to M.S.G, and though the European Commission through Marie Skłodowska-Curie Actions (MSCA) Innovative Training Network EUROoC (grant no. 812954) to A.S.M.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biomaterials.2019.119396.

References

[1] M. Knip, H. Siljander, The role of the intestinal microbiota in type 1 diabetes mellitus, Nat Rev Endocrinol 12 (2016) 154–167, https://doi.org/10.1038/nrendo.2015.218.

[2] L. Zhao, The gut microbiota and obesity: from correlation to causality, Nat Rev Microbiol 11 (2013) 639–647, https://doi.org/10.1038/nrmicro3089.

[3] X.C. Morgan, et al., Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment, Genome Biol 13 (2012) R79, https://doi.org/10.1186/gb-2012-13-9-r79.

[4] M. Valles-Colomer, et al., The neuroactive potential of the human gut microbiota in quality of life and depression, Nat Microbiol (2019), https://doi.org/10.1038/s41564-018-0337-x.

[5] I.D. Iliev, I. Leonardi, Fungal dysbiosis: immunity and interactions at mucosal barriers, Nat Rev Immunol 17 (2017) 635–646, https://doi.org/10.1038/nri.2017.55.

[6] T.L. Nguyen, S. Vieira-Silva, A. Liston, J. Raes, How informative is the mouse for human gut microbiota research? Dis Model Mech 8 (2015) 1–16, https://doi.org/10.1242/dmm.017400.

[7] J.L. Round, S.K. Mazmanian, The gut microbiota shapes intestinal immune responses during health and disease, Nat Rev Immunol 9 (2009) 313–323, https://doi.org/10.1038/nri2515.

[8] I. Mukhopadhya, R. Hansen, E.M. El-Omar, G.L. Hold, IBD-what role do Proteobacteria play? Nat Rev Gastroenterol Hepatol 9 (2012) 219–230, https://doi.org/10.1038/nrgastro.2012.14.

[9] T. Sato, H. Clevers, Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications, Science 340 (2013) 1190–1194, https://doi.org/10.1126/science.1234852.

[10] H.J. Kim, D. Huh, G. Hamilton, D.E. Ingber, Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow, Lab Chip 12 (2012) 2165–2174, https://doi.org/10.1039/c2lc40074j.

[11] H.J. Kim, D.E. Ingber, Gut-on-a-Chip microenvironment induces human intestinal cells to undergo villus differentiation, Integr Biol (Camb) 5 (2013) 1130–1140, https://doi.org/10.1039/c3ib40126j.

[12] B.M. Janelsins, M. Lu, S.K. Datta, Altered inactivation of commensal LPS due to acyloxyacyl hydrolase deficiency in colonic dendritic cells impairs mucosal Th17 immunity, Proc Natl Acad Sci U S A 111 (2014) 373–378, https://doi.org/10.1073/pnas.1311987111.

[13] U. Jaffer, R.G. Wade, T. Gourlay, Cytokines in the systemic inflammatory response syndrome: a review, HSR Proc Intensive Care Cardiovasc Anesth 2 (2010) 161–175.

[14] R.L. Chelvarajan, S.M. Collins, J.M. Van Willigen, S. Bondada, The unresponsiveness of aged mice to polysaccharide antigens is a result of a defect in macrophage function, J Leukoc Biol 77 (2005) 503–512, https://doi.org/10.1189/jlb.0804449.

[15] C.C. Bain, A.M. Mowat, Intestinal macrophages - specialised adaptation to a unique environment, Eur J Immunol 41 (2011) 2494–2498, https://doi.org/10.1002/eji.201141714.

[16] D.A. Hume, V.H. Perry, S. Gordon, The mononuclear phagocyte system of the mouse defined by immunohistochemical localisation of antigen F4/80: macrophages associated with epithelia, Anat Rec 210 (1984) 503–512, https://doi.org/10.1002/ar.1092100311.

[17] V. Rossini, et al., CX3CR1(+) cells facilitate the activation of CD4 T cells in the colonic lamina propria during antigen-driven colitis, Mucosal Immunol 7 (2014) 533–548, https://doi.org/10.1038/mi.2013.70.

[18] K.M. Schneider, et al., CX3CR1 is a gatekeeper for intestinal barrier integrity in mice: limiting steatohepatitis by maintaining intestinal homeostasis, Hepatology 62 (2015) 1405–1416, https://doi.org/10.1002/hep.27982.

[19] M. Kim, et al., Critical role for the microbiota in CX3CR1(+) intestinal mononuclear phagocyte regulation of intestinal T cell responses, Immunity 49 (2018) 151–163, https://doi.org/10.1016/j.immuni.2018.05.009 e155.

[20] S. Milling, U. Yrlid, V. Cerovic, G. MacPherson, Subsets of migrating intestinal dendritic cells, Immunol Rev 234 (2010) 259–267, https://doi.org/10.1111/j.0105-2896.2009.00866.x.

[21] J. Farache, et al., Luminal bacteria recruit CD103+ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation, Immunity 38 (2013) 581–595, https://doi.org/10.1016/j.immuni.2013.01.009.

[22] T. Worbs, et al., Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells, J Exp Med 203 (2006) 519–527, https://doi.org/10.1084/jem.20052016.

[23] M. Raasch, et al., Microfluidically supported biochip design for culture of endothelial cell layers with improved perfusion conditions, Biofabrication 7 (2015) 015013, , https://doi.org/10.1088/1758-5090/7/1/015013.

[24] H. Zalzali, et al., CEACAM1, a SOX9 direct transcriptional target identified in the colon epithelium, Oncogene 27 (2008) 7131–7138, https://doi.org/10.1038/onc.2008.331.

[25] S.W. Craig, L.D. Powell, Regulation of actin polymerization by villin, a 95,000 dalton cytoskeletal component of intestinal brush borders, Cell 22 (1980) 739–746.

[26] R. Noth, et al., Increased intestinal permeability and tight junction disruption by altered expression and localization of occludin in a murine graft versus host disease model, BMC Gastroenterol 11 (2011) 109, https://doi.org/10.1186/1471-230X-11-109.

[27] J. Sun, et al., Crosstalk between NF-kappaB and beta-catenin pathways in bacterial-colonized intestinal epithelial cells, Am J Physiol Gastrointest Liver Physiol 289 (2005) G129–G137, https://doi.org/10.1152/ajpgi.00515.2004.

[28] G. Luo, T. Guenthner, L.S. Gan, W.G. Humphreys, CYP3A4 induction by xenobiotics: biochemistry, experimental methods and impact on drug discovery and development, Curr Drug Metab 5 (2004) 483–505.

[29] O. Medina-Contreras, et al., CX3CR1 regulates intestinal macrophage homeostasis, bacterial translocation, and colitogenic Th17 responses in mice, J Clin Invest 121 (2011) 4787–4795, https://doi.org/10.1172/JCI59150.

[30] A.R. Tanner, M.J. Arthur, R. Wright, Macrophage activation, chronic inflammation and gastrointestinal disease, Gut 25 (1984) 760–783.

[31] F. Sanchez-Munoz, A. Dominguez-Lopez, J.K. Yamamoto-Furusho, Role of cytokines in inflammatory bowel disease, World J Gastroenterol 14 (2008) 4280–4288.

[32] A. Kaser, S. Zeissig, R.S. Blumberg, Inflammatory bowel disease, Annu Rev Immunol 28 (2010) 573–621, https://doi.org/10.1146/annurev-immunol-030409-101225.

[33] W.E. Cromer, J.M. Mathis, D.N. Granger, G.V. Chaitanya, J.S. Alexander, Role of the endothelium in inflammatory bowel diseases, World J Gastroenterol 17 (2011) 578–593, https://doi.org/10.3748/wjg.v17.i5.578.

[34] J. Zhang, A.F. Defelice, J.P. Hanig, T. Colatsky, Biomarkers of endothelial cell activation serve as potential surrogate markers for drug-induced vascular injury, Toxicol Pathol 38 (2010) 856–871, https://doi.org/10.1177/0192623310378866.

[35] A.L. Hart, et al., Homing of immune cells: role in homeostasis and intestinal inflammation, Inflamm Bowel Dis 16 (2010) 1969–1977, https://doi.org/10.1002/ibd.21304.

[36] L. Laval, et al., Lactobacillus rhamnosus CNCM I-3690 and the commensal bacterium Faecalibacterium prausnitzii A2-165 exhibit similar protective effects to induced barrier hyper-permeability in mice, Gut Microbes 6 (2015) 1–9, https://doi.org/10.4161/19490976.2014.990784.

[37] M.I. Alvarez-Olmos, R.A. Oberhelman, Probiotic agents and infectious diseases: a modern perspective on a traditional therapy, Clin Infect Dis 32 (2001) 1567–1576, https://doi.org/10.1086/320518.

[38] V.H. Matsubara, H.M. Bandara, M.P. Mayer, L.P. Samaranayake, Probiotics as antifungals in mucosal candidiasis, Clin Infect Dis 62 (2016) 1143–1153, https://doi.org/10.1093/cid/ciw038.

[39] T.M. Forster, et al., Enemies and brothers in arms: Candida albicans and gram-positive bacteria, Cell Microbiol 18 (2016) 1709–1715, https://doi.org/10.1111/cmi.12657.

[40] M.E. Bougnoux, et al., Multilocus sequence typing reveals intrafamilial transmission and microevolutions of Candida albicans isolates from the human digestive tract, J Clin Microbiol 44 (2006) 1810–1820, https://doi.org/10.1128/JCM.44.5.1810-1820.2006.

[41] A. Zaborin, et al., Membership and behavior of ultra-low-diversity pathogen communities present in the gut of humans during prolonged critical illness, MBio 5 (2014) 01314, https://doi.org/10.1128/mBio.01361-14 e01361.

[42] K.M. James, K.W. MacDonald, R.M. Chanyi, P.A. Cadieux, J.P. Burton, Inhibition of Candida albicans biofilm formation and modulation of gene expression by probiotic cells and supernatant, J Med Microbiol 65 (2016) 328–336, https://doi.org/10.1099/jmm.0.000226.

[43] G.A. Kohler, S. Assefa, G. Reid, Probiotic interference of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 with the opportunistic fungal pathogen Candida albicans, Infect Dis Obstet Gynecol (2012) 636474, https://doi.org/10.1155/2012/636474 2012.

[44] M. Strus, et al., The in vitro activity of vaginal Lactobacillus with probiotic properties against Candida, Infect Dis Obstet Gynecol 13 (2005) 69–75, https://doi.org/10.1080/10647440400028136.

[45] A. Rizzo, A. Losacco, C.R. Carratelli, Lactobacillus crispatus modulates epithelial cell defense against Candida albicans through Toll-like receptors 2 and 4, interleukin 8 and human beta-defensins 2 and 3, Immunol Lett 156 (2013) 102–109, https://doi.org/10.1016/j.imlet.2013.08.013.

[46] D. Mailander-Sanchez, et al., Antifungal defense of probiotic Lactobacillus rhamnosus GG is mediated by blocking adhesion and nutrient depletion, PLoS One 12 (2017) e0184438, , https://doi.org/10.1371/journal.pone.0184438.

[47] M.S. do Carmo, et al., Lactobacillus fermentum ATCC 23271 displays in vitro inhibitory activities against Candida spp, Front Microbiol 7 (2016) 1722, https://doi.org/10.3389/fmicb.2016.01722.

[48] M.D. Peterson, M.S. Mooseker, Characterization of the enterocyte-like brush border cytoskeleton of the C2BBe clones of the human intestinal cell line, Caco-2, J Cell Sci 102 (Pt 3) (1992) 581–600.

[49] B.J. van Klinken, et al., The human intestinal cell lines Caco-2 and LS174T as models to study cell-type specific mucin expression, Glycoconj J 13 (1996) 757–768.

[50] H.J. Kim, H. Li, J.J. Collins, D.E. Ingber, Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip, Proc Natl Acad Sci U S A 113 (2016) E7–E15, https://doi.org/10.1073/pnas.1522193112.

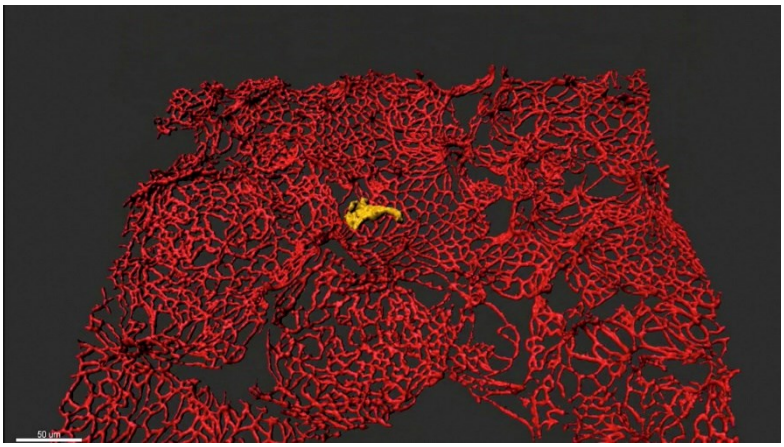
[51] P. Shah, et al., A microfluidics-based in vitro model of the gastrointestinal human-microbe interface, Nat Commun 7 (2016) 11535, https://doi.org/10.1038/ncomms11535.

[52] C.C. Bain, et al., Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice, Nat Immunol 15 (2014) 929–937, https://doi.org/10.1038/ni.2967.

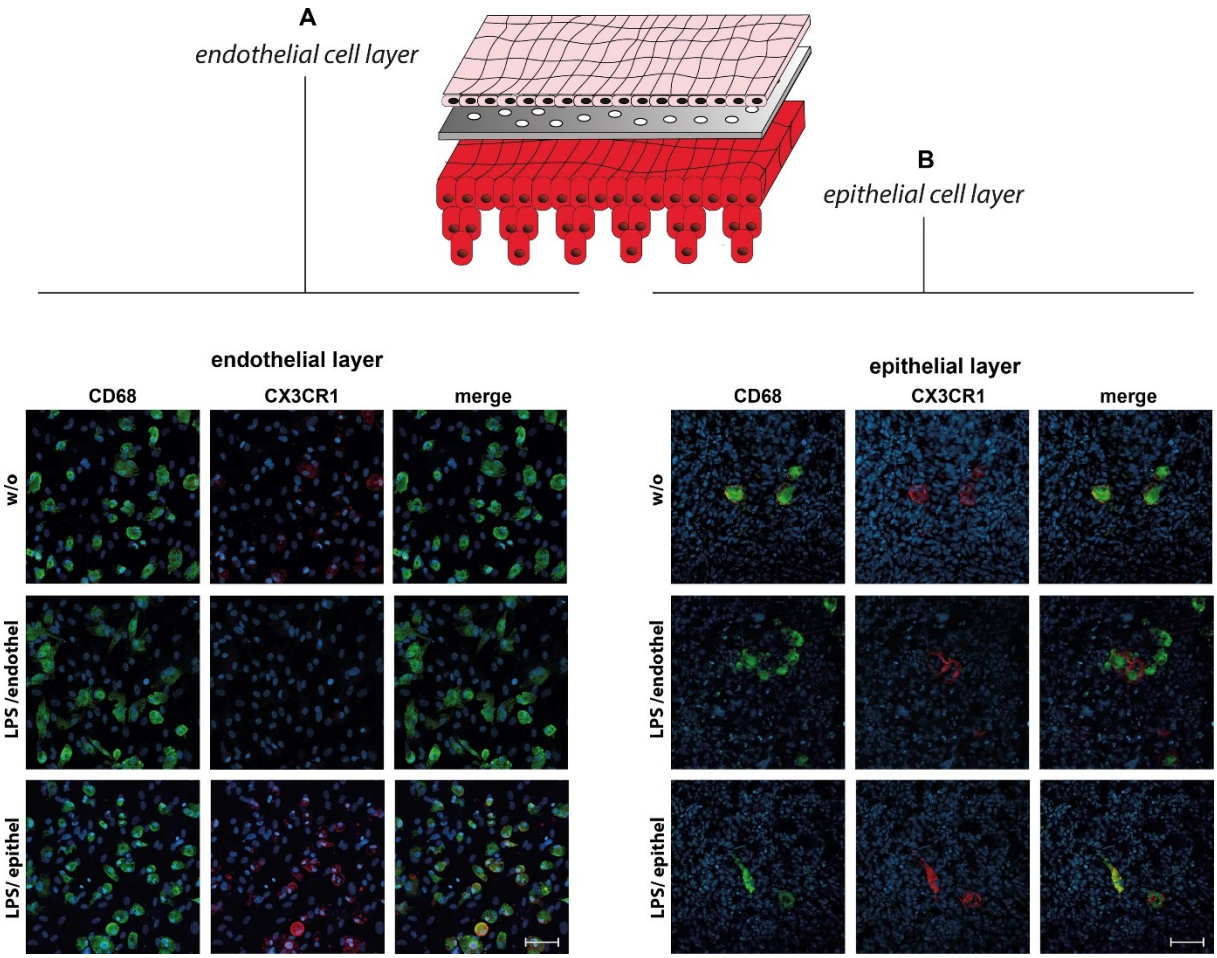
[53] I. Amit, D.R. Winter, S. Jung, The role of the local environment and epigenetics in shaping macrophage identity and their effect on tissue homeostasis, Nat Immunol 17 (2016) 18–25, https://doi.org/10.1038/ni.3325.

[54] F. Ginhoux, M. Guilliams, Tissue-resident macrophage ontogeny and homeostasis, Immunity 44 (2016) 439–449, https://doi.org/10.1016/j.immuni.2016.02.024.
[55] C.C. Bain, et al., Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors, Mucosal Immunol 6 (2013) 498–510, https://doi.org/10.1038/mi.2012.89.
[56] P. Krause, et al., IL-10-producing intestinal macrophages prevent excessive antibacterial innate immunity by limiting IL-23 synthesis, Nat Commun 6 (2015) 7055, https://doi.org/10.1038/ncomms8055.
[57] I. Leonardi, et al., CX3CR1(+) mononuclear phagocytes control immunity to intestinal fungi, Science 359 (2018) 232–236, https://doi.org/10.1126/science.aao1503.
[58] L.E. Smythies, et al., Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity, J Clin Invest 115 (2005) 66–75, https://doi.org/10.1172/JCI19229.
[59] J. Cosin-Roger, et al., The activation of Wnt signaling by a STAT6-dependent macrophage phenotype promotes mucosal repair in murine IBD, Mucosal Immunol 9 (2016) 986–998, https://doi.org/10.1038/mi.2015.123.
[60] J.E. Qualls, A.M. Kaplan, N. van Rooijen, D.A. Cohen, Suppression of experimental colitis by intestinal mononuclear phagocytes, J Leukoc Biol 80 (2006) 802–815, https://doi.org/10.1189/jlb.1205734.
[61] T.L. Denning, et al., Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization, J Immunol 187 (2011) 733–747, https://doi.org/10.4049/jimmunol.1002701.
[62] C.L. Scott, et al., Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells, Nat Commun 7 (2016) 10321, https://doi.org/10.1038/ncomms10321.
[63] M. Groger, et al., Monocyte-induced recovery of inflammation-associated hepatocellular dysfunction in a biochip-based human liver model, Sci Rep 6 (2016) 21868, https://doi.org/10.1038/srep21868.
[64] H.Y. Liu, et al., Effects of Lactobacillus johnsonii and Lactobacillus reuteri on gut barrier function and heat shock proteins in intestinal porcine epithelial cells, Physiol Rep 3 (2015), https://doi.org/10.14814/phy2.12355.
[65] G. Bamias, D.J. Clark, J. Rivera-Nieves, Leukocyte traffic blockade as a therapeutic strategy in inflammatory bowel disease, Curr Drug Targets 14 (2013) 1490–1500.
[66] S. Mosig, et al., Different functions of monocyte subsets in familial hypercholesterolemia: potential function of CD14+ CD16+ monocytes in detoxification of oxidized LDL, FASEB J 23 (2009) 866–874, https://doi.org/10.1096/fj.08-118240.

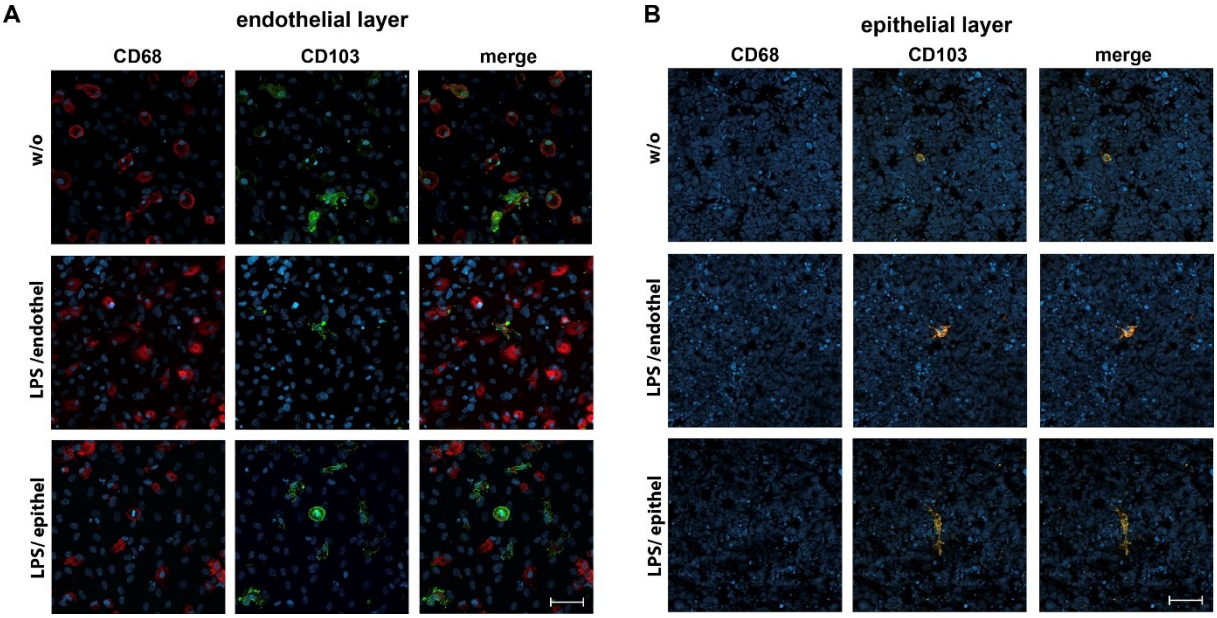
Manuscript I - Supplementary information



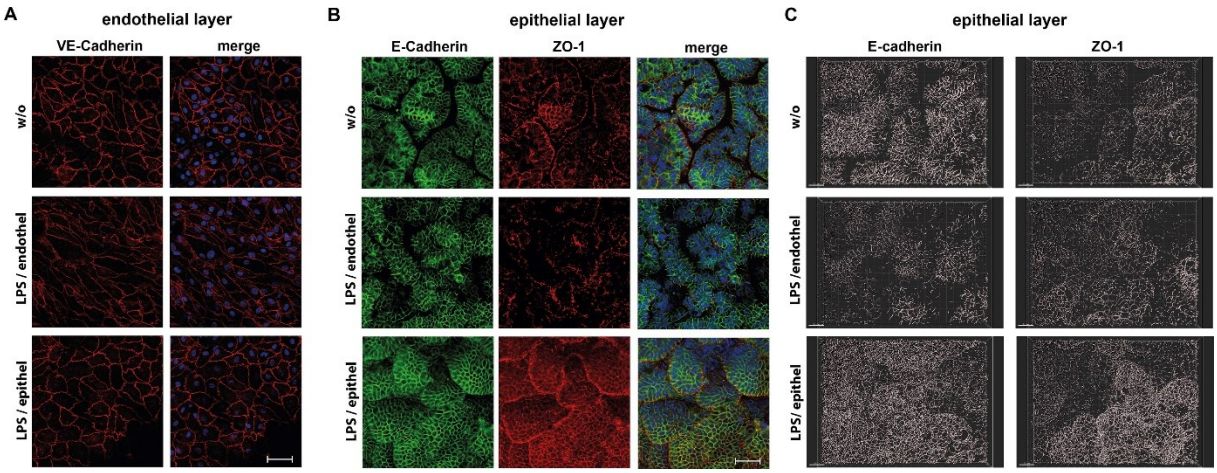
Supp. Fig. 1: CD103^{+} DC with dendrite formation into the luminal side of the epithelial cell layer. CD103 (yellow), ZO-1 (red). Scale bar 50 µm. Representative image of five independent experiments.



Supp. Fig. 2: Immunofluorescence staining of CD68 (green) and CX3CR1 (red) of mPCs within the A) endothelial and B) epithelial cell layer under non-stimulated conditions (w/o) and conditions of LPS exposure to the endothelial layer (LPS/endothel) or the epithelial cell layer (LPS/epithel). Nuclei were stained with DAPI (blue). Scale bar 50 µm. Representative images of five independent experiments.



Supp. Fig. 3: Immunofluorescence staining of CD68 (red) and CD103 (green) of mPCs within A) endothelial or B) epithelial cell layer of the intestinal model under non-stimulated conditions (w/o) and the intestinal model stimulated with LPS at the endothelial side (LPS/endothel) or the luminal side (LPS/epithel). Nuclei were stained with DAPI (blue). Scale bar 50 µm. Representative images of five independent experiments.



Supp. Fig. 4: Immunofluorescence images of VE-cadherin, E-cadherin and ZO-1 expression. A) Expression of VE-cadherin (red) at the endothelial cell layer. B) Expression of E-cadherin (green) and ZO-1 (red). A-B) Nuclei were stained with DAPI (blue). C) Extracted E-cadherin and ZO-1 protein expression volumes (calculated protein volume from z-stack images) from z-stack images for protein quantification. A-C) Scale bar 50 µm. Non-stimulated (w/o), LPS stimulation of the endothelial cell layer (LPS/endothel) or the epithelial cell layer (LPS/epithel). Images show representative results of four independent experiments.

3.2 Manuscript II

In vitro infection models to study fungal-host interactions

Antonia Last*, **Michelle Maurer***, Alexander S. Mosig, Mark S. Gresnigt, and Bernhard Hube

*These authors contributed equally to this work

Published in:
FEMS Microbiology Review, 2021, fuab005, 1-22

Fungal infections are on the rise worldwide and represent serious health threats. The species *Aspergillus fumigatus*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *Candida albicans* are responsible for the largest proportion of fungal infections. Mouse models have provided us with relevant insights into pathogenicity mechanisms employed by the fungi. However, due to physiological differences, the extrapolation of findings from mice studies to the human situation is difficult and hampers progress in finding adequate treatment strategies. Therefore, *in vitro* models are becoming increasingly important to understand and identify virulence factors, the interplay between the commensal microbiota, pathogens, and the host. Within the last decade, sophisticated *in vitro* models have been developed for several organs by the implementation of biomaterials and microfluidic technologies. These so-called microphysiological systems (MPS) represent promising tools to study fungi in environments closely resembling human physiology. We review *in vitro* models used for the investigation of fungal infections and discuss the potential of recently developed MPS for the research field.

FEMS Microbiology Reviews, fuab005, 1–22

doi: 10.1093/femsre/fuab005
Advance Access Publication Date: 1 February 2021
Review Article

REVIEW ARTICLE

In vitro infection models to study fungal–host interactions

Antonia Last[1,†], Michelle Maurer[2,3,†], Alexander S. Mosig[2,3], Mark S. Gresnigt[4,§] and Bernhard Hube[1,5,*,‡]

[1]Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology—Hans Knoell Institute, Beutenbergstrasse 11a, 07745, Jena, Germany, [2]Center for Sepsis Control and Care (CSCC), Jena University Hospital, Am Klinikum 1, 07747, Jena, Germany, [3]Institute of Biochemistry II, Jena University Hospital, Nonnenplan 2,07743, Jena, Germany, [4]Junior Research Group Adaptive Pathogenicity Strategies, Leibniz Institute for Natural Product Research and Infection Biology—Hans Knoell Institute, Beutenbergstrasse 11a, 07745, Jena, Germany and [5]Institute of Microbiology, Friedrich Schiller University, Neugasse 24, 07743, Jena, Germany

*Corresponding author: Adolf-Reichwein-Straße 23, 07745, Jena, Germany. Tel: +49 3641 532-1401; Fax: +49 3641 532-0810; E-mail: bernhard.hube@hki-jena.de

One sentence summary: From basic to complex: *in vitro* models to study interactions between human fungal pathogens and their host.

†These authors contributed equally to this work.

Editor: Gerhard Braus

‡Bernhard Hube, http://orcid.org/0000-0002-6028-0425
§Mark S. Gresnigt, https://orcid.org/0000-0002-9514-4634

ABSTRACT

Fungal infections (mycoses) affect over a billion people per year. Approximately, two million of these infections are life-threatening, especially for patients with a compromised immune system. Fungi of the genera *Aspergillus*, *Candida*, *Histoplasma* and *Cryptococcus* are opportunistic pathogens that contribute to a substantial number of mycoses. To optimize the diagnosis and treatment of mycoses, we need to understand the complex fungal–host interplay during pathogenesis, the fungal attributes causing virulence and how the host resists infection via immunological defenses. *In vitro* models can be used to mimic fungal infections of various tissues and organs and the corresponding immune responses at near-physiological conditions. Furthermore, models can include fungal interactions with the host–microbiota to mimic the *in vivo* situation on skin and mucosal surfaces. This article reviews currently used *in vitro* models of fungal infections ranging from cell monolayers to microfluidic 3D organ-on-chip (OOC) platforms. We also discuss how OOC models can expand the toolbox for investigating interactions of fungi and their human hosts in the future.

Keywords: *in vitro* model; fungal–host interaction; *Aspergillus*; *Candida*; *Histoplasma*; *Cryptococcus*

INTRODUCTION

Human fungal infections lead to approximately 1.5 million deaths worldwide each year, but receive little attention compared with malaria or tuberculosis, which kill a similar number of people on an annual basis (Brown *et al.* 2012; Bongomin *et al.* 2017). Over 70% of deaths resulting from fungal infections can be attributed to fungi of the genera *Aspergillus*, *Candida*, *Cryptococcus* and *Histoplasma* (Brown *et al.* 2012). These

Received: 19 August 2020; **Accepted:** 14 January 2021

opportunistic fungal pathogens are either normal commensals of the human microbiota or reside in the environment, resulting in constant exposure to pathogenic fungi for humans. Even in immunocompetent human hosts, superficial fungal infections are widespread. Among them, fungal skin diseases are the most common health complications (Vos *et al.* 2012), and vulvovaginal candidiasis (VVC) affects approximately 70% of women (Gonçalves *et al.* 2016; Rosati *et al.* 2020). Such infections are often connected to an imbalance of the bacterial microbiota, for example, after the use of antibiotics that favor fungal overgrowth (Weiss and Hennet 2017)). In addition to superficial infections, opportunistic fungal pathogens can also cause severe life-threatening systemic infections under certain predispositions, like surgery, stem cell transplantation, chemotherapy or HIV/AIDS (Perlroth, Choi and Spellberg 2007; Polvi *et al.* 2015; Vallabhaneni and Chiller 2016). Considering their clinical significance, suitable models to study opportunistic fungal infections are essential for obtaining insights into disease pathogenesis. Ideally, these models allow the dissection of the molecular details of host–pathogen interactions under physiologically relevant conditions. They should provide sufficient complexity to mimic the different types and stages of infections and predispositions of the host. These models should also be suitable to test experimental therapeutic interventions and allow the evaluation of clinically relevant biomarkers. Here, we review currently used *in vitro* models to study molecular mechanisms of fungal infections caused by common fungal pathogens, including *Aspergillus fumigatus*, *Candida* spp., *Cryptococcus neoformans* and *Histoplasma capsulatum*, and provide an outlook about models that will likely expand our toolbox to study fungal–host interactions in the near future.

DISEASE MODELING

To study fungal pathogens and their related diseases, a wide range of models can be used. Commonly, host–pathogen interactions are investigated in animal model organisms such as mice, rats, fish, insects or worms. *In vivo* models offer the advantage to study host–pathogen interactions in a whole organism, providing the most complex interactions that can be achieved experimentally. However, in addition to critical ethical issues associated with the use of animal models (Robinson *et al.* 2019), the translation of results from animal experiments to human disease can be hampered by differences in physiology. Another approach is the use of tissue samples or organs from living organisms and their culture in an *ex vivo* environment that resembles *in vivo* conditions. These *ex vivo* models offer the advantage that conditions can be easily manipulated and are often easier to handle than living organisms. A broad overview of *ex vivo* models to study fungal infection is given by Maciel Quatrin *et al.* (2019). *In vitro* experiments are also performed outside of the natural biological environment. Primary cells isolated from tissues and biopsies can be cultured for a limited time or can be immortalized and cultured as cell lines. *In vitro* models may lack the complexity of *in vivo* models, but allow ample control over external growth conditions of cells concerning O_2 and CO_2 saturation, temperature, pH and nutrients. Moreover, it is relatively easy to manipulate as well as to quantitatively and qualitatively assess the metabolism, transcription and protein function of cells, making it possible to work in and test conditions that cannot be studied in *in vivo* models. It is also possible to introduce or omit different cell types to study the individual impact of different kinds of cells within the system. *In vitro* models (Fig. 1) range from monolayers in well plates, to transwell systems, 3D tissue structures and complex organ-on-chip (OOC) models (Mosig 2017), which are used to mimic several organs such as the liver (Groger *et al.* 2016; Jang *et al.* 2019), lung (Benam *et al.* 2016; Deinhardt-Emmer *et al.* 2020) and gut (Shin and Kim 2018; Maurer *et al.* 2019). OOC models represent the smallest functional entity of an organ as well as a versatile and promising resource to study host–pathogen interactions (Ahadian *et al.* 2018). However, each model has its specific advantages and disadvantages. The most suitable model is the one that meets the actual needs with high predictability and robustness, depending on the pathogen, the host and the questions to be answered.

We discuss the fungal–host interactions in different biological niches (Fig. 2). We review *in vitro* models used to mimic infection routes and highlight relevant findings that contributed to expand our knowledge on fungal infections. Because the immune system plays a major role during fungal infections, the interplay of fungi and immune cells is discussed in the first part, followed by sections covering the respiratory tract, the gastrointestinal tract, the vaginal mucosa, the bloodstream and the blood–brain barrier (BBB).

STUDYING FUNGAL INTERACTIONS WITH THE IMMUNE SYSTEM

A properly functioning immune system is crucial for resistance against infections with fungal pathogens. Individuals with a compromised immune system are more susceptible to invasive fungal diseases, whereas detrimental, improper or hypersensitive immune reactions can also contribute to disease (Romani 2004; Wheeler, Limon and Underhill 2017). Thus, a protective host response against opportunistic fungal pathogens has to be specific, tightly regulated and effective. However, pathogenic fungi have evolved a series of mechanisms to deal with and evade the immune system. Knowledge of both aspects is crucial for the design of therapeutic strategies aiming to strengthen appropriate responses and suppress detrimental ones (Armstrong-James *et al.* 2017). We will discuss (i) the different immune cells involved in antifungal host defense, (ii) the different roles these cells play in antifungal immunity and (iii) different models and readouts that can be used to study the efficiency of the host response to pathogenic fungi.

Immune cells involved in antifungal host defense

A healthy and efficient immune system is fundamental to cope with the environmental fungi we encounter on a daily basis and to deal with the fungi we harbor as commensals. This antifungal immunity relies on the innate immune system represented by cells such as macrophages, monocytes, neutrophils, natural killer (NK) cells and dendritic cells (DCs) as well as the adaptive immune system, in particular on T helper cell responses. The importance of these different types of immune cells becomes apparent when they are dysfunctional or absent. For example, a compromised innate immune system due to immunosuppressive therapy predisposes not only to invasive candidiasis (Lionakis 2014) but also aspergillosis (Herbrecht *et al.* 2012). While the innate immune system plays a role in host defense against cryptococcosis (Voelz and May 2010), patients with a compromised adaptive immune response due to HIV infections are particularly susceptible (Warkentien and Crum-Cianflone 2010). In contrast to *Candida*, *Aspergillus* and *Cryptococcus* species, *Histoplasma* species more commonly cause infections in healthy individuals (Köhler *et al.* 2017). Nevertheless, a compromised innate as

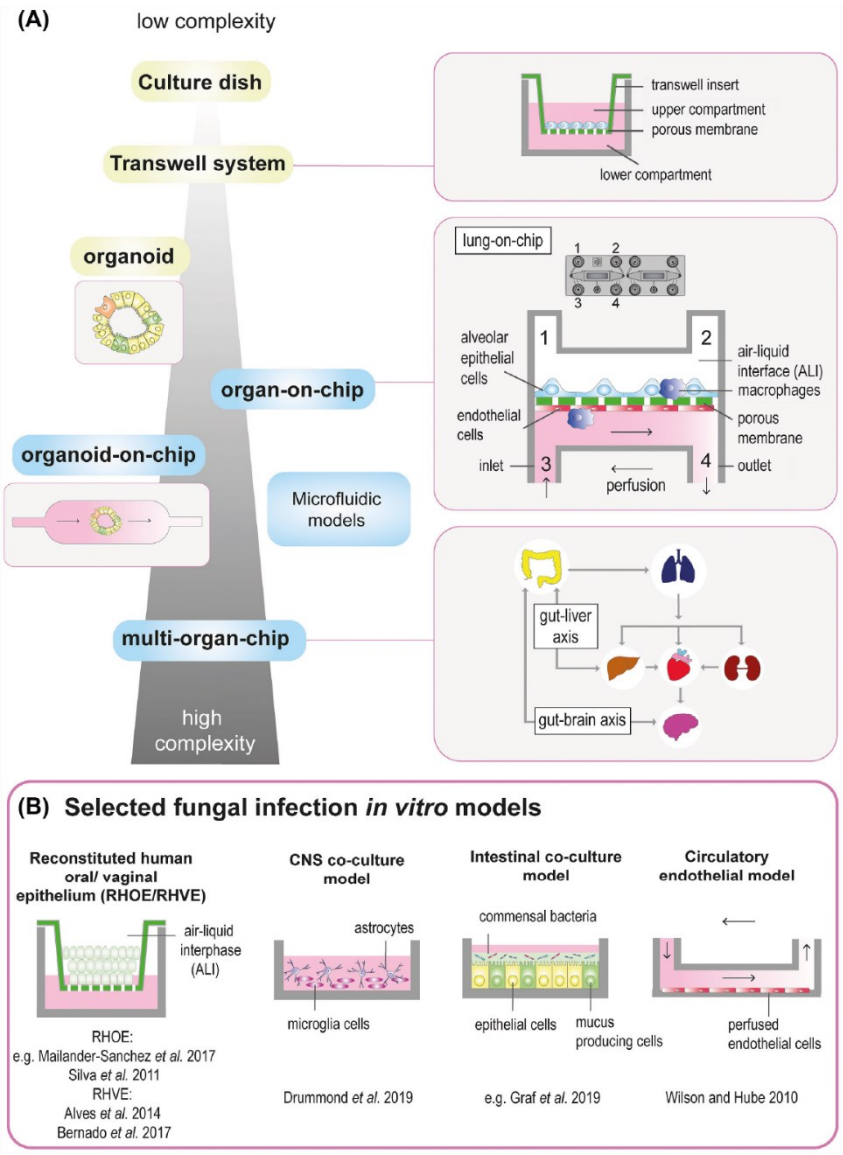


Figure 1. (A) Evolution of *in vitro* models from low to high complexity. Culture dish: one cell type cultured in media. Transwell system: transwell inserts separate the culture area into an upper and lower compartment; cells are cultured under static conditions on a porous membrane allowing apical-basal polarization. Organoid: 3D miniature organ generated out of intestinal stem cells. Organ-on-chip (example): 3D lung on-chip model on a microfluidic biochip holding a porous membrane and two individually accessible channels with one inlet and outlet each; pulmonary epithelial cells are cultured in the upper compartment in an air–liquid interface; and endothelial cells in the lower compartment are perfused with cell culture medium enabling the removal of metabolites. Organoid-on-chip: maturation of organoids within a dynamic culture environment. Principle of a multi-organ-on-chip: interconnected organ-on-chip models of gut and liver, or gut and brain or other combinations of lung, intestine, liver, brain and/or kidneys. Such combinations can, for example, mimic certain steps of fungal dissemination throughout the body. The intestine and lung serve as primary infection sites. (B) Selected *in vitro* models to study host–fungal interactions. 3D reconstituted human oral (RHOE) or vaginal (RHVE) epithelium grown at an air–liquid interface. Central nervous system (CNS) co-culture model including microglia cells and astrocytes. Intestinal co-culture model including epithelial cells, goblet cells and bacteria. Circulatory model with perfused endothelial cells.

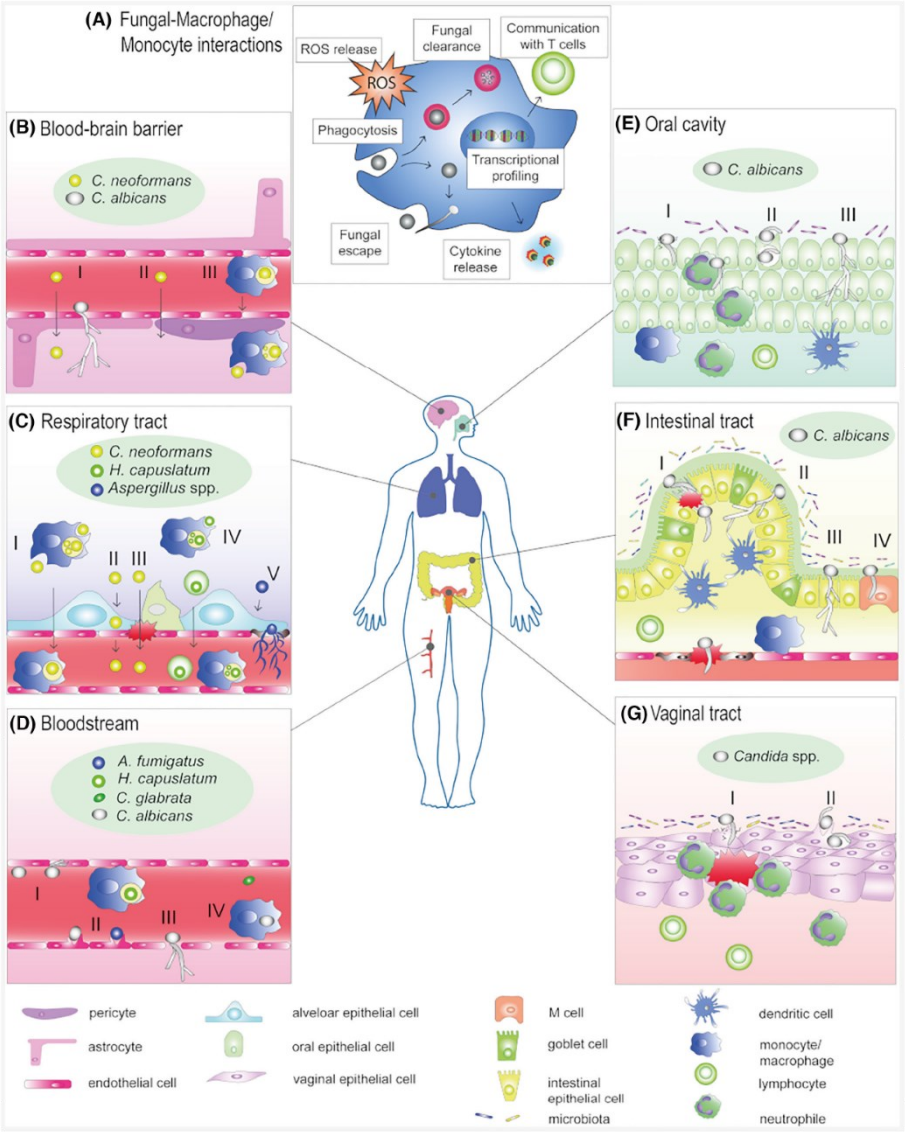


Figure 2. Fungal–host interactions during fungal diseases that are mimicked by *in vitro* infection models discussed in this review. (A) Fungal–monocyte/macrophage interactions resulting in several effector mechanisms that contribute to immunity against fungal infections (ROS: reactive oxygen species). (B) *C. neoformans* and *C. albicans* can cross the BBB via transcytosis (I); *C. neoformans* can overcome the barrier paracellularly (II) or use macrophages as shuttles (macrophages as 'Trojan horse') (III). (C) In the lung, *C. neoformans* and *H. capsulatum* induce their own phagocytosis by innate immune cells; they can replicate intracellularly and use host cells as shuttles to reach the blood stream and subsequently escape (I and IV); evasion of *C. neoformans* via transcytosis (II) or crossing of *C. neoformans* through a compromised epithelium (III). *Aspergillus* spp. form hyphae, can invade endothelial cells and enter the bloodstream (V). (D) *Candida* spp. can escape the blood circulation after adhesion to endothelial cells (I). *Candida* spp. and *A. fumigatus* can be endocytosed (II); *Candida* spp. can also use fenestrated endothelium as an escape route (III) or use leukocytes as shuttles (IV). (E) In the oral cavity, *C. albicans* hyphae can actively penetrate the epithelium (I) and/or invade via induced endocytosis (II) or translocate paracellularly (III). (F) In the intestine, *C. albicans* can actively penetrate the epithelium by hyphal growth (I), translocate paracellularly (II), invade without damaging the host cell (III) or translocate via M cells by inducing endocytosis (IV). (G) In the vaginal tract, *C. albicans* hyphae can actively penetrate the epithelium (I) or invade via induced endocytosis (II), thereby attracting neutrophils.

well as adaptive immune response increases the susceptibility to histoplasmosis (Akram and Koirala 2020).

Tissue-resident macrophages and monocyte-derived macrophages especially play an essential role against invasive candidiasis (Austermeier *et al.* 2020), whereas alveolar macrophages (AMs) are essential for clearance of fungi like *Aspergillus*, *Cryptococcus* or *Histoplasma* species that enter our body via the airways (Newman 2005; Xu and Shinohara 2017). Neutropenia is a common risk factor for aspergillosis and invasive candidiasis, showing the crucial role of neutrophils in antifungal host defense (Herbrecht *et al.* 2000). Dendritic cells (DCs) are crucial for activation of the adaptive immune system. Dysfunctions of the adaptive immune system like the reduced $CD4^+$ T cell function in AIDS patients increase the susceptibility for infections with *C. albicans*, *A. fumigatus*, *C. neoformans* or *H. capsulatum* (van de Veerdonk and Netea 2010). Interestingly, this predisposition manifests as mucosal *C. albicans* infections, in particular oropharyngeal candidiasis (OPC), but systemic *C. albicans* infections are also observed under such conditions (Fidel 2011). This is believed to be closely connected to the crucial roles of T helper responses in orchestrating oral mucosal resistance to infection (Gaffen and Moutsopoulos 2020; Scheffold, Bacher and LeibundGut-Landmann 2020). Antifungal immunity in the brain is connected to microglia that are the resident macrophage-like cells of the central nervous system (CNS), which show strong responses to fungal species like *C. albicans* (Blasi *et al.* 1991) and *C. neoformans* (Barluzzi *et al.* 1998). The C-type lectin receptor signaling adaptor CARD9 is expressed by microglia cells and its deficiency is associated with fungal brain infections (Drummond and Lionakis 2019). NK cells also exhibit antifungal effects (Schmidt, Tramsen and Lehrnbecher 2017) and a delayed NK cell reconstitution (e.g. after allogeneic stem cell transplantation) is associated with a higher risk of invasive aspergillosis (Weiss *et al.* 2020).

Antifungal effector functions during host defense against fungal pathogens

After the recognition of pathogen-associated molecular patterns (PAMPs) via pathogen recognition receptors (PRRs), supported by opsonization, innate immune cells mount responses to counteract the invading fungi. At early stages of infection, macrophages detect and engulf fungal pathogens (Gilbert, Wheeler and May 2014) (Fig. 2A). In addition, through the release of cytokines and chemokines they recruit and activate other immune cells. When neutrophils migrate to the site of infection, they act against fungal pathogens through phagocytosis, oxidative bursts and NETosis (Gazendam *et al.* 2016; Urban and Nett 2019). The production of reactive oxygen species (ROS) by phagocytes can kill fungal pathogens, like *C. albicans*, directly (Grondman *et al.* 2019) or impact LC3-mediated phagocytosis during defense against *A. fumigatus* (Sprenkeler, Gresnigt and van de Veerdonk 2016). This is highlighted, for example, by the fact that chronic granulomatous disease (CGD) patients, incapable of producing ROS, are highly susceptible to aspergillosis (Segal *et al.* 2000). DCs represent the bridge to activate the adaptive immune system via antigen processing and presentation to T-cells (LeibundGut-Landmann *et al.* 2007). T-cell differentiation can influence infection in different ways. Th1 cells augment the innate immune function through the release of IFNγ (Lionakis and Levitz 2018), which increases the microbicidal capacity of macrophages (Netea *et al.* 2015). Th17 cells release proinflammatory cytokines such as IL-17 and IL-22, which mediate recruitment of neutrophils and induce production of antimicrobial peptides (Khader, Gaffen and Kolls 2009; Conti *et al.* 2016) (Fig. 2A). The importance of these T-cell types for antifungal defense is evident in corresponding knock-out mice that have an increased susceptibility to disseminated *C. albicans* infections (Balish *et al.* 1998; Huang *et al.* 2004), but also show a striking susceptibility to mucosal infections. Th2 responses can result in a detrimental immune response, manifesting in a higher susceptibly to disseminated *C. albicans* infections (Haraguchi *et al.* 2010) or an aberrant immune response to *A. fumigatus* spores connected to allergic bronchopulmonary aspergillosis (ABPA) (Knutsen and Slavin 2011). T regulatory cells can suppress inflammatory responses and are highly beneficial to prevent immunopathology in the case of ABPA (Montagnoli *et al.* 2006), but also allow *C. albicans* persistence in the gastrointestinal tract (De Luca *et al.* 2007).

In vitro models to study interactions between fungi and immune cells

The interactions between fungi and the different effector functions of the immune system can be easily studied *in vitro* using cell lines (Table S1A, Supporting Information) and primary immune cells (Table S1B, Supporting Information). Cell lines have the advantage of easy handling and provide highly reproducible results. The availability of many reporter cell lines and the possibilities to generate transgenic/knockout cell lines represent valuable resources that allow the study of highly conserved mechanisms in the immunology against fungal infections. Nevertheless, central cellular processes such as pyroptosis, apoptosis and autophagy are considerably different or modified in cancer cell lines. Over the past few years, the essential role of these processes in shaping antifungal immunity has become increasingly clear (Kanayama and Shinohara 2016; Sprenkeler, Gresnigt and van de Veerdonk 2016; Dominguez-Andres *et al.* 2017; Evans, Sundaramurthy and Frickel 2018; O'Meara and Cowen 2018; Gonçalves *et al.* 2020; Thak *et al.* 2020; Weerasinghe and Traven 2020). Therefore, primary cells offer the highest similarity to the physiological situation. Primary immune cells are commonly isolated from peripheral human blood. By density gradient centrifugation, peripheral blood mononuclear cells (PBMCs) can be separated from erythrocytes and granulocytes (Munoz and Leff 2006). An important aspect to consider when using primary cells is that strong donor variation and even seasonal differences can influence experimental outcomes (Ter Horst *et al.* 2016). However, genetic differences between donors can also be exploited to analyze the impact of specific genotypes on the antifungal immune response (Lionakis *et al.* 2013; Smeekens *et al.* 2013; Matzaraki *et al.* 2017; Gresnigt *et al.* 2018b; Jaeger *et al.* 2019a,b). In these functional genomic approaches, immune cells of large cohorts of volunteers are screened for variation in specific immunological effectors such as cytokine release, ROS release or fungal killing. After genotyping the donors, the results of immunological phenotypes can be stratified based on the corresponding genotype. This knowledge on the influence of common genetic variations on the antifungal host response can provide valuable information about the role of certain genes in antifungal host defense. Combined with genetic association studies, functional genomics can be used to validate the impact of identified variants on immune pathways and susceptibilities to infections. In this way, crucial roles have been identified for CX3CR1 and

its role in host defense against of systemic candidiasis (Lionakis *et al.* 2013), as well as for the SIGLEC15 receptor in the susceptibility to vulvovaginal candidiasis (Jaeger *et al.* 2019b). Conversely, knowledge about genetic variations that influence critical antifungal host defense pathways can lead to the discovery of genetic susceptibilities. In this way NOD2 variants were found to increase resistance to invasive aspergillosis (Gresnigt *et al.* 2018b).

Macrophages

Interactions between macrophages/macrophage-like cells and fungal pathogens have been studied using cell lines like J774A.1, RAW, Ana-1, U937, BV-2 and THP-1 (Table S1A, Supporting Information). Such cell lines allow the generation of reporter constructs that can be used to monitor the activation of specific immune pathways. In this way, the importance of RAB-GTPases for maturation of *C. albicans*-containing phagosomes has been demonstrated (Bain *et al.* 2014; Okai *et al.* 2015). Another option is the use of macrophages derived from murine bone marrow cells and differentiated *in vitro* (BMDMs) (Table S1B, Supporting Information). A major advantage of this approach is the possibility to isolate BMDMs from mice with different genetic backgrounds (e.g. gene knockout or transgenic mice), thus providing a toolbox to obtain in-depth knowledge about key players of the host immune response during fungal infections. Such cells from knockout mice have been widely used to investigate, for example, inflammasome activation in the response to *C. albicans* (Kasper *et al.* 2018), *C. neoformans* (Guo *et al.* 2014) and *A. fumigatus* (Karki *et al.* 2015). In addition to BMDMs, human monocyte-derived macrophages (MDMs) can be used for *in vitro* studies. In such experiments, monocytes are isolated from PBMCs or whole blood and can be differentiated *in vitro* into a wide range of functionally different MDMs (Xue *et al.* 2014). MDMs have been used in numerous studies to dissect not only cytokine release, inflammasome activation, oxidative burst, phagocytosis and phagosome maturation after confrontation with fungi but also escape and survival mechanisms of fungi during these interactions (Smith, Dixon and May 2015; Gresnigt *et al.* 2018b; Kasper *et al.* 2018; O'Meara *et al.* 2018; Friedrich *et al.* 2019) (Table S1B, Supporting Information).

However, undifferentiated monocytes are also used to investigate how these cells are differentially activated (Halder *et al.* 2016; Dominguez-Andres *et al.* 2017; Klassert *et al.* 2017; Camilli *et al.* 2018; Leonhardt *et al.* 2018). The stimulation of monocytes using PAMPs such as β-glucan can induce epigenetic reprogramming, which alters the response to secondary *C. albicans* stimulation (Quintin *et al.* 2012), a concept known as innate immune memory or 'trained immunity'. In contrast, the response to *C. albicans* can also be hampered by the induction of innate immune tolerance by PAMPs such as lipopolysaccharide (Grondman *et al.* 2019). Over the past years it has become increasingly evident that cell metabolism is linked with immune cell functionality. Global as well as targeted profiling of metabolic pathways in primary immune cells, especially monocytes and macrophages, have been used to uncover immunometabolism in response to fungi (Dominguez-Andres *et al.* 2017; Gonçalves *et al.* 2020; Weerasinghe and Traven 2020).

Since pathogenic fungi often colonize and infect specific organs, the corresponding tissue macrophages offer the highest physiological relevance. For example, specific cells lines such as the murine alveolar macrophage cell lines MH-S (Mattern *et al.* 2015) and AMJ2-C11 (Pitangui Nde *et al.* 2015) are used to study fungal pathogens that cause pulmonary infections (Table S1A, Supporting Information). Alternatively, primary alveolar macrophages can be used to study the immune response of pulmonary fungal infections *ex vivo*. Though, the limited availability of these cells makes it challenging to obtain sufficient numbers for experiments. Nevertheless, protocols are available to obtain large numbers of AMs from bronchoalveolar lavage (BAL) (Busch *et al.* 2019) or resected lung tissue (Nayak *et al.* 2018). Similarly, peritoneal macrophages have been used to study the interactions with *Candida* spp. (Ifrim *et al.* 2016; Shimamura *et al.* 2019). Because peritoneal macrophages are easier to obtain in larger quantities than AMs, they have also been used for interaction studies with *H. capsulatum* (primarily infecting the lung) (Youseff *et al.* 2012; Huang *et al.* 2018; Shen *et al.* 2018) (Table S1B, Supporting Information). To dissect fungal interactions with immune cells in the brain, BV-2 microglia cells (Blasi *et al.* 1990) (Table S1A, Supporting Information) were co-cultured with astrocytes to demonstrate that candidalysin induces IL-1β release, which in turn mediates neutrophil recruitment (Drummond *et al.* 2019) (Fig. 1B).

Interaction studies with macrophages revealed mechanisms enabling fungal cells to evade macrophage phagocytosis or to escape from phagosomes. Masking of cell wall epitopes can prevent the detection of *A. fumigatus*, *C. albicans* and *H. capsulatum* by macrophages (Rappleye, Eissenberg and Goldman 2007; Aimanianda *et al.* 2009; Ballou *et al.* 2016). Morphological changes such as titan cell formation by *C. neoformans* (Okagaki and Nielsen 2012) or filamentation by *A. fumigatus* and *C. albicans* influence phagocytosis efficiency (Lewis *et al.* 2012; Erwig and Gow 2016; Maxson *et al.* 2018). Additionally, these fungi can inhibit phagosome acidification or phagosome maturation to prevent intracellular killing. These processes are reviewed in detail by Gilbert, Wheeler and May (2014) and Seider *et al.* (2010).

Irrespectively of the immune cell type used, numerous readouts are available to study interactions between fungi and cells of the immune system. Transcriptional profiling has provided indispensable insights into the interplay between immune cells and fungal pathogens. Specifically, dual-species transcriptional profiling has helped to elucidate key features of the adaptations of fungal cells in response to immune cells and *vice versa* (Niemiec *et al.* 2017; Munoz *et al.* 2019). Given the crucial role of phagocytes in fungal clearance, protocols established to investigate phagocytosis and phagosome maturation are common (Fig. 2A). Using live-cell microscopy, phagocytosis and viability dynamics can be studied on a kinetic scale involving multiple phagocytes (Smith, Dixon and May 2015; Gresnigt *et al.* 2018a; Kasper *et al.* 2018; Lim *et al.* 2018; Guimaraes *et al.* 2019; Seoane *et al.* 2020). For example, a struggle for glucose availability between macrophages and *C. albicans* was demonstrated to be crucial in dictating inflammasome activation (Tucey *et al.* 2020).*Candida albicans* cells however, can filament thereby complicating clearance through phagocytosis (Erwig and Gow 2016). Phagocytosis and phagosome maturation can also be examined in detail on a single-cell level (Bain *et al.* 2014; Okai *et al.* 2015; Westman *et al.* 2018). Such studies have contributed to the understanding of the role of phagosome–lysosome fusion in maintaining phagosome integrity while fungal cells filament inside the phagosome (Westman *et al.* 2020). Apart from live cell imaging, phagocytes can also be fixed at specific time-points to investigate the co-localization of proteins to the phagosome using immunofluorescence staining. In this way, LC3-associated phagocytosis has been investigated as a crucial pathway to improve phagocytosis efficiency of *H. capsulatum* and *A. fumigatus* (Huang *et al.* 2018; Kyrmizi *et al.* 2018). Using a similar approach, a key role has been shown for flotillin-dependent

microdomains or lipid rafts in phagosome formation for efficient host defense against *A. fumigatus* (Schmidt *et al.* 2020).

Natural killer (NK) cells

Primary NK cells can be obtained from PBMCs by different isolation kits (Wang *et al.* 2017). NK cells have been studied alone or in co-culture with other immune cells and have been observed to have direct antifungal capacity against *C. neoformans* through the release of perforins (Wiseman *et al.* 2007). The recognition of β1,3-glucan through the NKp30 receptor was identified to trigger and enhance the killing of *C. albicans* and *C. neoformans* by NK cells (Li *et al.* 2018). Other *in vitro* studies revealed an exhausted phenotype of NK cells, when they degranulate in contact with *A. fumigatus* (Santiago *et al.* 2018). NK cell activation in response to *Candida* species has been observed to occur indirectly by cross talk with monocytes (Marolda *et al.* 2020). Similarly, for *A. fumigatus*, crosstalk between NK cells and DCs was found to mediate DC activation (Weiss *et al.* 2018). Further, direct antifungal effects of NK-cells against *A. fumigatus* have been associated with release of IFNγ (Bouzani *et al.* 2011) (Table S1B, Supporting Information).

Neutrophils

Using hypotonic lysis of erythrocytes or other gradient solutions like PolymorphPrep© (Progen, Heidelberg, Germany) (Degel and Shokrani 2010), primary neutrophils can be isolated from PBMCs to investigate their interaction with fungi. Neutrophils can act as phagocytes, but can also form neutrophil extracellular traps (NETs) and release cytokines in the presence of fungal cells. These features were studied intensively *in vitro* (Urban *et al.* 2006; Bruns *et al.* 2010; Rocha *et al.* 2015; Sun and Shi 2016; Dasari *et al.* 2018; Thompson-Souza *et al.* 2020). By studying phagocytosis, killing, NETosis and cytokine release, spleen tyrosine kinase (Syk) was identified as a crucial mediator for inducing antifungal effector mechanisms against various *Candida* species (Negoro *et al.* 2020). Another aspect is to monitor how these phagocytes migrate to the site of infection. Chemotaxis assays using specialized *in vitro* systems (Richards *et al.* 2004; Chen 2005; Thunström Salzer *et al.* 2018) can be used to elucidate this process in the context of fungal infections (Coenjaerts *et al.* 2001; Drummond *et al.* 2015; Rieber *et al.* 2016) (Table S1B, Supporting Information). ROS release or oxidative bursts in response to fungal pathogens can be assessed not only in neutrophils (Boyle *et al.* 2011; Liu *et al.* 2018) but also in monocytes (Wellington, Dolan and Krysan 2009; Brunel *et al.* 2018) and macrophages (Wolf *et al.* 1987; Youseff *et al.* 2012; Sun *et al.* 2014; Arce Miranda *et al.* 2019) (Fig. 2A; Table S1B, Supporting Information). Using a modified model, in which *C. albicans* cells are grown in clusters on poly-L-lysine coated glass slides, neutrophils were observed to form 'swarms' to efficiently use oxidative stress mechanisms to attack *C. albicans* (Hopke *et al.* 2020).

Dendritic cells, T-cells and whole blood models

Virtually all immune cell types are being employed to study transcriptional responses to fungal pathogens (Smeekens *et al.* 2013; Hellwig *et al.* 2016; Van Prooyen *et al.* 2016; Niemiec *et al.* 2017) as well as cytokine and chemokine responses (Coady and Sil 2015; Becker *et al.* 2016; Marischen *et al.* 2018) to fungal pathogens (Fig. 2A). Often such studies involve crosstalk between different immune cell types such as antigen-presenting cells and cells of the adaptive immune system. PBMCs are frequently used due to their composition of innate and adaptive immune cells and allow the study of innate host responses (Becker *et al.* 2016; Alvarez-Rueda *et al.* 2020), but also T-cell mediated responses such as Th1, Th17, Th2 and Tregs (Zielinski *et al.* 2012; Gresnigt *et al.* 2013; Becker *et al.* 2015; Raijmakers *et al.* 2017; Page *et al.* 2018; Vogel *et al.* 2018) (Fig. 2A). For example, using PBMCs, the type I interferon pathway was identified to play a crucial role in *C. albicans* defense (Smeekens *et al.* 2013). Interactions between DCs and T-cells were used to investigate how the adaptive immune response is polarized through antigen presentation, co-stimulation and the cytokine environment (van der Does *et al.* 2012; Stephen-Victor *et al.* 2017). DC maturation can be examined in transwell systems (Lother *et al.* 2014) or by profiling maturation features via flow cytometry (Pietrella *et al.* 2005; Hefter *et al.* 2017; Vivas *et al.* 2019). For interaction studies including a wide range of immune cell types, whole blood models were used to gain information about fungal killing (Hunniger *et al.* 2014), transcriptional responses (Dix *et al.* 2015; Kämmer *et al.* 2020), cytokine release (Oesterreicher, Eberl and Zeitlinger 2019) and platelet interactions (Fréalle *et al.* 2018; Eberl *et al.* 2019) (Table S1B, Supporting Information).

STUDYING RESPIRATORY TRACT INFECTIONS WITH *ASPERGILLUS*, *HISTOPLASMA* AND *CRYPTOCOCCUS* SPP.

In the respiratory tract fungal pathogens such as *A. fumigatus*, *H. capsulatum* and *C. neoformans* can cause infections in predisposed hosts. Since the major biological niche of these fungi is the environment, fungal elements (mostly conidia or yeast) are frequently inhaled by the human host. The healthy immune system can clear these inhaled fungal elements, whereas immunocompromised individuals or patients with pre-existing pulmonary conditions may fail to clear fungi and have a higher risk to develop aspergillosis, histoplasmosis or cryptococcosis. The clinical manifestations of these fungal diseases, however, are very diverse. Infections with pathogenic *Aspergillus* species can develop differently, depending on the immune reaction and underlying lung pathology (Soubani and Chandrasekar 2002; van de Veerdonk *et al.* 2017). While a compromised immune response can result in invasive pulmonary aspergillosis, pre-existing lung injury can lead to the development of an aspergilloma and a chronic or hyper inflammatory response. Such responses can also provoke allergic bronchopulmonary aspergillosis (Kosmidis and Denning 2015). In immunocompromised patients, specifically patients suffering from AIDS, *C. neoformans* can cause either pulmonary cryptococcosis or can disseminate into other organs after an (asymptomatic) pulmonary infection (Setianingrum, Rautemaa-Richardson and Denning 2019). *Cryptococcus neoformans* cells can be engulfed by AMs and DCs and can survive within the phagolysosome, proliferate and eventually escape via non-lytic exocytosis (vomocytosis) (Fig. 2C I). Vomocytosis was also observed for *C. albicans* (Bain *et al.* 2012), *C. krusei* (García-Rodas *et al.* 2011), *A. nidulans* and *A. fumigatus* (Gresnigt *et al.* 2018a). Intracellular survival is one key strategy of *C. neoformans* to disseminate from the respiratory tract (Coelho, Bocca and Casadevall 2014). Other translocation routes involve fungal cells crossing the epithelial border via transcytosis (Fig. 2C II) or a direct migration through areas where the epithelial lining has been compromised (Fig. 2C III) (Denham and Brown 2018). *Histoplasma capsulatum* can cause pulmonary histoplasmosis, and similar to *C. neoformans*, it can evade the immune system by hiding inside AMs (Ray and Rappleye 2019). Following growth and

replication, it can induce apoptosis facilitating further dissemination within the bloodstream and lymphatic organs (Fig. 2C IV) (Long *et al.* 2003; Mihu and Nosanchuk 2012; Pitangui Nde *et al.* 2015). In contrast to *H. capsulatum* and *C. neoformans*, which grow as yeast during infection, *A. fumigatus* proliferates as hyphae in the lung, allowing deep tissue invasion (Fig. 2C V).

Simple *in vitro* models mimicking lung infections

To mimic the alveolar environment, the pulmonary epithelial cell line A549, originating from a human alveolar cell carcinoma (Lieber *et al.* 1976), is frequently used to study pathogenicity attributes including adhesion (Gravelat *et al.* 2010; Pitangui *et al.* 2012; Teixeira *et al.* 2014), endocytosis (Liu *et al.* 2016), epithelial detachment (Kogan *et al.* 2004; Bertuzzi *et al.* 2014) and epithelial damage (Ejzykowicz *et al.* 2010; Bertuzzi *et al.* 2014). These studies revealed crucial roles for the *A. fumigatus* transcription factors PacC (Bertuzzi *et al.* 2014) and DvrA (Ejzykowicz *et al.* 2010) to mediate tissue invasion and damage. In addition, A549 cells were used to dissect pulmonary epithelial IL-8 responses to *C. neoformans* and *H. capsulatum* (Barbosa *et al.* 2007; Alcantara *et al.* 2020), and shed light on how different *A. fumigatus* isolates differentially regulate gene expression of epithelial cells (Watkins *et al.* 2018) (Table S2, Supporting Information). To examine the fungal translocation through the pulmonary epithelium, transwell models with different modifications have been employed (Fig. 1A).

Complex *in vitro* models mimicking lung infections

Models that combine A549 cells with DCs (Morton *et al.* 2018) or a bilayer of human pulmonary artery endothelial cells (HPAECs) with (Morton *et al.* 2014) or without DCs (Hope *et al.* 2007; Belic *et al.* 2018) were utilized to model the cellular complexity in the alveolus and the cellular cytokine response to fungal infections. The translational capacity of such a model was reflected in a study that validated the measurement of galactomannan as a biomarker of fungal infection and antifungal efficacy *in* vitro (Hope *et al.* 2007). These models have also been employed for microscopy-based analyses, gene expression analysis and analysis of immune activation to gain insights into the host–*Aspergillus* interactions at the alveolar epithelial interface (Table S2, Supporting Information).

To more closely resemble the physiological situation, primary human bronchial or small airway epithelial (HBE, SAE) cells were used to study proinflammatory epithelial cytokine responses to *C. neoformans* infections (Guillot *et al.* 2008). These cells differentiate when cultured at an air-liquid interphase (ALI) into lung epithelium and were also used to assess the host response to *A. fumigatus* conidia. Transcriptome and proteome analyses revealed the upregulation of apoptosis, autophagy, translation and cell cycle pathways as well as the downregulation of complement and coagulation pathways (Toor *et al.* 2018). The combination of differentiated pulmonary epithelial cells with DCs and macrophages provides an even more complex model, which allows the study of the interplay between fungal cells, the epithelium and the immune system (Chandorkar *et al.* 2017). As an alternative strategy to investigate *Aspergillus* spp. infections, bronchial mucosal tissue resected from cancer patients was used. Using this *ex vivo* model, adhesion, invasion, damage and structural changes of the epithelium were investigated (Amitani and Kawanami 2009). Although the latter model represents human physiology, its applicability is limited by the difficulty of obtaining patient material. Besides confounding factors, such as therapies and medication, inter-individual differences may impact the validity of this model and the ability to obtain reproducible results.

Lung-on-chip models

Most lung models used so far are cultured statically and thus are not subjected to shear stress. Further, these models rarely consider the impact of additional members of the microbial community, such as the lung microbiota in the infection process. A number of lung-on-chip models have been established that reflect additional physiological key features of the lung. A 'breathing' alveolus-on-chip is mimicked by stretching and contraction of a membrane using a vacuum, which leads to an increased uptake of nanoparticles of the epithelium and transport to the vasculature (Huh *et al.* 2010; Stucki *et al.* 2018). Mechanostimulation represents an important biophysical cue since the stretching of the lungs influences repair mechanisms in damaged epithelial cells and might also play a significant role during fungal invasion (Desai, Chapman and Waters 2008). Deinhardt-Emmer and colleagues established an alveolus-on-chip model that harbored immune cells and consisted of two compartments. In the upper compartment, lung epithelial cells differentiated into the two types of alveolar epithelial cells and were separated by a porous membrane from an endothelial lining, subjected to flow in the lower compartment (Deinhardt-Emmer *et al.* 2020) (Fig. 1A). Although this model was not used to dissect fungal–host interactions so far, it revealed new insights about the interplay of *S. aureus* and influenza virus at the alveolar–capillary interface. During co-infection, increased inflammatory responses were observed including cytokine expression and loss of barrier function similar to severe clinical outcomes of patients with bacterial-viral superinfections (Deinhardt-Emmer *et al.* 2020). Other platforms have used human alveolar epithelial cells (hAEpCs), and also integrated neutrophils (Huh *et al.* 2010; Benam *et al.* 2016; Jain *et al.* 2018; Zhang *et al.* 2018). Future models can be colonized with (additional) members of the pulmonary microbiome to investigate the interplay with fungi, which can contribute to progression of pulmonary fungal infections (Kolwijck and van de Veerdonk 2014). Taken together, current lung-on-chip models can produce a microenvironment resembling the *in vivo* physiology by imitating an ALI, mechanical strain and immune responses. This can facilitate the establishment of sophisticated pulmonary-infection models.

STUDYING COLONIZATION AND INFECTION OF THE ORAL CAVITY, THE INTESTINAL TRACT AND VAGINAL TRACT BY *CANDIDA* SPP.

In the oral cavity, the intestinal-, and vaginal tract, *Candida* spp. normally live as harmless commensal yeasts. However, some opportunistic *Candida* spp. can cause infections. These range from mucocutaneous infections such as OPC (Millsop and Fazel 2016) and VVC (Rosati *et al.* 2020) to invasive candidiasis (Pappas *et al.* 2018). Diverse predispositions, like immunosuppression, an impaired barrier function and an imbalanced microbiota, are prerequisites to enable infection of *Candida* species. However, both predisposition and protection by an adjusted immune response differ between the specific types of infections. In the following sections we discuss *in vitro* models used to study *C.*

albicans and *C. glabrata* interactions with the host in three different niches of the human body.

Studying *Candida* spp. infections of the oral cavity

OPC occurs mostly in combination with the use of broad-spectrum antibiotic therapy and immune suppression, e.g. through HIV/AIDS, chemotherapy or radiation therapy. Further, neonates, diabetic and elderly individuals are more susceptible (Patil *et al.* 2015). *Candida albicans* is the most prevalent species, but also other *Candida* species like *C. glabrata*, *C. dubliniensis*, *C. krusei*, *C. kefyr*, *C. parapsilosis*, *C. stellatoidea* and *C. tropicalis* can be found in oral lesions (Millsop and Fazel 2016). *Candida albicans* mainly interacts with the oral epithelium by invading cells via active penetration (Fig. 2E I) and/or induced endocytosis (Fig. 2E II) (Phan *et al.* 2007; Dalle *et al.* 2010; Wachtler *et al.* 2011a; Sheppard and Filler 2014; Naglik *et al.* 2017), or invasion of the tissue by degradation of E-cadherin, thereby disrupting the epithelial barrier (Fig. 2E III) (Villar *et al.* 2007). *In vivo*, the uppermost layer of the oral epithelium consists of stratified squamous epithelium, followed by a basal membrane and fibroblasts in the lamina propria.

Simple *in vitro* models mimicking oral infections

To study *Candida*–host interactions of the oral cavity, oral epithelial cells are commonly used. TR146 cells are derived from a squamous cell carcinoma of the buccal mucosa (Rupniak *et al.* 1985) and used to investigate invasion (Puri *et al.* 2019), damage (Wilson *et al.* 2014; Meir *et al.* 2018) and gene expression (Schaller *et al.* 1998; McCall, Kumar and Edgerton 2018; Meir *et al.* 2018). The TR146 model has contributed significantly to the understanding of *C. albicans* pathogenicity by showing that the peptide toxin candidalysin is responsible for the capacity of *C. albicans* hyphae to cause damage (Moyes *et al.* 2016). The same model was used to demonstrate that candidalysin also activates epithelial proinflammatory responses through the epithelial growth factor receptor (Ho *et al.* 2019) and its synergistic signaling with IL-17 (Verma *et al.* 2017). Immortalized oral mucosal cells (OKF6/TERT-2) (Dickson *et al.* 2000) have also been used to study epithelial transcriptional responses (Liu *et al.* 2015), to visualize *C. albicans* invasion (Wollert *et al.* 2012) and to demonstrate that invasion is, in part, mediated through endocytosis (Solis *et al.* 2017; Swidergall *et al.* 2018). The same cell line was used to show that damage is mediated through white cells in contrast to opaque cells (Solis *et al.* 2018). Furthermore, Epha2 was identified as an epithelial cell pattern recognition receptor for fungal β-glucans, activating a signal cascade that results in a proinflammatory and antifungal response (Swidergall *et al.* 2018).

Tongue cells derived from a squamous cell carcinoma (SCC15) represent a third cell type used to dissect interactions of *C. albicans* with the oral epithelium (Lindberg and Rheinwald 1990). Similar to the studies discussed above, SCC15 cells were used to investigate epithelial damage (Kumar *et al.* 2015), invasion (Villar *et al.* 2007) and cytokine release (Dongari-Bagtzoglou and Kashleva 2003) (Table S3A, Supporting Information).

Complex *in vitro* models mimicking oral infections

In addition to monolayer models (Fig. 1A), organotypic 3D models known as reconstituted human oral epithelium (RHOE) are commonly used to study oral *Candida* spp. infections due to their histological similarity to physiological oral epithelium. In these RHOE models, TR146 cells are cultured on a polycarbonate filter at an ALI with culture medium on the basal side, resulting in a multilayer model with differentiated cells (Fig. 1B). This model has been used to study epithelial damage (Silva *et al.* 2011; Mailander-Sanchez *et al.* 2017) and fungal (Spiering *et al.* 2010) or host cell gene expression (Wagener, Mailander-Sanchez and Schaller 2012) (Table S3A, Supporting Information). In addition, the model was used to show enhanced invasion and tissue damage during co-infection of *C. albicans* and *C. glabrata* (Silva *et al.* 2011). Because fungal biofilm formation is crucial for the development of caries and OPC, the RHOE model has also been used to analyze the expression of *C. albicans* virulence genes associated with biofilm formation (Nailis *et al.* 2010). Similar RHOE models exist, containing collagen embedded fibroblasts from mice and oral mucosal cells OKF6/TERT-2 cells, differentiated at an ALI (Dongari-Bagtzoglou and Kashleva 2006a,b). Since the interplay with the oral microbiota plays an essential role for the maintenance of a commensal state of *C. albicans* or for development of OPC (Montelongo-Jauregui and Lopez-Ribot 2018), the organotypic 3D models were also used to study interactions between *C. albicans* and bacteria. For example, antagonistic interactions between *Lactobacillus rhamnosus* and *C. albicans* were dissected (Mailander-Sanchez *et al.* 2017). Furthermore, fungal-induced dysbiosis after chemotherapy (Bertolini *et al.* 2019) and synergistically increased tissue damage during interactions with *S. mutans* (Diaz *et al.* 2012) were observed. Additionally, biofilm formation of *C. albicans* and *C. glabrata* after chemotherapeutic treatment was examined in the latter organotypic 3D model (Sobue *et al.* 2018). The model was further 'humanized' by using human fibroblasts and spontaneously immortalized keratinocytes to analyze interactions between *C. albicans* and *S. aureus* (de Carvalho Dias *et al.* 2018) (Table S3A, Supporting Information).

In vitro modeling of *C. albicans* stomatitis

C. albicans mediated stomatitis, an inflammatory reaction of the oral mucosa, is a major complication for users of removable dental prostheses, but also common in smokers or patients suffering from diabetes mellitus (Salerno *et al.* 2011; Javed *et al.* 2017; Alzayer *et al.* 2018). To model this oral infection, primary human palate epithelial cells (HPECs) were used to study the host response to *C. albicans* in terms of apoptosis, nitric oxide production (Casaroto *et al.* 2019) and mucosal gene expression (Offenbacher *et al.* 2019). Similarly, a combination of TR146 cells and primary fibroblasts was used for adhesion and gene expression studies (Morse *et al.* 2018) (Table S3A, Supporting Information).

Mucosa-on-chip models

Monolayer or multilayered mucosal models commonly feature a perpendicular configuration. This vertical culture arrangement hampers the individual monitoring of different cell layers by microscopy, and resolution decreases in deeper layers. A horizontal organization of cell layers was applied in a mucosa-on-chip model (Rahimi *et al.* 2018) consisting of microchambers, which were aligned in parallel and interconnected by pores. A central subepithelial chamber harbored a collagen hydrogel with gingival fibroblasts, while keratinocytes were seeded into the pores connecting the luminal and subepithelial compartment. The luminal chamber can be microfluidically perfused to imitate saliva and saliva flow, which is an important contributor to epithelial barrier integrity. A further refinement for both

static and microfluidic models can include an endothelial lining and immune cells such as dendritic Langerhans cells, which are almost exclusively found in stratified squamous epithelium and have been shown to react to *Candida* species (Upadhyay *et al.* 2013).

Studying *Candida* spp. colonization of the intestinal tract and intestinal translocation

Both *C. albicans* and *C. glabrata* colonize the human intestinal tract (Hallen-Adams and Suhr 2017). The gut represents the main reservoir of fungi, especially *C. albicans*, that can cause disseminated and systemic infections (Gouba and Drancourt 2015). In these life-threatening infections, the fungus overcomes the intestinal epithelium, which forms a barrier between the intestinal lumen and the sterile tissues of the human body. During this process, termed translocation, the fungus employs several mechanisms including active penetration (Fig. 2F I), paracellular translocation (Fig. 2F II) or migration through the intestinal epithelial layer without damaging the host cells (Fig. 2F III) (Allert *et al.* 2018; Basmaciyan *et al.* 2019). Certain predispositions favor fungal overgrowth and translocation: antibiotics induce an imbalance of the microbiota and cytostatic therapy or abdominal surgery, which compromise the barrier function (Pfaller and Diekema 2007). To better understand the conditions that keep *C. albicans* commensal or drive the commensal-to-pathogen shift, the interactions between *C. albicans* and the intestinal barrier are studied extensively to find ways to prevent or reverse this shift (Kumamoto, Gresnigt and Hube 2020).

Simple *in vitro* models mimicking intestinal infections

Monolayers of cell lines originating from colorectal adenocarcinomas are widely used (Fig. 1A). The most common cell lines are Caco-2 and HT-29. Caco-2 cells differentiate spontaneously into a polarized monolayer with characteristic villi and tight junctions after 12 days of culture (Fogh, Wright and Loveless 1977). These cells were used to demonstrate that damage to the intestinal epithelium induced by *C. albicans* relies on a combination of adhesion-mediated contact sensing, tissue invasion through hyphal extension and damage by the expression of pathogenicity factors (Wachtler *et al.* 2011a). Interactions with non-pathogenic yeast cells that can antagonize *C. albicans* pathogenicity were examined (Lohith and Anu-Appaiah 2018; Kunyeit *et al.* 2019). Furthermore, receptor signaling pathways (Mao *et al.* 2019), induction of defensins (Gacser *et al.* 2014), impact on tight junctions (Goyer *et al.* 2016) and the potential of epithelial cells to discriminate between yeast and hyphal morphologies (Schirbel *et al.* 2018) are processes that can be analyzed in this model. A subclone of the Caco-2 cell line, C2BBe1, was often used in *in vitro* systems due to its more homogeneous brush boarder expression (Peterson and Mooseker 1992). A model of C2BBe1 cells cultured in transwell systems (Fig. 1A) was instrumental to elucidate important virulence requirements of translocation through the epithelial barrier and revealed a key role for candidalysin by mediating necrotic cell damage that allowed transcellular translocation (Allert *et al.* 2018). Additionally, using this model, a MAPK/NFκB mediated epithelial response to *C. albicans* infection was shown to increase epithelial resistance (Bohringer *et al.* 2016) (Table S3B, Supporting Information).

Essential features of *C. albicans* pathogenicity like adhesion, invasion and damage were also studied using the HT-29 cell line (Deng *et al.* 2015; Garcia *et al.* 2018). A methotrexate treatment of HT-29 cells, transformed these cells into mucus-secreting goblet cells (HT-29-MTX) (Lesuffleur *et al.* 1990). These mucus-secreting cells were instrumental in demonstrating the role of mucus in suppressing virulence-associated attributes of *C. albicans*, such as hypha formation (Kavanaugh *et al.* 2014).

Complex *in vitro* models mimicking intestinal infections

As the intestinal epithelium consists of a myriad of cell types, combinations of different cell lines have been employed to more accurately mimic the *in vivo* situation. For example, a combination of Caco-2 cells and Raji B cells (human Burkitt's lymphoma) was used to study the interaction of *C. albicans* with an epithelial barrier including M-cells, which demonstrated M-cells as a preferred cell type for translocation via induced endocytosis (Fig. 2F IV) (Albac *et al.* 2016). In general, most *in vitro* models investigate *C. albicans* in its pathogenic state. To limit the pathogenicity of *C. albicans* and mimic commensalism, a mixture of C2BBe1 cells and the mucus-producing HT-29-MTX cells were colonized with *L. rhamnosus* to establish a basic 'commensal' model (Fig. 1B). Using this model, a damage reduction was observed in the presence of mucus and bacteria, both antagonizing *C. albicans* pathogenicity by reducing filamentation, proliferation and inducing shedding that physically separates hyphae from host cells (Graf *et al.* 2019) (Table S3B, Supporting Information).

Intestine-on-chip models

Although 2D intestinal models mimic the fundamental physiological structures of the intestinal tissue such as mucus production, M-cells and brush border epithelium, they do not reflect the unique 3D architecture of the intestinal epithelial tissue consisting of villi and crypts. Cells in these models are cultured statically and are not subjected to the peristaltic movement characteristic for the intestine. In addition, *in vitro* models often lack immune cells, which convey tolerance towards commensals and trigger inflammatory responses when pathogens inflict damage to the intestinal lining. A number of intestine-on-chip models have been developed that recapitulate some of these key physiological features (Bein *et al.* 2018). In these models, Caco-2 cells grow out and form villi-like structures when grown on a membrane and exposed to shear stress (Kim and Ingber 2013). Microfluidic intestine models often include endothelial cells adjacent to epithelial cells in an individually perfused compartment. The luminal and the vascular compartment are separated by a porous membrane to facilitate transmigration of cells and cell communication. Innate immune cells such as monocytes can be implemented in the endothelial layer and differentiated into macrophages and DC-like cells, which tolerate inflammatory triggers in the intestinal lumen, but elicit a strong inflammatory response when a systemic infection is mimicked (Maurer *et al.* 2019). In this model, *C. albicans* invasion of the epithelial layer and subsequent invasion of the bloodstream compartment in the presence and absence of the commensal bacterium *L. rhamnosus* were investigated. Patient-derived colon epithelial cells are difficult to access, but can sufficiently be maintained in microfluidic platforms and produce a mucus layer resembling the *in vivo* thickness (Sontheimer-Phelps *et al.* 2020). 3D intestine-on-chip models will be valuable tools to uncover the role of commensals and their products, as well as host immune responses in the yeast-to-hypha transition of *C. albicans* in the future (Table S3B, Supporting Information).

Intestinal organoids

Apart from intestine-on-chip models, human intestinal organoids have emerged as a valuable disease-modeling tool. Human intestinal organoids can be grown from adult stem cells extracted from intestine biopsies or induced pluripotent stem cells (iPSCs) (Rahmani *et al.* 2019) to form 3D organotypic structures by self-organization and resemblance of key embryonic signaling *in vitro* (Clevers 2016) (Fig. 1A). Intestinal organoids show a villus and crypt-like architecture with epithelial cells facing inwards, creating a lumen as an enclosed space (Sato *et al.* 2009; Spence *et al.* 2011). Organoid models face similar challenges like OOC platforms, such as additional cell types, immune cells, endothelial cells and extracellular matrix components that need to be incorporated to create a physiological microenvironment for cell differentiation and tissue development. However, mesenchymal cells and neural crest cells have already been successfully implemented in these models (Workman *et al.* 2017). Unlike microfluidic OOC models, stem cell-derived organoids currently lack perfusion and therefore deprive epithelial cells of shear stress and removal of metabolites. An idea has emerged that aims at combining self-assembling organoids with microfluidic OOC techniques, termed 'Organoids-on-a-Chip' (Park, Georgescu and Huh 2019) (Fig. 1A). The technique encompasses the maturation of organoids within a dynamic culture environment allowing the control of nutrient supply, establishment of biochemical gradients vital for self-organization of the organoids and the introduction of additional cell types.

Studying *Candida* spp. infections of the vaginal mucosa

The vaginal mucosa represents another commensal niche of *Candida* spp. in the human body. VVC affects 70–75% of women in their reproductive age (Sobel 2007). Antibiotic treatment is a strong predisposing factor for VVC (Shukla and Sobel 2019), most likely due to the induced dysbiosis of the vaginal microbiome. *C. albicans* is the most prominent species isolated from VVC, followed by *C. glabrata* (Makanjuola, Bongomin and Fayemiwo 2018). The interactions between *Candida* spp. and the vaginal epithelium, as well as the vaginal microbiota, are complex (Pekmezovic *et al.* 2019; Kalia, Singh and Kaur 2020), and invasion of the epithelium occurs through active penetration (Fig. 2G I) and induced endocytosis (Fig. 2G II), while neutrophils are attracted simultaneously.

Simple *in vitro* models mimicking vaginal infections

The VK2/E6E7 cell line originates from healthy human vaginal mucosal tissue and was immortalized by retroviral transduction (Fichorova, Rheinwald and Anderson 1997). This cell line was used to demonstrate synergistic interactions between *C. albicans* and streptococci (Pidwill *et al.* 2018) and a role for autophagy machinery in the survival of epithelial cells during *C. albicans* infection (Shroff and Reddy 2018). In addition, Type-I IFN signaling was elucidated to increase resistance of the epithelium to *C. albicans* infection (Li *et al.* 2017). By introducing high glucose conditions, this model has been used to demonstrate that the association of VVC in diabetes patients might be related to increased adhesion of *C. albicans* through a potential interaction with ICAM-1 (Mikamo *et al.* 2018). Another cell line, A431, originates from a vaginal epidermoid carcinoma. This cell line was used to investigate inflammatory cytokine responses and damage of A431 cells induced by candidalysin (Richardson *et al.* 2018). Additionally, the cell line was utilized to evaluate the impact of azole antifungal treatment on damage induced by *C. albicans* spp. (Wachtler, Wilson and Hube 2011b) (Table S3C, Supporting Information).

Complex *in vitro* models mimicking vaginal infections

A reconstituted vaginal epithelium (RHVE) is available as an alternative model. RHVE is based on A431 cells, cultivated at an ALI, similar to the previously described RHOE (Fig. 1B). RHVE was used to demonstrate that *C. albicans* facilitates interactions of *C. glabrata* with the vaginal epithelium by increasing fungal colonization, invasion and damage of epithelial cells during co-infection (Alves *et al.* 2014). Furthermore, the adaptation of *C. glabrata* to an acidic vaginal environment was investigated using RHVE (Bernardo *et al.* 2017) (Table S3C, Supporting Information).

Organ-on-chip models mimicking vaginal infections

Several OOC models for the female reproductive tract are available, predominantly to mimic the physiology of the endometrium, the uterus or the placenta (Mancini and Pensabene 2019). Possible OOC models of the vaginal mucosa should comprise stratified squamous epithelium and perfused endothelial cells, separated by a porous membrane. Immune cells can easily be integrated to recapitulate relevant inflammatory responses during hyphal invasion of the epithelium such as neutrophil recruitment.

In vivo, the vaginal tract harbors a microbiota that consist to a large extent of *Lactobacillus* species. Although predicted, it is not entirely clear whether the microbiota actually has a protective effect against *Candida* spp. infection and if so, whether diversity among microbial communities leads to a higher degree of protection (Cassone 2015).

STUDYING FUNGAL BLOODSTREAM INFECTION AND CROSSING OF THE BBB

Vascular infection models

Fungal dissemination into the bloodstream is a major driver for the development of multi-organ infections or sepsis. *Aspergillus fumigatus*, *H. capsulatum* and *C. neoformans* can enter the bloodstream after crossing the pulmonary alveolar epithelium (Fig. 2C), whereas *C. albicans* reaches the bloodstream mostly via the intestinal tract (Fig. 2F). Central venous catheters, surgery and parenteral nutrition represent additional entry routes, especially for *Candida* species (Hashemi Fesharaki *et al.* 2018). To exit the blood circulation and invade other organs, fungi interact with the endothelial lining of the blood vessels (Fig. 2D), which can be simulated by human umbilical vein endothelial cells (HUVECs) (Jaffe *et al.* 1973). Although access to umbilical cords is limited, high amounts of cells can be isolated from a single umbilical cord and stored frozen for several experiments (Crampton, Davis and Hughes 2007). HUVECs were used to dissect *C. albicans* adhesion to the endothelial lining (Fig. 2D I), for example, it was shown that a certain hyphal length is crucial for adhesion in a circulatory *in vitro* model that simulated physiological capillary blood pressure (Wilson and Hube 2010) (Fig. 1B). Following adhesion, three mechanisms to pass the endothelial barrier were discovered. Attached *Candida* cells can be endocytosed by endothelial cells (Phan *et al.* 2005; Liu *et al.* 2016) (Fig. 2D II), a process that depends on a complex formation including

endothelial cell septin 7 (SEP7) and N-cadherin (Phan *et al.* 2013). Endocytosis was also described for *A. fumigatus*, independent of its morphology (Kamai *et al.* 2006) (Fig. 2D II). In addition, *Candida* spp. can cross the endothelial barrier via paracellular translocation (Fig. 2D III) or via leucocytes following engulfment (Fig. 2D IV) (Filler and Sheppard 2006; Grubb *et al.* 2008). It is likely that similar Trojan horse transport mechanisms following engulfment by mononuclear cells are exploited by intracellularly persistent *H. capsulatum* (Gilbert, Wheeler and May 2014) (Fig. 2D IV) as it already has been shown for *C. neoformans* (Coelho *et al.* 2019).

The ability of different *C. albicans* mutants to damage HUVECs was leveraged to identify virulence factors that are important for fungal dissemination (Sanchez *et al.* 2004). Similarly, the transcription factor DvrA was identified as crucial for endothelial damage induced by *A. fumigatus* (Ejzykowicz *et al.* 2010). Besides, the proteome profile of HUVECs was investigated during infection with *A. fumigatus* (Neves *et al.* 2017) and *C. neoformans* (Wang *et al.* 2011), indicating alterations that contribute to fungal invasion. Transcriptional profiling of HUVECs revealed the upregulation of genes involved in chemotaxis, stress response, angiogenesis and inhibition of apoptosis in response to *C. albicans* (Barker *et al.* 2008). A proinflammatory immune response associated with the release of TNF in HUVECs was reported after infections with *C. albicans* (Orozco, Zhou and Filler 2000) and *A. fumigatus* (Kamai *et al.* 2009; Neves *et al.* 2017). In addition, it was shown that neutrophils protect endothelial cells against *C. albicans*-induced damage in a co-culture model with HUVECs and neutrophils (Edwards *et al.* 1987) (Table S4, Supporting Information).

Blood–brain barrier

Whereas cerebral infections with *Candida* spp. (Drummond *et al.* 2015), *Aspergillus* spp. (Rieber *et al.* 2016) or *Histoplasma* spp. (Schestatsky *et al.* 2006) are rare, meningitis is the most prominent complication during cryptococcosis (Srikanta, Santiago-Tirado and Doering 2014). Cerebral infections are induced when fungi cross the BBB, a part of the neurovascular unit (NVU). Other than the endothelial lining, the NVU consists of pericytes, forming a scaffold for endothelial cells together with the basal lamina. Endfeet of astrocytes provide a connection to neurons and microglia (van der Helm *et al.* 2016). A physical barrier between the blood circulation and the brain tissue is maintained by an intact NVU via zona occludens proteins and claudins.

Simple *in vitro* models mimicking the BBB

Immortalized human brain vascular endothelial cells (HBMEC and HCMEC/D3) are commonly used for BBB models, whereas primary cells are not frequently used due to insufficient availability and loss of phenotype during culturing (Oddo *et al.* 2019). The HBMEC and HCMEC/D3 cell lines are especially suitable to model the BBB because of their expression of tight junction proteins, receptors and transporters (Weksler, Romero and Couraud 2013; Oddo *et al.* 2019). They can be cultured as monolayers on transwell inserts or cell culture plates and infected with *C. albicans* (Jong *et al.* 2001), *A. fumigatus* (Patel *et al.* 2018) or *C. neoformans* (Aaron *et al.* 2018) and used for transcytosis (Aaron *et al.* 2018), gene expression (Lahiri *et al.* 2019) and barrier integrity studies (Patel *et al.* 2018). For example, it was demonstrated that *C. neoformans* and *C. albicans* can pass the BBB via transcytosis (Fig. 2B I). True hyphae of *C. albicans* are associated with endocytosis by endothelial cells (Liu *et al.* 2011) (Fig. 2B I). *Cryptococcus neoformans*, however, was shown to also translocate paracellularly (Fig. 2B II) and use macrophages as a shuttle to cross the BBB using the Trojan horse mechanism mentioned above (Charlier *et al.* 2009; Santiago-Tirado *et al.* 2017) (Fig. 2B III). This mechanism was visualized and analyzed in detail using a co-culture model of HCMEC/D3 cells and THP-1 cells or primary monocytes (He *et al.* 2016; Santiago-Tirado *et al.* 2017) (Table S4, Supporting Information).

BBB-on-chip models

2D transwell models of the BBB can be valuable tools to gain insights into how fungi invade the CNS. However, current models lack some key properties of the NVU. For example, endothelial cells need to experience shear stress to trigger the establishment of a barrier that limits Na^+ and Cl^- ions efflux and influx (Oddo *et al.* 2019). Furthermore, to mimic the physiological situation more closely, the model should contain multiple cell types of the NVU such as astrocytes, pericytes and neurons since their communication influences each other's growth, differentiation and permeability (Abbott, Ronnback and Hansson 2006). A range of microfluidic BBB-on-Chip models has recently been developed, recapitulating the blood flow by perfusion of the endothelium in realistic dimensions and geometry and integration of various NVU cell types (Griep *et al.* 2013; Raasch *et al.* 2016; Maoz *et al.* 2018). In models using one cell type, HUVECs in astrocyte-conditioned medium or HCMEC/D3 cells have been cultured in a single perfused channel (Yeon *et al.* 2012; Griep *et al.* 2013; Englert *et al.* 2016). Using a CNS angiogenesis model comprising endothelial cells, pericytes, astrocytes and lung fibroblasts, it was demonstrated that a low vascular permeability can be achieved by co-culturing the different NVU cell types (Lee *et al.* 2020). These microfluidic BBB models can contribute to investigating the role of additional cell types of the NVU and shear stress in the transmigration of fungi across the BBB. Moreover, the implementation of innate immune cells would enable the simulation of inflammatory responses in the brain tissue following fungal invasion (Table S4, Supporting Information).

FUTURE DIRECTIONS

Interconnecting organ-on-chip systems to study fungal dissemination

Although the multiple infection models reviewed here have been and will be very useful tools to study fungal infections, we can expect a new generation of complex *in vitro* system based on OOC platforms. In fact, individual OOC systems can be combined to recapitulate multi-organ cross communication in an enclosed microfluidic network (Luni, Serena and Elvassore 2014). These platforms have the potential to investigate fungal infections not only at a single-organ level, but also at the multi-organ level, including systemic immune responses (Fig. 1A). The complexity of systemic immune reactions was only addressed in animal models until recently. Multi-organ-on-chip (MOC) models expand the toolbox with systems having a purely human genetic background to circumvent the problem of interspecies transferability. A range of MOC platforms have been developed that connect two or more organs such as the liver and intestine (Zhang *et al.* 2009; Chen, Miller and Shuler 2018; Ramme *et al.* 2019). MOC models provide the opportunity to study the dissemination of fungi throughout the body. It will allow (to mimic) tracking dissemination of *Candida* spp. from the intestine to the liver and kidney, the key target organs of disseminated candidiasis

(Lionakis *et al*. 2011), or dissemination of *A. fumigatus*, *C. neoformans* and *H. capsulatum* from the lung to the brain, which has not been possible *in vitro* so far. An additional aspect to be elucidated using MOC models is the relationship between dysbiosis in the intestine resulting in overgrowth of *C. albicans* and concomitant biochemical changes in the brain or the liver (gut–brain axis and gut–liver axis, respectively) (Burrus 2012; Yang *et al*. 2017). However, MOC systems are still in their infancy and there are many obstacles to overcome. A current challenge is to scale the organs to their relative physiological size (Lee and Sung 2017; Rogal, Probst and Loskill 2017). Current MOC systems are mostly used for toxicity screening of drugs and chemicals and are constructed in a way to be suitable for this particular application (Rogal, Probst and Loskill 2017). MOC models dedicated for fungal studies may take into account other criteria, e.g. the distance between distinct tissues, the number of integrated immune cells, and possibilities to prevent adherence of fungi to tubing and subsequent clogging, to be applicable as tools.

Human induced pluripotent stem cells as another cell source for fungal *in vitro* systems

The *in vitro* models discussed in this review rely on primary cells and cell lines. Human induced pluripotent stem cells (hiPSC) are an alternative source of cells and are highly relevant for biomedical research (Raasch *et al*. 2019). hiPSC can be generated by reprogramming adult tissue cells, such as fibroblasts, to an embryonic-like pluripotent state (Takahashi and Yamanaka 2006). Once reprogrammed, they can be differentiated into virtually all cell types except extra-embryonic cell types. Therefore, they offer the opportunity to establish OOC systems containing various cell types originating from a single donor. However, current models often combine hiPSC with primary cells and cell lines. Taking the BBB as an example, Brown and colleagues cultured HBMEC, glutamatergic neurons differentiated from iPSC, primary pericytes and astrocytes in a two-chamber model. The resulting system consisted of a brain compartment, which is separated from perfused vasculature by a porous membrane (Brown *et al*. 2015).

hiPSC are also utilized for the establishment of 'patient-on-chip' models to mimic genetic predispositions. Aspergillosis is a common complication of patients suffering from asthma and cystic fibrosis (CF) (Knutsen and Slavin 2011) or CGD (Leiding and Holland 1993); CARD9 and STAT1 mutations predispose for *C. albicans* CNS (Drummond *et al*. 2019) and mucocutaneous infections (van de Veerdonk *et al*. 2011), respectively, and diabetes mellitus is a common predisposition for histoplasmosis (Lockhart and Guarner 2019). Furthermore, intestinal fungi have been tightly connected to inflammatory bowel diseases (Leonardi, Li and Iliev 2018). Future OOC models might be able to reflect these predispositions by implementing hiPSC generated from patients bearing these diseases. Alternatively, specific mutations associated with the disease can be reproduced in hiPSC. For example, they have been successfully differentiated into macrophages and lung epithelial cells that carry mutations associated with CF (Pollard and Pollard 2018) and CGD (Brault *et al*. 2017). Although there has been substantial progress in OOC systems incorporating hiPSC, caution should be exercised: Protocols for differentiation require optimization and standardization, especially the understanding of factors promoting differentiation needs improvement. Differentiation might differ under static and dynamic conditions (Luni, Serena and Elvassore 2014; Rogal, Probst and Loskill 2017). Standardization of these aspects is crucial to guarantee reproducibility of findings from different labs.

CONCLUDING REMARKS

To study human fungal infections on a higher level of complexity, expertise of fungal infection biology and the OOC platforms needs to be combined. This will ensure studies in the most suitable *in vitro* model, providing conditions akin to the *in vivo* situation. For example, 3D intestine-on-chip models will be valuable tools to uncover the role of microbial commensals and their products, as well as the host immune responses to a local yeast-to-hypha transition of *C. albicans*. In the future, it would be favorable to make use of experience gained with MOC systems to mimic and follow fungal dissemination throughout the body and evaluate novel therapeutic strategies addressing fungal infections.

ACKNOWLEDGEMENT

We thank Jakob Sprague for critical reading of the manuscript.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSRE* online.

FUNDING

MSG was supported by a Humboldt Research Fellowship for Postdoctoral Researchers by the Alexander von Humboldt Foundation, a Research Grant 2019 from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), and the German Research Foundation (Deutsche Forschungsgemeinschaft—DFG) Emmy Noether Programm (Project no. 434385622/GR5617/1-1). BH was supported by the European Union Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement no. 812969 (FunHoMic), the DFG project Hu 532/20-1, project C1 within the Collaborative Research Centre (CRC)/Transregio (TRR) 124 FungiNet, the Leibniz Association Campus InfectoOptics SAS-2015-HKI-LWC, the Leibniz Research Alliance Infections'21 and the Wellcome Trust (grant 215599/Z/19/Z). BH, ASM, MM and AL were supported by the Center for Sepsis Control and Care (CSCC)/Bundesministerium für Bildung und Forschung (BMBF, grant no. 01EO1002). ASM received funding by the European Commission through Actions Marie Skłodowska-Curie (MSCA) Innovative Training Network EUROoC (grant no. 812954). BH and ASM were supported by funding through the Cluster of Excellence 'Balance of the Microverse', funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy—EXC 2051, Project ID 390713860.

Conflict of Interest. None declared.

REFERENCES

Aaron PA, Jamklang M, Uhrig JP *et al*. The blood–brain barrier internalises Cryptococcus neoformans via the EphA2-tyrosine kinase receptor. *Cell Microbiol* 2018;**20**.

Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood–brain barrier. *Nat Rev Neurosci* 2006;**7**: 41–53.

Ahadian S, Civitarese R, Bannerman D *et al.* Organ-on-a-chip platforms: a convergence of advanced materials, cells, and microscale technologies. *Adv Healthc Mater* 2018;**7**.

Aimanianda V, Bayry J, Bozza S *et al.* Surface hydrophobin prevents immune recognition of airborne fungal spores. *Nature* 2009;**460**:1117–21.

Akram SM, Koirala J. *Histoplasmosis*. Treasure Island, FL: StatPearls Publishing, 2020.

Albac S, Schmitz A, Lopez-Alayon C *et al.* Candida albicans is able to use M cells as a portal of entry across the intestinal barrier *in vitro*. *Cell Microbiol* 2016;**18**:195–210.

Alcantara C, Almeida BR, Barros B *et al.* Histoplasma capsulatum chemotypes I and II induce IL-8 secretion in lung epithelial cells in distinct manners. *Med Mycol* 2020;**58**:1169–77.

Allert S, Forster TM, Svensson CM *et al.* Candida albicans-induced epithelial damage mediates translocation through intestinal barriers. *mBio* 2018;**9**:e00915–8.

Alvarez-Rueda N, Rouges C, Touahri A *et al.* *In vitro* immune responses of human PBMCs against Candida albicans reveals fungal and leucocyte phenotypes associated with fungal persistence. *Sci Rep* 2020;**10**:6211.

Alves CT, Wei XQ, Silva S *et al.* Candida albicans promotes invasion and colonisation of Candida glabrata in a reconstituted human vaginal epithelium. *J Infect* 2014;**69**:396–407.

Alzayer YM, Gomez GF, Eckert GJ *et al.* The impact of nicotine and cigarette smoke condensate on metabolic activity and biofilm formation of Candida albicans on acrylic denture material. *J Prosthodont* 2018;**29**:173–8.

Amitani R, Kawanami R. Interaction of Aspergillus with human respiratory mucosa: a study with organ culture model. *Med Mycol* 2009;**47 Suppl 1**:S127–31.

Arce Miranda JE, Baronetti JL, Sotomayor CE *et al.* Oxidative and nitrosative stress responses during macrophage–Candida albicans biofilm interaction. *Med Mycol* 2019;**57**:101–13.

Armstrong-James D, Brown GD, Netea MG *et al.* Immunotherapeutic approaches to treatment of fungal diseases. *Lancet Infect Dis* 2017;**17**:e393–402.

Austermeier S, Kasper L, Westman J *et al.* I want to break free: macrophage strategies to recognize and kill Candida albicans, and fungal counter-strategies to escape. *Curr Opin Microbiol* 2020;**58**:15–23.

Bain JM, Lewis LE, Okai B *et al.* Non-lytic expulsion/exocytosis of Candida albicans from macrophages. *Fungal Genet Biol* 2012;**49**:677–8.

Bain JM, Louw J, Lewis LE *et al.* Candida albicans hypha formation and mannan masking of β-glucan inhibit macrophage phagosome maturation. *mBio* 2014;**5**:e01874.

Balish E, Wagner RD, Vázquez-Torres A *et al.* Candidiasis in interferon-gamma knockout (IFN-gamma-/-) mice. *J Infect Dis* 1998;**178**:478–87.

Ballou ER, Avelar GM, Childers DS *et al.* Lactate signalling regulates fungal β-glucan masking and immune evasion. *Nat Microbiol* 2016;**2**:16238.

Barbosa FM, Fonseca FL, Figueiredo RT *et al.* Binding of glucuronoxylomannan to the CD14 receptor in human A549 alveolar cells induces interleukin-8 production. *Clin Vaccine Immunol* 2007;**14**:94–8.

Barker KS, Park H, Phan QT *et al.* Transcriptome profile of the vascular endothelial cell response to Candida albicans. *J Infect Dis* 2008;**198**:193–202.

Barluzzi R, Brozzetti A, Delfino D *et al.* Role of the capsule in microglial cell–Cryptococcus neoformans interaction: impairment of antifungal activity but not of secretory functions. *Med Mycol* 1998;**36**:189–97.

Basmaciyan L, Bon F, Paradis T *et al.*"Candida albicans interactions with the host: crossing the intestinal epithelial barrier". *Tissue Barriers* 2019;**7**:1612661.

Becker KL, Aimanianda V, Wang X *et al.* Aspergillus cell wall chitin induces anti- and proinflammatory cytokines in human PBMCs via the Fc-γ receptor/Syk/PI3K pathway. *mBio* 2016;**7**:e01823–15.

Becker KL, Gresnigt MS, Smeekens SP *et al.* Pattern recognition pathways leading to a Th2 cytokine bias in allergic bronchopulmonary aspergillosis patients. *Clin Exp Allergy* 2015;**45**:423–37.

Bein A, Shin W, Jalili-Firoozinezhad S *et al.* Microfluidic organ-on-a-chip models of human intestine. *Cell Mol Gastroenterol Hepatol* 2018;**5**:659–68.

Belic S, Page L, Lazariotou M *et al.* Comparative analysis of inflammatory cytokine release and alveolar epithelial barrier invasion in a transwell((R)) bilayer model of mucormycosis. *Front Microbiol* 2018;**9**:3204.

Benam KH, Villenave R, Lucchesi C *et al.* Small airway-on-a-chip enables analysis of human lung inflammation and drug responses *in vitro*. *Nat Methods* 2016;**13**:151–7.

Bernardo RT, Cunha DV, Wang C *et al.* The CgHaa1-regulon mediates response and tolerance to acetic acid stress in the human pathogen Candida glabrata. *G3 (Bethesda)* 2017;**7**:1–18.

Bertolini M, Ranjan A, Thompson A *et al.* Candida albicans induces mucosal bacterial dysbiosis that promotes invasive infection. *PLoS Pathog* 2019;**15**:e1007717.

Bertuzzi M, Schrettl M, Alcazar-Fuoli L *et al.* The pH-responsive PacC transcription factor of Aspergillus fumigatus governs epithelial entry and tissue invasion during pulmonary aspergillosis. *PLoS Pathog* 2014;**10**:e1004413.

Blasi E, Barluzzi R, Bocchini V *et al.* Immortalization of murine microglial cells by a v-raf/v-myc carrying retrovirus. *J Neuroimmunol* 1990;**27**:229–37.

Blasi E, Mazzolla R, Barluzzi R *et al.* Microglial cell-mediated anti-Candida activity: temperature, ions, protein kinase C as crucial elements. *J Neuroimmunol* 1991;**34**:53–60.

Bohringer M, Pohlers S, Schulze S *et al.* Candida albicans infection leads to barrier breakdown and a MAPK/NF-κB mediated stress response in the intestinal epithelial cell line C2BBe1. *Cell Microbiol* 2016;**18**:889–904.

Bongomin F, Gago S, Oladele RO *et al.* Global and multi-national prevalence of fungal diseases: estimate precision. *J Fungi (Basel)* 2017;**3**:57.

Bouzani M, Ok M, McCormick A *et al.* Human NK cells display important antifungal activity against Aspergillus fumigatus, which is directly mediated by IFN-γ release. *J Immunol* 2011;**187**:1369–76.

Boyle KB, Gyori D, Sindrilaru A *et al.* Class IA phosphoinositide 3-kinase β and δ regulate neutrophil oxidase activation in response to Aspergillus fumigatus hyphae. *J Immunol* 2011;**186**:2978–89.

Brault J, Vaganay G, Le Roy A *et al.* Therapeutic effects of proteoliposomes on X-linked chronic granulomatous disease: proof of concept using macrophages differentiated from patient-specific induced pluripotent stem cells. *Int J Nanomedicine* 2017;**12**:2161–77.

Brown GD, Denning DW, Gow NA *et al.* Hidden killers: human fungal infections. *Sci Transl Med* 2012;**4**:165rv13.

Brown JA, Pensabene V, Markov DA *et al.* Recreating blood–brain barrier physiology and structure on chip: a novel neurovascular microfluidic bioreactor. *Biomicrofluidics* 2015;**9**:054124.

Brunel SF, Willment JA, Brown GD *et al.* Aspergillus-induced superoxide production by cystic fibrosis phagocytes is associated with disease severity. *ERJ Open Res* 2018;**4**:00068–2017.

Bruns S, Kniemeyer O, Hasenberg M *et al.* Production of extracellular traps against Aspergillus fumigatus *in vitro* and in infected lung tissue is dependent on invading neutrophils and influenced by hydrophobin RodA. *PLoS Pathog* 2010;**6**:e1000873.

Burrus CJ. A biochemical rationale for the interaction between gastrointestinal yeast and autism. *Med Hypotheses* 2012;**79**:784–5.

Busch CJ, Favret J, Geirsdottir L *et al.* Isolation and long-term cultivation of mouse alveolar macrophages. *Bio Protoc* 2019;**9**:e3302.

Camilli G, Eren E, Williams DL *et al.* Impaired phagocytosis directs human monocyte activation in response to fungal derived β-glucan particles. *Eur J Immunol* 2018;**48**:757–70.

Casaroto AR, da Silva RA, Salmeron S *et al.* Candida albicans–cell interactions activate innate immune defense in human palate epithelial primary cells via nitric oxide (NO) and β-defensin 2 (hBD-2). *Cells* 2019;**8**:707.

Cassone A. Vulvovaginal Candida albicans infections: pathogenesis, immunity and vaccine prospects. *BJOG* 2015;**122**:785–94.

Chandorkar P, Posch W, Zaderer V *et al.* Fast-track development of an *in vitro* 3D lung/immune cell model to study Aspergillus infections. *Sci Rep* 2017;**7**:11644.

Charlier C, Nielsen K, Daou S *et al.* Evidence of a role for monocytes in dissemination and brain invasion by Cryptococcus neoformans. *Infect Immun* 2009;**77**:120–7.

Chen HC. Boyden chamber assay. *Methods Mol Biol* 2005;**294**:15–22.

Chen HJ, Miller P, Shuler ML. A pumpless body-on-a-chip model using a primary culture of human intestinal cells and a 3D culture of liver cells. *Lab Chip* 2018;**18**:2036–46.

Clevers H. Modeling development and disease with organoids. *Cell* 2016;**165**:1586–97.

Coady A, Sil A. MyD88-dependent signaling drives host survival and early cytokine production during Histoplasma capsulatum infection. *Infect Immun* 2015;**83**:1265–75.

Coelho C, Bocca AL, Casadevall A. The tools for virulence of Cryptococcus neoformans. *Adv Appl Microbiol* 2014;**87**:1–41.

Coelho C, Camacho E, Salas A *et al.* Intranasal inoculation of Cryptococcus neoformans in mice produces nasal infection with rapid brain dissemination. *mSphere* 2019;**4**:e00483–19.

Coenjaerts FE, Walenkamp AM, Mwinzi PN *et al.* Potent inhibition of neutrophil migration by cryptococcal mannoprotein-4-induced desensitization. *J Immunol* 2001;**167**:3988–95.

Conti HR, Bruno VM, Childs EE *et al.* IL-17 receptor signaling in oral epithelial cells is critical for protection against oropharyngeal candidiasis. *Cell Host Microbe* 2016;**20**:606–17.

Crampton SP, Davis J, Hughes CC. Isolation of human umbilical vein endothelial cells (HUVEC). *J Vis Exp* 2007;**3**:183.

Dalle F, Wachtler B, L'Ollivier C *et al.* Cellular interactions of Candida albicans with human oral epithelial cells and enterocytes. *Cell Microbiol* 2010;**12**:248–71.

Dasari P, Shopova IA, Stroe M *et al.* Aspf2 From Aspergillus fumigatus recruits human immune regulators for immune evasion and cell damage. *Front Immunol* 2018;**9**:1635.

de Carvalho Dias K, de Sousa DL, Barbugli PA *et al.* Development and characterization of a 3D oral mucosa model as a tool for host–pathogen interactions. *J Microbiol Methods* 2018;**152**:52–60.

Degel J, Shokrani M. Validation of the efficacy of a practical method for neutrophils isolation from peripheral blood. *Clin Lab Sci* 2010;**23**:94–8.

Deinhardt-Emmer S, Rennert K, Schicke E *et al.* Co-infection with Staphylococcus aureus after primary influenza virus infection leads to damage of the endothelium in a human alveolus-on-a-chip model. *Biofabrication* 2020;**12**:025012.

De Luca A, Montagnoli C, Zelante T *et al.* Functional yet balanced reactivity to Candida albicans requires TRIF, MyD88, and IDO-dependent inhibition of Rorc. *J Immunol* 2007;**179**:5999–6008.

Deng K, Chen T, Wu Q *et al.* *In vitro* and *in vivo* examination of anticolonization of pathogens by Lactobacillus paracasei FJ861111.1. *J Dairy Sci* 2015;**98**:6759–66.

Denham ST, Brown JCS. Mechanisms of pulmonary escape and dissemination by Cryptococcus neoformans. *J Fungi (Basel)* 2018;**4**:25.

Desai LP, Chapman KE, Waters CM. Mechanical stretch decreases migration of alveolar epithelial cells through mechanisms involving Rac1 and Tiam1. *Am J Physiol Lung Cell Mol Physiol* 2008;**295**:L958–65.

Diaz PI, Xie Z, Sobue T *et al.* Synergistic interaction between Candida albicans and commensal oral streptococci in a novel *in vitro* mucosal model. *Infect Immun* 2012;**80**:620–32.

Dickson MA, Hahn WC, Ino Y *et al.* Human keratinocytes that express hTERT and also bypass a p16(INK4a)-enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics. *Mol Cell Biol* 2000;**20**:1436–47.

Dix A, Hunniger K, Weber M *et al.* Biomarker-based classification of bacterial and fungal whole-blood infections in a genome-wide expression study. *Front Microbiol* 2015;**6**:171.

Dominguez-Andres J, Arts RJW, Ter Horst R *et al.* Rewiring monocyte glucose metabolism via C-type lectin signaling protects against disseminated candidiasis. *PLoS Pathog* 2017;**13**:e1006632.

Dongari-Bagtzoglou A, Kashleva H. Candida albicans triggers interleukin-8 secretion by oral epithelial cells. *Microb Pathog* 2003;**34**:169–77.

Dongari-Bagtzoglou A, Kashleva H. Development of a highly reproducible three-dimensional organotypic model of the oral mucosa. *Nat Protoc* 2006a;**1**:2012–8.

Dongari-Bagtzoglou A, Kashleva H. Development of a novel three-dimensional *in vitro* model of oral Candida infection. *Microb Pathog* 2006b;**40**:271–8.

Drummond RA, Collar AL, Swamydas M *et al.* CARD9-dependent neutrophil recruitment protects against fungal invasion of the central nervous system. *PLoS Pathog* 2015;**11**:e1005293.

Drummond RA, Lionakis MS. Organ-specific mechanisms linking innate and adaptive antifungal immunity. *Semin Cell Dev Biol* 2019;**89**:78–90.

Drummond RA, Swamydas M, Oikonomou V *et al.* CARD9(+) microglia promote antifungal immunity via IL-1β- and CXCL1-mediated neutrophil recruitment. *Nat Immunol* 2019;**20**:559–70.

Eberl C, Speth C, Jacobsen ID *et al.* Candida: platelet interaction and platelet activity *in vitro*. *J Innate Immun* 2019;**11**:52–62.

Edwards JE, Jr, Rotrosen D, Fontaine JW *et al.* Neutrophil-mediated protection of cultured human vascular endothelial cells from damage by growing Candida albicans hyphae. *Blood* 1987;**69**:1450–7.

Ejzykowicz DE, Solis NV, Gravelat FN *et al.* Role of Aspergillus fumigatus DvrA in host cell interactions and virulence. *Eukaryot Cell* 2010;**9**:1432–40.

Englert C, Trutzschler AK, Raasch M *et al.* Crossing the blood–brain barrier: glutathione-conjugated poly(ethylene imine) for gene delivery. *J Control Release* 2016;**241**:1–14.

Erwig LP, Gow NA. Interactions of fungal pathogens with phagocytes. *Nat Rev Microbiol* 2016;**14**:163–76.

Evans RJ, Sundaramurthy V, Frickel EM. The interplay of host autophagy and eukaryotic pathogens. *Front Cell Dev Biol* 2018;**6**:118.

Fichorova RN, Rheinwald JG, Anderson DJ. Generation of papillomavirus-immortalized cell lines from normal human ectocervical, endocervical, and vaginal epithelium that maintain expression of tissue-specific differentiation proteins. *Biol Reprod* 1997;**57**:847–55.

Fidel PL, Jr. Candida-host interactions in HIV disease: implications for oropharyngeal candidiasis. *Adv Dent Res* 2011;**23**:45–9.

Filler SG, Sheppard DC. Fungal invasion of normally non-phagocytic host cells. *PLoS Pathog* 2006;**2**:e129.

Fogh J, Wright WC, Loveless JD. Absence of HeLa cell contamination in 169 cell lines derived from human tumors. *J Natl Cancer Inst* 1977;**58**:209–14.

Friedrich D, Zapf D, Lohse B *et al.* The HIF-1α/LC3-II axis impacts fungal immunity in human macrophages. *Infect Immun* 2019;**87**:e00125–19.

Fréalle E, Gosset P, Leroy S *et al. In vitro* coagulation triggers anti-Aspergillus fumigatus neutrophil response. *Future Microbiol* 2018;**13**:659–69.

Gacser A, Tiszlavicz Z, Nemeth T *et al.* Induction of human defensins by intestinal Caco-2 cells after interactions with opportunistic Candida species. *Microbes Infect* 2014;**16**:80–5.

Gaffen SL, Moutsopoulos NM. Regulation of host–microbe interactions at oral mucosal barriers by type 17 immunity. *Sci Immunol* 2020;**5**:eaau4594.

Garcia C, Burgain A, Chaillot J *et al.* A phenotypic small-molecule screen identifies halogenated salicylanilides as inhibitors of fungal morphogenesis, biofilm formation and host cell invasion. *Sci Rep* 2018;**8**:11559.

García-Rodas R, González-Camacho F, Rodríguez-Tudela JL *et al.* The interaction between Candida krusei and murine macrophages results in multiple outcomes, including intracellular survival and escape from killing. *Infect Immun* 2011;**79**:2136–44.

Gazendam RP, van de Geer A, Roos D *et al.* How neutrophils kill fungi. *Immunol Rev* 2016;**273**:299–311.

Gilbert AS, Wheeler RT, May RC. Fungal pathogens: survival and replication within macrophages. *Cold Spring Harb Perspect Med* 2014;**5**:a019661.

Gonçalves B, Ferreira C, Alves CT *et al.* Vulvovaginal candidiasis: epidemiology, microbiology and risk factors. *Crit Rev Microbiol* 2016;**42**:905–27.

Gonçalves SM, Duarte-Oliveira C, Campos CF *et al.* Phagosomal removal of fungal melanin reprograms macrophage metabolism to promote antifungal immunity. *Nat Commun* 2020;**11**:2282.

Gouba N, Drancourt M. Digestive tract mycobiota: a source of infection. *Med Mal Infect* 2015;**45**:9–16.

Goyer M, Loiselet A, Bon F *et al.* Intestinal cell tight junctions limit invasion of Candida albicans through active penetration and endocytosis in the early stages of the interaction of the fungus with the intestinal barrier. *PLoS One* 2016;**11**:e0149159.

Graf K, Last A, Gratz R *et al.* Keeping Candida commensal: how lactobacilli antagonize pathogenicity of Candida albicans in an *in vitro* gut model. *Dis Model Mech* 2019;**12**:dmm039719.

Gravelat FN, Ejzykowicz DE, Chiang LY *et al.* Aspergillus fumigatus MedA governs adherence, host cell interactions and virulence. *Cell Microbiol* 2010;**12**:473–88.

Gresnigt MS, Becker KL, Leenders F *et al.* Differential kinetics of Aspergillus nidulans and Aspergillus fumigatus phagocytosis. *J Innate Immun* 2018a;**10**:145–60.

Gresnigt MS, Becker KL, Smeekens SP *et al.* Aspergillus fumigatus-induced IL-22 is not restricted to a specific Th cell subset and is dependent on complement receptor 3. *J Immunol* 2013;**190**:5629–39.

Gresnigt MS, Cunha C, Jaeger M *et al.* Genetic deficiency of NOD2 confers resistance to invasive aspergillosis. *Nat Commun* 2018b;**9**:2636.

Griep LM, Wolbers F, de Wagenaar B *et al.* BBB on chip: microfluidic platform to mechanically and biochemically modulate blood–brain barrier function. *Biomed Microdevices* 2013;**15**:145–50.

Groger M, Rennert K, Giszas B *et al.* Monocyte-induced recovery of inflammation-associated hepatocellular dysfunction in a biochip-based human liver model. *Sci Rep* 2016;**6**:21868.

Grondman I, Arts RJW, Koch RM *et al.* Frontline science: endotoxin-induced immunotolerance is associated with loss of monocyte metabolic plasticity and reduction of oxidative burst. *J Leukoc Biol* 2019;**106**:11–25.

Grubb SE, Murdoch C, Sudbery PE *et al.* Candida albicans–endothelial cell interactions: a key step in the pathogenesis of systemic candidiasis. *Infect Immun* 2008;**76**:4370–7.

Guillot L, Carroll SF, Badawy M *et al.* Cryptococcus neoformans induces IL-8 secretion and CXCL1 expression by human bronchial epithelial cells. *Respir Res* 2008;**9**:9.

Guimaraes AJ, de Cerqueira MD, Zamith-Miranda D *et al.* Host membrane glycosphingolipids and lipid microdomains facilitate Histoplasma capsulatum internalisation by macrophages. *Cell Microbiol* 2019;**21**:e12976.

Guo C, Chen M, Fa Z *et al.* Acapsular Cryptococcus neoformans activates the NLRP3 inflammasome. *Microbes Infect* 2014;**16**:845–54.

Halder LD, Abdelfatah MA, Jo EA *et al.* Factor H binds to extracellular DNA traps released from human blood monocytes in response to Candida albicans. *Front Immunol* 2016;**7**:671.

Hallen-Adams HE, Suhr MJ. Fungi in the healthy human gastrointestinal tract. *Virulence* 2017;**8**:352–8.

Haraguchi N, Ishii Y, Morishima Y *et al.* Impairment of host defense against disseminated candidiasis in mice overexpressing GATA-3. *Infect Immun* 2010;**78**:2302–11.

Hashemi Fesharaki S, Aghili SR, Shokohi T *et al.* Catheter-related candidemia and identification of causative Candida species in patients with cardiovascular disorder. *Curr Med Mycol* 2018;**4**:7–13.

Hefter M, Lother J, Weiss E *et al.* Human primary myeloid dendritic cells interact with the opportunistic fungal pathogen Aspergillus fumigatus via the C-type lectin receptor Dectin-1. *Med Mycol* 2017;**55**:573–8.

Hellwig D, Voigt J, Bouzani M *et al.* Candida albicans induces metabolic reprogramming in human NK cells and responds to perforin with a zinc depletion response. *Front Microbiol* 2016;**7**:750.

Herbrecht R, Bories P, Moulin JC *et al.* Risk stratification for invasive aspergillosis in immunocompromised patients. *Ann N Y Acad Sci* 2012;**1272**:23–30.

Herbrecht R, Neuville S, Letscher-Bru V *et al.* Fungal infections in patients with neutropenia: challenges in prophylaxis and treatment. *Drugs Aging* 2000;**17**:339–51.

He X, Shi X, Puthiyakunnon S *et al.* CD44-mediated monocyte transmigration across Cryptococcus neoformans-infected brain microvascular endothelial cells is enhanced by HIV-1 gp41-I90 ectodomain. *J Biomed Sci* 2016; **23**:28.

Ho J, Yang X, Nikou SA *et al.* Candidalysin activates innate epithelial immune responses via epidermal growth factor receptor. *Nat Commun* 2019;**10**:2297.

Hope WW, Kruhlak MJ, Lyman CA *et al.* Pathogenesis of Aspergillus fumigatus and the kinetics of galactomannan in an *in vitro* model of early invasive pulmonary aspergillosis: implications for antifungal therapy. *J Infect Dis* 2007;**195**: 455–66.

Hopke A, Scherer A, Kreuzburg S *et al.* Neutrophil swarming delays the growth of clusters of pathogenic fungi. *Nat Commun* 2020;**11**:2031.

Huang JH, Liu CY, Wu SY *et al.* NLRX1 facilitates Histoplasma capsulatum-induced LC3-associated phagocytosis for cytokine production in macrophages. *Front Immunol* 2018;**9**:2761.

Huang W, Na L, Fidel PL *et al.* Requirement of interleukin-17A for systemic anti-Candida albicans host defense in mice. *J Infect Dis* 2004;**190**:624–31.

Huh D, Matthews BD, Mammoto A *et al.* Reconstituting organ-level lung functions on a chip. *Science* 2010;**328**:1662–8.

Hunniger K, Lehnert T, Bieber K *et al.* A virtual infection model quantifies innate effector mechanisms and Candida albicans immune escape in human blood. *PLoS Comput Biol* 2014;**10**:e1003479.

Ifrim DC, Quintin J, Courjol F *et al.* The role of Dectin-2 for host defense against disseminated candidiasis. *J Interferon Cytokine Res* 2016;**36**:267–76.

Jaeger M, Matzaraki V, Aguirre-Gamboa R *et al.* A genome-wide functional genomics approach identifies susceptibility pathways to fungal bloodstream infection in humans. *J Infect Dis* 2019a;**220**:862–72.

Jaeger M, Pinelli M, Borghi M *et al.* A systems genomics approach identifies SIGLEC15 as a susceptibility factor in recurrent vulvovaginal candidiasis. *Sci Transl Med* 2019b;**11**:eaar3558.

Jaffe EA, Nachman RL, Becker CG *et al.* Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J Clin Invest* 1973;**52**:2745–56.

Jain A, Barrile R, van der Meer AD *et al.* Primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics. *Clin Pharmacol Ther* 2018;**103**:332–40.

Jang KJ, Otieno MA, Ronxhi J *et al.* Reproducing human and cross-species drug toxicities using a Liver-Chip. *Sci Transl Med* 2019;**11**:eaax5516.

Javed F, Al-Kheraif AA, Kellesarian SV *et al.* Oral Candida carriage and species prevalence in denture stomatitis patients with and without diabetes. *J Biol Regul Homeost Agents* 2017;**31**: 343–6.

Jong AY, Stins MF, Huang SH *et al.* Traversal of Candida albicans across human blood–brain barrier *in vitro*. *Infect Immun* 2001;**69**:4536–44.

Kalia N, Singh J, Kaur M. Microbiota in vaginal health and pathogenesis of recurrent vulvovaginal infections: a critical review. *Ann Clin Microbiol Antimicrob* 2020;**19**:5.

Kamai Y, Chiang LY, Lopes Bezerra LM *et al.* Interactions of Aspergillus fumigatus with vascular endothelial cells. *Med Mycol* 2006;**44 Suppl 1**:S115–7.

Kamai Y, Lossinsky AS, Liu H *et al.* Polarized response of endothelial cells to invasion by Aspergillus fumigatus. *Cell Microbiol* 2009;**11**:170–82.

Kanayama M, Shinohara ML. Roles of autophagy and autophagy-related proteins in antifungal immunity. *Front Immunol* 2016;**7**:47.

Karki R, Man SM, Malireddi RK *et al.* Concerted activation of the AIM2 and NLRP3 inflammasomes orchestrates host protection against Aspergillus infection. *Cell Host Microbe* 2015;**17**:357–68.

Kasper L, Konig A, Koenig PA *et al.* The fungal peptide toxin Candidalysin activates the NLRP3 inflammasome and causes cytolysis in mononuclear phagocytes. *Nat Commun* 2018;**9**:4260.

Kavanaugh NL, Zhang AQ, Nobile CJ *et al.* Mucins suppress virulence traits of Candida albicans. *mBio* 2014;**5**:e01911.

Khader SA, Gaffen SL, Kolls JK. Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. *Mucosal Immunol* 2009;**2**:403–11.

Kim HJ, Ingber DE. Gut-on-a-Chip microenvironment induces human intestinal cells to undergo villus differentiation. *Integr Biol (Camb)* 2013;**5**:1130–40.

Klassert TE, Brauer J, Holzer M *et al.* Differential effects of vitamins A and D on the transcriptional landscape of human monocytes during infection. *Sci Rep* 2017;**7**:40599.

Knutsen AP, Slavin RG. Allergic bronchopulmonary aspergillosis in asthma and cystic fibrosis. *Clin Dev Immunol* 2011;**2011**:843763.

Kogan TV, Jadoun J, Mittelman L *et al.* Involvement of secreted Aspergillus fumigatus proteases in disruption of the actin fiber cytoskeleton and loss of focal adhesion sites in infected A549 lung pneumocytes. *J Infect Dis* 2004;**189**:1965–73.

Kolwijck E, van de Veerdonk FL. The potential impact of the pulmonary microbiome on immunopathogenesis of Aspergillus-related lung disease. *Eur J Immunol* 2014;**44**:3156–65.

Kosmidis C, Denning DW. The clinical spectrum of pulmonary aspergillosis. *Thorax* 2015;**70**:270–7.

Kumamoto CA, Gresnigt MS, Hube B. The gut, the bad and the harmless: Candida albicans as a commensal and opportunistic pathogen in the intestine. *Curr Opin Microbiol* 2020;**56**:7–15.

Kumar R, Saraswat D, Tati S *et al.* Novel aggregation properties of Candida albicans secreted aspartyl proteinase Sap6 mediate virulence in oral candidiasis. *Infect Immun* 2015;**83**:2614–26.

Kunyeit L, Kurrey NK, Anu-Appaiah KA *et al.* Probiotic yeasts inhibit virulence of non-albicans Candida species. *mBio* 2019;**10**.

Kyrmizi I, Ferreira H, Carvalho A *et al.* Calcium sequestration by fungal melanin inhibits calcium-calmodulin signalling to prevent LC3-associated phagocytosis. *Nat Microbiol* 2018;**3**:791–803.

Kämmer P, McNamara S, Wolf T *et al.* Survival strategies of pathogenic Candida species in human blood show independent and specific adaptations. *mBio* 2020;**11**:e02435–20.

Köhler JR, Hube B, Puccia R *et al.* Fungi that infect humans. *Microbiology Spectr* 2017;**5**:0014–2016.

Lahiri S, Banerjee A, Bhutda S *et al.* *In vitro* expression of vital virulent genes of clinical and environmental isolates of Cryptococcus neoformans/gattii in endothelial cells of human blood–brain barrier. *J Mycol Med* 2019;**29**:239–44.

Lee S, Chung M, Lee SR *et al.* 3D brain angiogenesis model to reconstitute functional human blood–brain barrier *in vitro*. *Biotechnol Bioeng* 2020;**117**:748–62.

Lee SH, Sung JH. Microtechnology-based multi-organ models. *Bioengineering (Basel)* 2017;**4**:46.

LeibundGut-Landmann S, Gross O, Robinson MJ *et al.* Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat Immunol* 2007;**8**:630–8.

Leiding JW, Holland SM. Chronic granulomatous disease. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mefford HC, Stephens K, Amemiya A, Ledbetter N (eds). *GeneReviews(R)*. Seattle (WA), 1993.

Leonardi I, Li X, Iliev ID. Macrophage interactions with fungi and bacteria in inflammatory bowel disease. *Curr Opin Gastroenterol* 2018;**34**:392–7.

Leonhardt J, Grosse S, Marx C *et al.* Candida albicans β-glucan differentiates human monocytes into a specific subset of macrophages. *Front Immunol* 2018;**9**:2818.

Lesuffleur T, Barbat A, Dussaulx E *et al.* Growth adaptation to methotrexate of HT-29 human colon carcinoma cells is associated with their ability to differentiate into columnar absorptive and mucus-secreting cells. *Cancer Res* 1990;**50**:6334–43.

Lewis LE, Bain JM, Lowes C *et al.* Stage specific assessment of Candida albicans phagocytosis by macrophages identifies cell wall composition and morphogenesis as key determinants. *PLoS Pathog* 2012;**8**:e1002578.

Lieber M, Smith B, Szakal A *et al.* A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. *Int J Cancer* 1976;**17**:62–70.

Lim J, Coates CJ, Seoane PI *et al.* Characterizing the mechanisms of nonopsonic uptake of cryptococci by macrophages. *J Immunol* 2018;**200**:3539–46.

Lindberg K, Rheinwald JG. Three distinct keratinocyte subtypes identified in human oral epithelium by their patterns of keratin expression in culture and in xenografts. *Differentiation* 1990;**45**:230–41.

Lionakis MS, Levitz SM. Host control of fungal infections: lessons from basic studies and human cohorts. *Annu Rev Immunol* 2018;**36**:157–91.

Lionakis MS, Lim JK, Lee CC *et al.* Organ-specific innate immune responses in a mouse model of invasive candidiasis. *J Innate Immun* 2011;**3**:180–99.

Lionakis MS, Swamydas M, Fischer BG *et al.* CX3CR1-dependent renal macrophage survival promotes Candida control and host survival. *J Clin Invest* 2013;**123**:5035–51.

Lionakis MS. New insights into innate immune control of systemic candidiasis. *Med Mycol* 2014;**52**:555–64.

Li SS, Ogbomo H, Mansour MK *et al.* Identification of the fungal ligand triggering cytotoxic PRR-mediated NK cell killing of Cryptococcus and Candida. *Nat Commun* 2018;**9**:751.

Li T, Niu X, Zhang X *et al.* Recombinant human IFNα-2b response promotes vaginal epithelial cells defense against Candida albicans. *Front Microbiol* 2017;**8**:697.

Liu H, Lee MJ, Solis NV *et al.* Aspergillus fumigatus CalA binds to integrin $\alpha_5\beta_1$ and mediates host cell invasion. *Nat Microbiol* 2016;**2**:16211.

Liu NN, Uppuluri P, Broggi A *et al.* Intersection of phosphate transport, oxidative stress and TOR signalling in Candida albicans virulence. *PLoS Pathog* 2018;**14**:e1007076.

Liu Y, Mittal R, Solis NV *et al.* Mechanisms of Candida albicans trafficking to the brain. *PLoS Pathog* 2011;**7**:e1002305.

Liu Y, Shetty AC, Schwartz JA *et al.* New signaling pathways govern the host response to C. albicans infection in various niches. *Genome Res* 2015;**25**:679–89.

Lockhart SR, Guarner J. Emerging and reemerging fungal infections. *Semin Diagn Pathol* 2019;**36**:177–81.

Lohith K, Anu-Appaiah KA. Antagonistic effect of Saccharomyces cerevisiae KTP and Issatchenkia occidentalis ApC on hyphal development and adhesion of Candida albicans. *Med Mycol* 2018;**56**:1023–32.

Long KH, Gomez FJ, Morris RE *et al.* Identification of heat shock protein 60 as the ligand on Histoplasma capsulatum that mediates binding to CD18 receptors on human macrophages. *J Immunol* 2003;**170**:487–94.

Lother J, Breitschopf T, Krappmann S *et al.* Human dendritic cell subsets display distinct interactions with the pathogenic mould Aspergillus fumigatus. *Int J Med Microbiol* 2014;**304**:1160–8.

Luni C, Serena E, Elvassore N. Human-on-chip for therapy development and fundamental science. *Curr Opin Biotechnol* 2014;**25**:45–50.

Maciel Quatrin P, Flores Dalla Lana D, Andrzejewski Kaminski TF *et al.* Fungal infection models: current progress of *ex vivo* methods. *Mycoses* 2019;**62**:860–73.

Mailander-Sanchez D, Braunsdorf C, Grumaz C *et al.* Antifungal defense of probiotic Lactobacillus rhamnosus GG is mediated by blocking adhesion and nutrient depletion. *PLoS One* 2017;**12**:e0184438.

Makanjuola O, Bongomin F, Fayemiwo SA. An update on the roles of non-albicans Candida species in vulvovaginitis. *J Fungi (Basel)* 2018;**4**:121.

Mancini V, Pensabene V. Organs-on-chip models of the female reproductive system. *Bioengineering (Basel)* 2019;**6**:103.

Mao X, Qiu X, Jiao C *et al.* Candida albicans SC5314 inhibits NLRP3/NLRP6 inflammasome expression and dampens human intestinal barrier activity in Caco-2 cell monolayer model. *Cytokine* 2019;**126**:154882.

Maoz BM, Herland A, FitzGerald EA *et al.* A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells. *Nat Biotechnol* 2018;**36**:865–74.

Marischen L, Englert A, Schmitt AL *et al.* Human NK cells adapt their immune response towards increasing multiplicities of infection of Aspergillus fumigatus. *BMC Immunol* 2018;**19**:39.

Marolda A, Hünniger K, Böttcher S *et al.* Candida species-dependent release of IL-12 by dendritic cells induces different levels of NK cell stimulation. *J Infect Dis* 2020, DOI: 10.1093/infdis/jiaa035.

Mattern DJ, Schoeler H, Weber J *et al.* Identification of the antiphagocytic trypacidin gene cluster in the human-pathogenic fungus Aspergillus fumigatus. *Appl Microbiol Biotechnol* 2015;**99**:10151–61.

Matzaraki V, Gresnigt MS, Jaeger M *et al.* An integrative genomics approach identifies novel pathways that influence candidaemia susceptibility. *PLoS One* 2017;**12**:e0180824.

Maurer M, Gresnigt MS, Last A *et al.* A three-dimensional immunocompetent intestine-on-chip model as *in vitro* platform for functional and microbial interaction studies. *Biomaterials* 2019;**220**:119396.

Maxson ME, Naj X, O'Meara TR *et al.* Integrin-based diffusion barrier separates membrane domains enabling the formation of microbiostatic frustrated phagosomes. *eLife* 2018;**7**:e34798.

McCall AD, Kumar R, Edgerton M. Candida albicans Sfl1/Sfl2 regulatory network drives the formation of pathogenic microcolonies. *PLoS Pathog* 2018;**14**:e1007316.

Meir J, Hartmann E, Eckstein MT *et al.* Identification of Candida albicans regulatory genes governing mucosal infection. *Cell Microbiol* 2018;**20**:e12841.

Mihu MR, Nosanchuk JD. Histoplasma virulence and host responses. *Int J Microbiol* 2012;**2012**:268123.

Mikamo H, Yamagishi Y, Sugiyama H *et al.* High glucose-mediated overexpression of ICAM-1 in human vaginal epithelial cells increases adhesion of Candida albicans. *J Obstet Gynaecol* 2018; **38**:226–30.

Millsop JW, Fazel N. Oral candidiasis. *Clin Dermatol* 2016;**34**: 487–94.

Montagnoli C, Fallarino F, Gaziano R *et al.* Immunity and tolerance to Aspergillus involve functionally distinct regulatory T cells and tryptophan catabolism. *J Immunol* 2006;**176**:1712–23.

Montelongo-Jauregui D, Lopez-Ribot JL. Candida interactions with the oral bacterial microbiota. *J Fungi (Basel)* 2018;**4**.

Morse DJ, Wilson MJ, Wei X *et al.* Denture-associated biofilm infection in three-dimensional oral mucosal tissue models. *J Med Microbiol* 2018;**67**:364–75.

Morton CO, Fliesser M, Dittrich M *et al.* Gene expression profiles of human dendritic cells interacting with Aspergillus fumigatus in a bilayer model of the alveolar epithelium/endothelium interface. *PLoS One* 2014;**9**:e98279.

Morton CO, Wurster S, Fliesser M *et al.* Validation of a simplified *in vitro* Transwell((R)) model of the alveolar surface to assess host immunity induced by different morphotypes of Aspergillus fumigatus. *Int J Med Microbiol* 2018;**308**:1009–17.

Mosig AS. Organ-on-chip models: new opportunities for biomedical research. *Future Sci OA* 2017;**3**:Fso130.

Moyes DL, Wilson D, Richardson JP *et al.* Candidalysin is a fungal peptide toxin critical for mucosal infection. *Nature* 2016;**532**:64–8.

Munoz JF, Delorey T, Ford CB *et al.* Coordinated host–pathogen transcriptional dynamics revealed using sorted subpopulations and single macrophages infected with Candida albicans. *Nat Commun* 2019;**10**:1607.

Munoz NM, Leff AR. Highly purified selective isolation of eosinophils from human peripheral blood by negative immunomagnetic selection. *Nat Protoc* 2006;**1**:2613–20.

Naglik JR, Konig A, Hube B *et al.* Candida albicans-epithelial interactions and induction of mucosal innate immunity. *Curr Opin Microbiol* 2017;**40**:104–12.

Nailis H, Kucharikova S, Ricicova M *et al.* Real-time PCR expression profiling of genes encoding potential virulence factors in Candida albicans biofilms: identification of model-dependent and -independent gene expression. *BMC Microbiol* 2010;**10**:114.

Nayak DK, Mendez O, Bowen S *et al.* Isolation and *in vitro* culture of murine and human alveolar macrophages. *J Vis Exp* 2018;**134**: e57287.

Negoro PE, Xu S, Dagher Z *et al.* Spleen tyrosine kinase is a critical regulator of neutrophil responses to Candida species. *mBio* 2020;**11**:e02043–19.

Netea MG, Joosten LA, van der Meer JW *et al.* Immune defence against Candida fungal infections. *Nat Rev Immunol* 2015;**15**:630–42.

Neves GW, Curty NA, Kubitschek-Barreira PH *et al.* Modifications to the composition of the hyphal outer layer of Aspergillus fumigatus modulates HUVEC proteins related to inflammatory and stress responses. *J Proteomics* 2017;**151**: 83–96.

Newman SL. Interaction of Histoplasma capsulatum with human macrophages, dendritic cells, and neutrophils. *Methods Mol Med* 2005;**118**:181–91.

Niemiec MJ, Grumaz C, Ermert D *et al.* Dual transcriptome of the immediate neutrophil and Candida albicans interplay. *BMC Genomics* 2017;**18**:696.

O'Meara TR, Cowen LE. Insights into the host–pathogen interaction: C. albicans manipulation of macrophage pyroptosis. *Microb Cell* 2018;**5**:566–8.

O'Meara TR, Duah K, Guo CX *et al.* High-throughput screening identifies genes required for Candida albicans induction of macrophage pyroptosis. *mBio* 2018;**9**.

Oddo A, Peng B, Tong Z *et al.* Advances in microfluidic blood–brain barrier (BBB) models. *Trends Biotechnol* 2019;**37**: 1295–314.

Oesterreicher Z, Eberl S, Zeitlinger M. Impact of different antimycotics on cytokine levels in an *in vitro* aspergillosis model in human whole blood. *Infection* 2019;**48** :65–73.

Offenbacher S, Barros SP, Bencharit S *et al.* Differential mucosal gene expression patterns in Candida-associated, chronic oral denture stomatitis. *J Prosthodont* 2019;**28**:202–8.

Okagaki LH, Nielsen K. Titan cells confer protection from phagocytosis in Cryptococcus neoformans infections. *Eukaryot Cell* 2012;**11**:820–6.

Okai B, Lyall N, Gow NA *et al.* Rab14 regulates maturation of macrophage phagosomes containing the fungal pathogen Candida albicans and outcome of the host–pathogen interaction. *Infect Immun* 2015;**83**:1523–35.

Orozco AS, Zhou X, Filler SG. Mechanisms of the proinflammatory response of endothelial cells to Candida albicans infection. *Infect Immun* 2000;**68**:1134–41.

Page L, Weis P, Muller T *et al.* Evaluation of Aspergillus and Mucorales specific T-cells and peripheral blood mononuclear cell cytokine signatures as biomarkers of environmental mold exposure. *Int J Med Microbiol* 2018;**308**:1018–26.

Pappas PG, Lionakis MS, Arendrup MC *et al.* Invasive candidiasis. *Nat Rev Dis Primers* 2018;**4**:18026.

Park SE, Georgescu A, Huh D. Organoids-on-a-chip. *Science* 2019;**364**:960–5.

Patel R, Hossain MA, German N *et al.* Gliotoxin penetrates and impairs the integrity of the human blood–brain barrier *in vitro*. *Mycotoxin Res* 2018;**34**:257–68.

Patil S, Rao RS, Majumdar B *et al.* Clinical appearance of oral Candida infection and therapeutic strategies. *Front Microbiol* 2015;**6**:1391.

Pekmezovic M, Mogavero S, Naglik JR *et al.* Host–pathogen interactions during female genital tract infections. *Trends Microbiol* 2019;**27**:982–96.

Perlroth J, Choi B, Spellberg B. Nosocomial fungal infections: epidemiology, diagnosis, and treatment. *Med Mycol* 2007;**45**: 321–46.

Peterson MD, Mooseker MS. Characterization of the enterocyte-like brush border cytoskeleton of the C2BBe clones of the human intestinal cell line, Caco-2. *J Cell Sci* 1992;**102 (Pt 3)**:581–600.

Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 2007;**20**:133–63.

Phan QT, Eng DK, Mostowy S *et al.* Role of endothelial cell septin 7 in the endocytosis of Candida albicans. *mBio* 2013;**4**: e00542–13.

Phan QT, Fratti RA, Prasadarao NV *et al.* N-cadherin mediates endocytosis of Candida albicans by endothelial cells. *J Biol Chem* 2005;**280**:10455–61.

Phan QT, Myers CL, Fu Y *et al.* Als3 is a Candida albicans invasin that binds to cadherins and induces endocytosis by host cells. *PLoS Biol* 2007;**5**:e64.

Pidwill GR, Rego S, Jenkinson HF *et al.* Coassociation between group B Streptococcus and Candida albicans promotes interactions with vaginal epithelium. *Infect Immun* 2018;**86**.

Pietrella D, Corbucci C, Perito S *et al*. Mannoproteins from Cryptococcus neoformans promote dendritic cell maturation and activation. *Infect Immun* 2005;**73**:820–7.
Pitangui Nde S, Sardi Jde C, Voltan AR *et al*. An intracellular arrangement of Histoplasma capsulatum yeast-aggregates generates nuclear damage to the cultured murine alveolar macrophages. *Front Microbiol* 2015;**6**:1526.
Pitangui NS, Sardi JC, Silva JF *et al*. Adhesion of Histoplasma capsulatum to pneumocytes and biofilm formation on an abiotic surface. *Biofouling* 2012;**28**:711–8.
Pollard BS, Pollard HB. Induced pluripotent stem cells for treating cystic fibrosis: state of the science. *Pediatr Pulmonol* 2018;**53**:S12–29.
Polvi EJ, Li X, O'Meara TR *et al*. Opportunistic yeast pathogens: reservoirs, virulence mechanisms, and therapeutic strategies. *Cellular Mol Life Sci* 2015;**72**:2261–87.
Puri S, Kumar R, Rojas IG *et al*. Iron chelator deferasirox reduces Candida albicans invasion of oral epithelial cells and infection levels in murine oropharyngeal candidiasis. *Antimicrob Agents Chemother* 2019;**63**.
Quintin J, Saeed S, Martens JHA *et al*. Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. *Cell Host Microbe* 2012;**12**:223–32.
Raasch M, Fritsche E, Kurtz A *et al*. Microphysiological systems meet hiPSC technology: new tools for disease modeling of liver infections in basic research and drug development. *Adv Drug Deliv Rev* 2019;**140**:51–67.
Raasch M, Rennert K, Jahn T *et al*. An integrative microfluidically supported *in vitro* model of an endothelial barrier combined with cortical spheroids simulates effects of neuroinflammation in neocortex development. *Biomicrofluidics* 2016;**10**:044102.
Rahimi C, Rahimi B, Padova D *et al*. Oral mucosa-on-a-chip to assess layer-specific responses to bacteria and dental materials. *Biomicrofluidics* 2018;**12**:054106.
Rahmani S, Breyner NM, Su HM *et al*. Intestinal organoids: a new paradigm for engineering intestinal epithelium *in vitro*. *Biomaterials* 2019;**194**:195–214.
Raijmakers RPH, Sprenkeler EGG, Aleva FE *et al*. Toll-like receptor 2 induced cytotoxic T-lymphocyte-associated protein 4 regulates Aspergillus-induced regulatory T-cells with pro-inflammatory characteristics. *Sci Rep* 2017;**7**:11500.
Ramme AP, Koenig L, Hasenberg T *et al*. Autologous induced pluripotent stem cell-derived four-organ-chip. *Future Sci OA* 2019;**5**:Fso413.
Rappleye CA, Eissenberg LG, Goldman WE. Histoplasma capsulatum α-(1,3)-glucan blocks innate immune recognition by the β-glucan receptor. *Proc Natl Acad Sci USA* 2007;**104**:1366–70.
Ray SC, Rappleye CA. Flying under the radar: Histoplasma capsulatum avoidance of innate immune recognition. *Semin Cell Dev Biol* 2019;**89**:91–8.
Richards GR, Millard RM, Leveridge M *et al*. Quantitative assays of chemotaxis and chemokinesis for human neural cells. *Assay Drug Dev Technol* 2004;**2**:465–72.
Richardson JP, Willems HME, Moyes DL *et al*. Candidalysin drives epithelial signaling, neutrophil recruitment, and immunopathology at the vaginal mucosa. *Infect Immun* 2018;**86**.
Rieber N, Gazendam RP, Freeman AF *et al*. Extrapulmonary Aspergillus infection in patients with CARD9 deficiency. *JCI Insight* 2016;**1**:e89890.
Robinson NB, Krieger K, Khan FM *et al*. The current state of animal models in research: a review. *Int J Surg* 2019;**72**:9–13.
Rocha JD, Nascimento MT, Decote-Ricardo D *et al*. Capsular polysaccharides from Cryptococcus neoformans modulate production of neutrophil extracellular traps (NETs) by human neutrophils. *Sci Rep* 2015;**5**:8008.
Rogal J, Probst C, Loskill P. Integration concepts for multi-organ chips: how to maintain flexibility?! *Future Sci OA* 2017;**3**:Fso180.
Romani L. Immunity to fungal infections. *Nat Rev Immunol* 2004;**4**:1–23.
Rosati D, Bruno M, Jaeger M *et al*. Recurrent vulvovaginal candidiasis: an immunological perspective. *Microorganisms* 2020;**8**.
Rupniak HT, Rowlatt C, Lane EB *et al*. Characteristics of four new human cell lines derived from squamous cell carcinomas of the head and neck. *J Natl Cancer Inst* 1985;**75**:621–35.
Salerno C, Pascale M, Contaldo M *et al*. Candida-associated denture stomatitis. *Med Oral Patol Oral Cir Bucal* 2011;**16**:e139–43.
Sanchez AA, Johnston DA, Myers C *et al*. Relationship between Candida albicans virulence during experimental hematogenously disseminated infection and endothelial cell damage *in vitro*. *Infect Immun* 2004;**72**:598–601.
Santiago-Tirado FH, Onken MD, Cooper JA *et al*. Trojan horse transit contributes to blood–brain barrier crossing of a eukaryotic pathogen. *mBio* 2017;**8**.
Santiago V, Rezvani K, Sekine T *et al*. Human NK cells develop an exhaustion phenotype during polar degranulation at the Aspergillus fumigatus hyphal synapse. *Front Immunol* 2018;**9**:2344.
Sato T, Vries RG, Snippert HJ *et al*. Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. *Nature* 2009;**459**:262–5.
Schaller M, Schafer W, Korting HC *et al*. Differential expression of secreted aspartyl proteinases in a model of human oral candidosis and in patient samples from the oral cavity. *Mol Microbiol* 1998;**29**:605–15.
Scheffold A, Bacher P, LeibundGut-Landmann S. T cell immunity to commensal fungi. *Curr Opin Microbiol* 2020;**58**:116–23.
Schestatsky P, Chedid MF, Amaral OB *et al*. Isolated central nervous system histoplasmosis in immunocompetent hosts: a series of 11 cases. *Scand J Infect Dis* 2006;**38**:43–8.
Schirbel A, Shouval DS, Hebecker B *et al*. Intestinal epithelial cells and T cells differentially recognize and respond to Candida albicans yeast and hypha. *Eur J Immunol* 2018;**48**:1826–37.
Schmidt F, Thywißen A, Goldmann M *et al*. Flotillin-dependent membrane microdomains are required for functional phagolysosomes against fungal infections. *Cell Rep* 2020;**32**:108017.
Schmidt S, Tramsen L, Lehrnbecher T. Natural killer cells in antifungal immunity. *Front Immunol* 2017;**8**:1623.
Segal BH, Leto TL, Gallin JI *et al*. Genetic, biochemical, and clinical features of chronic granulomatous disease. *Medicine (Baltimore)* 2000;**79**:170–200.
Seider K, Heyken A, Lüttich A *et al*. Interaction of pathogenic yeasts with phagocytes: survival, persistence and escape. *Curr Opin Microbiol* 2010;**13**:392–400.
Seoane PI, Taylor-Smith LM, Stirling D *et al*. Viral infection triggers interferon-induced expulsion of live Cryptococcus neoformans by macrophages. *PLoS Pathog* 2020;**16**:e1008240.
Setianingrum F, Rautemaa-Richardson R, Denning DW. Pulmonary cryptococcosis: a review of pathobiology and clinical aspects. *Med Mycol* 2019;**57**:133–50.
Shen Q, Beucler MJ, Ray SC *et al*. Macrophage activation by IFN-gamma triggers restriction of phagosomal copper from intracellular pathogens. *PLoS Pathog* 2018;**14**:e1007444.

Sheppard DC, Filler SG. Host cell invasion by medically important fungi. *Cold Spring Harb Perspect Med* 2014;**5**:a019687.
Shimamura S, Miyazaki T, Tashiro M *et al.* Autophagy-inducing factor Atg1 is required for virulence in the pathogenic fungus Candida glabrata. *Front Microbiol* 2019;**10**:27.
Shin W, Kim HJ. Pathomimetic modeling of human intestinal diseases and underlying host–gut microbiome interactions in a gut-on-a-chip. *Methods Cell Biol* 2018;**146**: 135–48.
Shroff A, Reddy KVR. Autophagy gene ATG5 knockdown upregulates apoptotic cell death during Candida albicans infection in human vaginal epithelial cells. *American J Reprod Immunol* 2018;**80**:e13056.
Shukla A, Sobel JD. Vulvovaginitis caused by Candida species following antibiotic exposure. *Curr Infect Dis Rep* 2019;**21**:44.
Silva S, Henriques M, Hayes A *et al.* Candida glabrata and Candida albicans co-infection of an *in vitro* oral epithelium. *J Oral Pathol Med* 2011;**40**:421–7.
Smeekens SP, Ng A, Kumar V *et al.* Functional genomics identifies type I interferon pathway as central for host defense against Candida albicans. *Nat Commun* 2013;**4**:1342.
Smith LM, Dixon EF, May RC. The fungal pathogen Cryptococcus neoformans manipulates macrophage phagosome maturation. *Cell Microbiol* 2015;**17**:702–13.
Sobel JD. Vulvovaginal candidosis. *Lancet (London, England)* 2007;**369**:1961–71.
Sobue T, Bertolini M, Thompson A *et al.* Chemotherapy-induced oral mucositis and associated infections in a novel organotypic model. *Mol Oral Microbiol* 2018;**33**:212–23.
Solis NV, Park YN, Swidergall M *et al.* Candida albicans white-opaque switching influences virulence but not mating during oropharyngeal candidiasis. *Infect Immun* 2018;**86**: e00774–17.
Solis NV, Swidergall M, Bruno VM *et al.* The aryl hydrocarbon receptor governs epithelial cell invasion during oropharyngeal candidiasis. *mBio* 2017;**8**:e00025–17.
Sontheimer-Phelps A, Chou DB, Tovaglieri A *et al.* Human colon-on-a-chip enables continuous *in vitro* analysis of colon mucus layer accumulation and physiology. *Cell Mol Gastroenterol Hepatol* 2020;**9**:507–26.
Soubani AO, Chandrasekar PH. The clinical spectrum of pulmonary aspergillosis. *Chest* 2002;**121**:1988–99.
Spence JR, Mayhew CN, Rankin SA *et al.* Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro*. *Nature* 2011;**470**:105–9.
Spiering MJ, Moran GP, Chauvel M *et al.* Comparative transcript profiling of Candida albicans and Candida dubliniensis identifies SFL2, a C. albicans gene required for virulence in a reconstituted epithelial infection model. *Eukaryot Cell* 2010;**9**:251–65.
Sprenkeler EG, Gresnigt MS, van de Veerdonk FL. LC3-associated phagocytosis: a crucial mechanism for antifungal host defence against Aspergillus fumigatus. *Cell Microbiol* 2016;**18**:1208–16.
Srikanta D, Santiago-Tirado FH, Doering TL. Cryptococcus neoformans: historical curiosity to modern pathogen. *Yeast* 2014;**31**:47–60.
Stephen-Victor E, Karnam A, Fontaine T *et al.* Aspergillus fumigatus cell wall α-(1,3)-glucan stimulates regulatory T-cell polarization by inducing PD-L1 expression on human dendritic cells. *J Infect Dis* 2017;**216**:1281–94.
Stucki JD, Hobi N, Galimov A *et al.* Medium throughput breathing human primary cell alveolus-on-chip model. *Sci Rep* 2018;**8**:14359.
Sun D, Shi M. Neutrophil swarming toward Cryptococcus neoformans is mediated by complement and leukotriene B4. *Biochem Biophys Res Commun* 2016;**477**:945–51.
Sun H, Xu XY, Tian XL *et al.* Activation of NF-κB and respiratory burst following Aspergillus fumigatus stimulation of macrophages. *Immunobiology* 2014;**219**:25–36.
Swidergall M, Solis NV, Lionakis MS *et al.* EphA2 is an epithelial cell pattern recognition receptor for fungal β-glucans. *Nat Microbiol* 2018;**3**:53–61.
Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;**126**:663–76.
Teixeira PA, Penha LL, Mendonca-Previato L *et al.* Mannoprotein MP84 mediates the adhesion of Cryptococcus neoformans to epithelial lung cells. *Front Cell Infect Microbiol* 2014;**4**:106.
Ter Horst R, Jaeger M, Smeekens SP *et al.* Host and environmental factors influencing individual human cytokine responses. *Cell* 2016;**167**:1111–24.e13.
Thak EJ, Lee SB, Xu-Vanpala S *et al.* Core *N*-glycan structures are critical for the pathogenicity of Cryptococcus neoformans by modulating host cell death. *mBio* 2020;**11**:e00711–20.
Thompson-Souza GA,Santos GMP, Silva JC *et al.* Histoplasma capsulatum-induced extracellular DNA trap release in human neutrophils. *Cell Microbiol* 2020;**22**:e13195.
Thunström Salzer A, Niemiec MJ, Hosseinzadeh A *et al.* Assessment of neutrophil chemotaxis upon G-CSF treatment of healthy stem cell donors and in allogeneic transplant recipients. *Front Immunol* 2018;**9**:1968.
Toor A, Culibrk L, Singhera GK *et al.* Transcriptomic and proteomic host response to Aspergillus fumigatus conidia in an air–liquid interface model of human bronchial epithelium. *PLoS One* 2018;**13**:e0209652.
Tucey TM, Verma J, Olivier FAB *et al.* Metabolic competition between host and pathogen dictates inflammasome responses to fungal infection. *PLoS Pathog* 2020;**16**:e1008695.
Upadhyay J, Upadhyay RB, Agrawal P *et al.* Langerhans cells and their role in oral mucosal diseases. *N Am J Med Sci* 2013;**5**: 505–14.
Urban CF, Nett JE. Neutrophil extracellular traps in fungal infection. *Semin Cell Dev Biol* 2019;**89**:47–57.
Urban CF, Reichard U, Brinkmann V *et al.* Neutrophil extracellular traps capture and kill Candida albicans yeast and hyphal forms. *Cell Microbiol* 2006;**8**:668–76.
Vallabhaneni S, Chiller TM. Fungal infections and new biologic therapies. *Curr Rheumatol Rep* 2016;**18**:29.
van der Does AM, Joosten SA, Vroomans E *et al.* The antimicrobial peptide hLF1-11 drives monocyte-dendritic cell differentiation toward dendritic cells that promote antifungal responses and enhance Th17 polarization. *J Innate Immun* 2012;**4**:284–92.
van der Helm MW, van der Meer AD, Eijkel JC *et al.* Microfluidic organ-on-chip technology for blood–brain barrier research. *Tissue Barriers* 2016;**4**:e1142493.
van de Veerdonk FL, Gresnigt MS, Romani L *et al.* Aspergillus fumigatus morphology and dynamic host interactions. *Nat Rev Microbiol* 2017;**15**:661–74.
van de Veerdonk FL, Netea MG. T-cell subsets and antifungal host defenses. *Curr Fungal Infect Rep* 2010;**4**:238–43.
van de Veerdonk FL, Plantinga TS, Hoischen A *et al.* STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. *N Engl J Med* 2011;**365**:54–61.
Van Prooyen N, Henderson CA, Hocking Murray D *et al.* CD103+ conventional dendritic cells are critical for TLR7/9-dependent host defense against Histoplasma capsulatum,

an endemic fungal pathogen of humans. *PLoS Pathog* 2016;**12**:e1005749.
Verma AH, Richardson JP, Zhou C *et al.* Oral epithelial cells orchestrate innate type 17 responses to Candida albicans through the virulence factor candidalysin. *Sci Immunol* 2017;**2**:eaam8834.
Villar CC, Kashleva H, Nobile CJ *et al.* Mucosal tissue invasion by Candida albicans is associated with E-cadherin degradation, mediated by transcription factor Rim101p and protease Sap5p. *Infect Immun* 2007;**75**:2126–35.
Vivas W, Leonhardt I, Hünniger K *et al.* Multiple signaling pathways involved in human dendritic cell maturation are affected by the fungal quorum-sensing molecule farnesol. *J Immunol* 2019;**203**:2959–69.
Voelz K, May RC. Cryptococcal interactions with the host immune system. *Eukaryot Cell* 2010;**9**:835–46.
Vogel K, Pierau M, Arra A *et al.* Developmental induction of human T-cell responses against Candida albicans and Aspergillus fumigatus. *Sci Rep* 2018;**8**:16904.
Vos T, Flaxman AD, Naghavi M *et al.* Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet (London, England)* 2012;**380**:2163–96.
Wachtler B, Wilson D, Haedicke K *et al.* From attachment to damage: defined genes of Candida albicans mediate adhesion, invasion and damage during interaction with oral epithelial cells. *PLoS One* 2011a;**6**:e17046.
Wachtler B, Wilson D, Hube B. Candida albicans adhesion to and invasion and damage of vaginal epithelial cells: stage-specific inhibition by clotrimazole and bifonazole. *Antimicrob Agents Chemother* 2011b;**55**:4436–9.
Wagener J, Mailander-Sanchez D, Schaller M. Immune responses to Candida albicans in models of *in vitro* reconstituted human oral epithelium. *Methods Mol Biol* 2012;**845**:333–44.
Wang G, Yu G, Wang D *et al.* Comparison of the purity and vitality of natural killer cells with different isolation kits. *Exp Ther Med* 2017;**13**:1875–83.
Wang XJ, Zhu YJ, Cui JG *et al.* Proteomic analysis of human umbilical vein endothelial cells incubated with Cryptococcus neoformans var. neoformans. *Mycoses* 2011;**54**:e336–43.
Warkentien T, Crum-Cianflone NF. An update on Cryptococcus among HIV-infected patients. *Int J STD AIDS* 2010;**21**:679–84.
Watkins TN, Liu H, Chung M *et al.* Comparative transcriptomics of Aspergillus fumigatus strains upon exposure to human airway epithelial cells. *Microb Genom* 2018;**4**:e000154.
Weerasinghe H, Traven A. Immunometabolism in fungal infections: the need to eat to compete. *Curr Opin Microbiol* 2020;**58**:32–40.
Weiss E, Schlegel J, Terpitz U *et al.* Reconstituting NK cells after allogeneic stem cell transplantation show impaired response to the fungal pathogen Aspergillus fumigatus. *Front Immunol* 2020;**11**:2117.
Weiss E, Ziegler S, Fliesser M *et al.* First insights in NK-DC crosstalk and the importance of soluble factors during infection with Aspergillus fumigatus. *Front Cell Infect Microbiol* 2018;**8**:288.
Weiss GA, Hennet T. Mechanisms and consequences of intestinal dysbiosis. *Cell Mol Life Sci* 2017;**74**:2959–77.
Weksler B, Romero IA, Couraud PO. The hCMEC/D3 cell line as a model of the human blood brain barrier. *Fluids Barriers CNS* 2013;**10**:16.
Wellington M, Dolan K, Krysan DJ. Live Candida albicans suppresses production of reactive oxygen species in phagocytes. *Infect Immun* 2009;**77**:405–13.
Westman J, Moran G, Mogavero S *et al.* Candida albicans hyphal expansion causes phagosomal membrane damage and luminal alkalinization. *mBio* 2018;**9**:e01226–18.
Westman J, Walpole GFW, Kasper L *et al.* Lysosome fusion maintains phagosome integrity during fungal infection. *Cell Host Microbe* 2020;**28**:798–812.e6.
Wheeler ML, Limon JJ, Underhill DM. Immunity to commensal fungi: detente and disease. *Annu Rev Pathol* 2017;**12**:359–85.
Wilson D, Hube B. Hgc1 mediates dynamic Candida albicans–endothelium adhesion events during circulation. *Eukaryot Cell* 2010;**9**:278–87.
Wilson D, Mayer FL, Miramon P *et al.* Distinct roles of Candida albicans-specific genes in host–pathogen interactions. *Eukaryot Cell* 2014;**13**:977–89.
Wiseman JC, Ma LL, Marr KJ *et al.* Perforin-dependent cryptococcal microbicidal activity in NK cells requires PI3K-dependent ERK1/2 signaling. *J Immunol* 2007;**178**:6456–64.
Wolf JE, Kerchberger V, Kobayashi GS *et al.* Modulation of the macrophage oxidative burst by Histoplasma capsulatum. *J Immunol* 1987;**138**:582–6.
Wollert T, Rollenhagen C, Langford GM *et al.* Human oral keratinocytes: a model system to analyze host–pathogen interactions. *Methods Mol Biol* 2012;**845**:289–302.
Workman MJ, Mahe MM, Trisno S *et al.* Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. *Nat Med* 2017;**23**:49–59.
Xue J, Schmidt SV, Sander J *et al.* Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* 2014;**40**:274–88.
Xu S, Shinohara ML. Tissue-resident macrophages in fungal infections. *Front Immunol* 2017;**8**:1798.
Yang AM, Inamine T, Hochrath K *et al.* Intestinal fungi contribute to development of alcoholic liver disease. *J Clin Invest* 2017;**127**:2829–41.
Yeon JH, Na D, Choi K *et al.* Reliable permeability assay system in a microfluidic device mimicking cerebral vasculatures. *Biomed Microdevices* 2012;**14**:1141–8.
Youseff BH, Holbrook ED, Smolnycki KA *et al.* Extracellular superoxide dismutase protects Histoplasma yeast cells from host-derived oxidative stress. *PLoS Pathog* 2012;**8**:e1002713.
Zhang C, Zhao Z, Abdul Rahim NA *et al.* Towards a human-on-chip: culturing multiple cell types on a chip with compartmentalized microenvironments. *Lab Chip* 2009;**9**:3185–92.
Zhang M, Xu C, Jiang L *et al.* A 3D human lung-on-a-chip model for nanotoxicity testing. *Toxicol Res (Camb)* 2018;**7**:1048–60.
Zielinski CE, Mele F, Aschenbrenner D *et al.* Pathogen-induced human TH17 cells produce IFN-γ or IL-10 and are regulated by IL-1β. *Nature* 2012;**484**:514–8.

Table S1: Overview about the different immune cell *in vitro* models, their structure, possible read outs and applications for the different fungi

Model	Structure	Readout	Reference
A) Macrophage (like) cell lines			
J774A.1 (Mouse cell line)	Monoculture	Fungal killing	*A. fumigatus*: (Gresnigt *et al.* 2018a)
		Phagocytosis	*A. fumigatus*: (Gresnigt *et al.* 2018a) *C. albicans*: (Cottier *et al.* 2019; Duvenage *et al.* 2019; Loureiro *et al.* 2019; Rudkin *et al.* 2018) *C. neoformans*: (Bryan *et al.* 2014)
		Cytokine release	*A. fumigatus:* (Cho *et al.* 2016)
RAW (Mouse cell line)	Monoculture	Fungal killing	*C. glabrata:* (Chew *et al.* 2019)
		Proteomics	*A. fumigatus*: (Schmidt *et al.* 2018)
		ROS production	*C. albicans*: (Arce Miranda *et al.* 2019)
		Phagosome maturation	*C. albicans:* (Bain *et al.* 2014; Okai *et al.* 2015)
Ana-1 (Mouse cell line)	Monoculture	Apoptosis	*C. albicans*: (Jiang *et al.* 2019)
THP-1 (Human cell line)	Monoculture	Phagocytosis	*C. albicans* : (Liu *et al.* 2019; Vaz *et al.* 2019)
		Proteomics	*C. albicans*: (Vaz *et al.* 2019)
		Cytokine release	*A. fumigatus:* (Oya *et al.* 2019) *C. albicans*: (de Albuquerque *et al.* 2018)
		ROS release	*A. fumigatus:* (Sun *et al.* 2014)
U937 (Human cell line)	Monoculture	Adhesion	*C. albicans:* (Lopez et al. 2014) *C. glabrata:* (Kuhn and Vyas 2012)
		Internalization	*H. capsulatum:* (Scott and Woods 2000)
		Cytokine release	*C. albicans:* (Kaya et al. 2011)
MH-S (Mouse alveolar macrophage cell line)	Monoculture	Phagocytosis	*A. fumigatus:* (Mattern *et al.* 2015)
AMJ2-C11 (Mouse alveolar macrophage cell line)	Monoculture	Intracellular yeast arrangement	*H. capsulatum:* (Pitangui Nde et al., 2015)
BV-2 (Mouse microglial cell line)	Monoculture	Cytokine release	*C. albicans:* (Wu et al. 2019b) *C. neoformans*: (Barluzzi *et al.* 1998)
BV-2 (Mouse cell line)	Co-Culture	Cytokine release	*C. albicans:* (Drummond et al. 2019)

+ C8-D1A Astrocytes (Mouse cell line)			
B) Primary cells			
Bone marrow derived macrophages BMDMs (Murine primary cells)	Monoculture	Phagocytosis	*A. fumigatus:* (Gresnigt *et al.* 2018a) *C. albicans:* (Haider *et al.* 2019; Okai *et al.* 2015) *H. capsulatum*: (Baltazar *et al.* 2018; Guimaraes *et al.* 2019)
		Cytokine release	*A. fumigatus*: (Rubino *et al.* 2012) *C. albicans*: (Alsina-Beauchamp *et al.* 2018; Thompson *et al.* 2019; Wang *et al.* 2019) *C. neoformans*: (Veloso Júnior *et al.* 2019)
		Exocytosis	*C. neoformans*: (Stukes and Casadevall 2014)
		Transcriptomics	*C. albicans:* (Muñoz et al. 2019)
Alveolar macrophages (Murine/Human primary cells)	Monoculture	Phagocytosis	*A. fumigatus:* (Grimm *et al.* 2014; Wu *et al.* 2016) *C. neoformans*: (Hansakon *et al.* 2019; Walsh *et al.* 2017) *H. capsulatum*: (Pereira *et al.* 2018; Tagliari *et al.* 2012)
		Autophagy	*A. fumigatus:* (Dai *et al.* 2018)
		Apoptosis	*H. capsulatum:* (Deepe and Buesing 2012)
		Cytokine release	*A. fumigatus:* (Zhang *et al.* 2017a) *H. capsulatum*: (Coady and Sil 2015)
Peritoneal macrophages (Murine primary cells)	Monoculture	Phagocytosis	*H. capsulatum*: (Huang et al., 2018)
		Autophagy	*C. albicans:* (Ifrim et al. 2016) *C. glabrata*: (Shimamura *et al.* 2019)
		Cytokine release	*H. capsulatum*: (Shen *et al.* 2018)
		ROS release	*H. capsulatum:* (Youseff *et al.* 2012)
Monocyte derived macrophages MDMs (Human primary cells)	Monoculture	Phagocytosis	*C. albicans:* (Behrens *et al.* 2019; Munawara *et al.* 2017)
		Phagosome maturation	*C. neoformans*: (Smith *et al.* 2015)
		Cytokine release	*A. fumigatus:* (Gresnigt *et al.* 2018b)

			C. albicans: (Kasper *et al.* 2018; O'Meara *et al.* 2018) *H. capsulatum*: (Friedrich *et al.* 2019)
		ROS release	*A. fumigatus:* (Gresnigt *et al.* 2018b) *H. capsulatum:* (Wolf *et al.* 1987)
		Immuno-metabolism	*A. fumigatus:* (Gonçalves et al. 2020)
Monocytes (Human primary cells)	Monoculture	Phagocytosis	*A. fumigatus:* (Brunel *et al.* 2017; Kyrmizi *et al.* 2018) *C. albicans:* (Camilli *et al.* 2018; Halder *et al.* 2016) *C. neoformans*: (Charlier *et al.* 2009)
		Metabolomics	*C. albicans:* (Grondman *et al.* 2019)
		Cytokine release	*C. albicans:* (Dominguez-Andres *et al.* 2017; Leonhardt *et al.* 2018)
		ROS release	*A. fumigatus:* (Brunel *et al.* 2018) *C. albicans*: (Camilli *et al.* 2018; Wellington *et al.* 2009)
Monocytes (Human primary cells) **+** **DCs** (Human primary differentiated cells)	Co-culture	Phagocytosis Cytokine release	*C. neoformans*: (Alvarez *et al.* 2009; Kelly *et al.* 2005)
Neutrophils (Human primary cells)	Monoculture	Fungal killing	*A. fumigatus*: (Dasari *et al.* 2018; Gazendam *et al.* 2016; Jones *et al.* 2019) *C. albicans*: (Essig *et al.* 2015; Gazendam *et al.* 2016; Jones *et al.* 2019; Salvatori *et al.* 2018)
		Phagocytosis	*A. fumigatus:* (Brunel *et al.* 2017) *C. neoformans*: (Sun *et al.* 2016)
		NET formation	*A. fumigatus*: (Bruns *et al.* 2010; Clark *et al.* 2018; Ellett *et al.* 2017; Röhm *et al.* 2014; Silva *et al.* 2019) *C. albicans*: (Campos-Garcia *et al.* 2019; Guiducci *et al.* 2018; Johnson *et al.* 2017; Negoro *et al.* 2020; Urban *et al.* 2006; Wu *et al.* 2019a; Zawrotniak *et al.* 2019) *C. neoformans*: (Rocha *et al.* 2015)

			H. capsulatum: (Thompson-Souza *et al.* 2020)
		Transcriptomics	*C. albicans*: (Niemiec *et al.* 2017)
		ROS release	*A. fumigatus:* (Boyle *et al.* 2011) *C. albicans:* (Liu *et al.* 2018; Miramón *et al.* 2012; Salvatori *et al.* 2018)
	Monoculture on poly-L-lysine coated glass slides	Swarming	*C. albicans:* (Hopke et al. 2020)
	Monoculture on Transwell (top: neutrophils Bottom: chemoattractant)	Chemotaxis	*A. fumigatus:* (Rieber *et al.* 2016) *C. albicans*: (Drummond *et al.* 2015) *C. neoformans:* (Coenjaerts *et al.* 2001)
Dendritic cells DCs (Human primary differentiated cells)	Monoculture	Phagocytosis	*A. fumigatus*: (Lother *et al.* 2014) *H. capsulatum*: (Gildea *et al.* 2001; Nguyen *et al.* 2018)
		Maturation	*A. fumigatus*: (Fliesser *et al.* 2016; Hefter *et al.* 2017; Lother *et al.* 2014) *C. albicans:* (Roudbary *et al.* 2009; Vivas *et al.* 2019) *C. glabrata:* (Bazan *et al.* 2018) *C. neoformans:* (Pietrella *et al.* 2005)
		Transcriptomics	*A. fumigatus*: (Srivastava *et al.* 2019) *H. capsulatum*: (Van Prooyen *et al.* 2016)
		Cytokine release	*C. albicans*: (Maher *et al.* 2015) *H. capsulatum*: (Chang *et al.* 2017; Garfoot *et al.* 2016)
Natural killer cells NK cells (Human primary cells)	Monoculture	Fungal killing	*A. fumigatus:* (Weiss *et al.* 2018) *C. albicans*: (Li *et al.* 2018) *C. neoformans:* (Ma *et al.* 2004; Wiseman *et al.* 2007)
		Transcriptomics	*C. albicans*: (Hellwig *et al.* 2016)
		Chemokines	*A. fumigatus:* (Marischen *et al.* 2018)

		Cytokine release	*A. fumigatus:* (Bouzani *et al.* 2011; Santiago *et al.* 2018) *C. albicans:* (Marolda *et al.* 2020; Voigt *et al.* 2014)
NK cells (Human primary cells) **+ DCs** (Human primary differentiated cells)	Co-culture	NK-DC cross talk	*A. fumigatus:* (Weiss *et al.* 2018)
DCs (Human primary differentiated cells) **+ T-cells** (Human primary cells)	Co-culture	Maturation	*A. fumigatus:* (Stephen-Victor *et al.* 2017)
		Cytokine release	*C. albicans:* (van der Does *et al.* 2012)
		Cytokine release	*A. fumigatus:* (Becker *et al.* 2016; Page *et al.* 2018) *C. albicans*: (Alvarez-Rueda *et al.* 2020; Estrada-Mata *et al.* 2015; Li *et al.* 2016) *C. neoformans:* (Mora *et al.* 2017; Siddiqui *et al.* 2006)
Whole blood model (Human/Mouse)	Multiculture	Fungal killing	*C. albicans*: (Duggan *et al.* 2015; Hunniger *et al.* 2014)
		Transcriptomics	*A. fumigatus:* (Dix *et al.* 2015) *C. albicans:* (Fradin *et al.* 2005; Kämmer *et al.* 2020)
		Cytokine release	*A. fumigatus:* (Oesterreicher *et al.* 2019)
		Platelet interaction	*A. fumigatus:* (Fréalle *et al.* 2018) *C. albicans:* (Eberl *et al.* 2019)

References Tab. S1:

Alsina-Beauchamp D, Escos A, Fajardo P *et al.* Myeloid cell deficiency of p38gamma/p38delta protects against candidiasis and regulates antifungal immunity. *EMBO molecular medicine* 2018;**10**.

Alvarez-Rueda N, Rouges C, Touahri A *et al.* In vitro immune responses of human PBMCs against Candida albicans reveals fungal and leucocyte phenotypes associated with fungal persistence. *Scientific reports* 2020;**10**: 6211.

Alvarez M, Burn T, Luo Y *et al.* The outcome of Cryptococcus neoformans intracellular pathogenesis in human monocytes. *BMC microbiology* 2009;**9**: 51.

Arce Miranda JE, Baronetti JL, Sotomayor CE *et al.* Oxidative and nitrosative stress responses during macrophage-Candida albicans biofilm interaction. *Medical mycology* 2019;**57**: 101-13.

Bain JM, Louw J, Lewis LE *et al.* Candida albicans hypha formation and mannan masking of β-glucan inhibit macrophage phagosome maturation. *mBio* 2014;**5**: e01874.

Baltazar LM, Zamith-Miranda D, Burnet MC *et al.* Concentration-dependent protein loading of extracellular vesicles released by Histoplasma capsulatum after

antibody treatment and its modulatory action upon macrophages. *Scientific reports* 2018;**8**: 8065.
Barluzzi R, Brozzetti A, Delfino D *et al.* Role of the capsule in microglial cell-Cryptococcus neoformans interaction: impairment of antifungal activity but not of secretory functions. *Medical mycology* 1998;**36**: 189-97.
Bazan SB, Walch-Rückheim B, Schmitt MJ *et al.* Maturation and cytokine pattern of human dendritic cells in response to different yeasts. *Medical microbiology and immunology* 2018;**207**: 75-81.
Becker KL, Aimanianda V, Wang X *et al.* Aspergillus Cell Wall Chitin Induces Anti- and Proinflammatory Cytokines in Human PBMCs via the Fc-gamma Receptor/Syk/PI3K Pathway. *mBio* 2016;**7**.
Behrens NE, Lipke PN, Pilling D *et al.* Serum Amyloid P Component Binds Fungal Surface Amyloid and Decreases Human Macrophage Phagocytosis and Secretion of Inflammatory Cytokines. *mBio* 2019;**10**.
Bouzani M, Ok M, McCormick A *et al.* Human NK cells display important antifungal activity against Aspergillus fumigatus, which is directly mediated by IFN-γ release. *J Immunol* 2011;**187**: 1369-76.
Boyle KB, Gyori D, Sindrilaru A *et al.* Class IA phosphoinositide 3-kinase β and δ regulate neutrophil oxidase activation in response to Aspergillus fumigatus hyphae. *J Immunol* 2011;**186**: 2978-89.
Brunel SF, Bain JM, King J *et al.* Live Imaging of Antifungal Activity by Human Primary Neutrophils and Monocytes in Response to A. fumigatus. *Journal of visualized experiments : JoVE* 2017, DOI 10.3791/55444.
Brunel SF, Willment JA, Brown GD *et al.* Aspergillus-induced superoxide production by cystic fibrosis phagocytes is associated with disease severity. *ERJ open research* 2018;**4**.
Bruns S, Kniemeyer O, Hasenberg M *et al.* Production of extracellular traps against Aspergillus fumigatus in vitro and in infected lung tissue is dependent on invading neutrophils and influenced by hydrophobin RodA. *PLoS pathogens* 2010;**6**: e1000873.
Bryan AM, Farnoud AM, Mor V *et al.* Macrophage cholesterol depletion and its effect on the phagocytosis of Cryptococcus neoformans. *Journal of visualized experiments : JoVE* 2014, DOI 10.3791/52432.
Camilli G, Eren E, Williams DL *et al.* Impaired phagocytosis directs human monocyte activation in response to fungal derived beta-glucan particles. *European journal of immunology* 2018;**48**: 757-70.
Campos-Garcia L, Jimenez-Valdes RJ, Hernandez-Bello R *et al.* Candida albicans and non-albicans Isolates from Bloodstream Have Different Capacities to Induce Neutrophil Extracellular Traps. *Journal of fungi (Basel, Switzerland)* 2019;**5**.
Chang TH, Huang JH, Lin HC *et al.* Dectin-2 is a primary receptor for NLRP3 inflammasome activation in dendritic cell response to Histoplasma capsulatum. *PLoS pathogens* 2017;**13**: e1006485.
Charlier C, Nielsen K, Daou S *et al.* Evidence of a role for monocytes in dissemination and brain invasion by Cryptococcus neoformans. *Infection and immunity* 2009;**77**: 120-7.
Chew SY, Ho KL, Cheah YK *et al.* Glyoxylate cycle gene ICL1 is essential for the metabolic flexibility and virulence of Candida glabrata. *Scientific reports* 2019;**9**: 2843.

Cho SY, Kwon EY, Choi SM *et al.* Immunomodulatory effect of mesenchymal stem cells on the immune response of macrophages stimulated by Aspergillus fumigatus conidia. *Medical mycology* 2016;**54**: 377-83.
Clark HL, Abbondante S, Minns MS *et al.* Protein Deiminase 4 and CR3 Regulate Aspergillus fumigatus and beta-Glucan-Induced Neutrophil Extracellular Trap Formation, but Hyphal Killing Is Dependent Only on CR3. *Frontiers in immunology* 2018;**9**: 1182.
Coady A, Sil A. MyD88-dependent signaling drives host survival and early cytokine production during Histoplasma capsulatum infection. *Infection and immunity* 2015;**83**: 1265-75.
Coenjaerts FE, Walenkamp AM, Mwinzi PN *et al.* Potent inhibition of neutrophil migration by cryptococcal mannoprotein-4-induced desensitization. *J Immunol* 2001;**167**: 3988-95.
Cottier F, Sherrington S, Cockerill S *et al.* Remasking of Candida albicans beta-Glucan in Response to Environmental pH Is Regulated by Quorum Sensing. *mBio* 2019;**10**.
Dai J, Liang Y, Li H *et al.* Vitamin D enhances resistance to aspergillus fumigatus in mice via inhibition of excessive autophagy. *American journal of translational research* 2018;**10**: 381-91.
Dasari P, Shopova IA, Stroe M *et al.* Aspf2 From Aspergillus fumigatus Recruits Human Immune Regulators for Immune Evasion and Cell Damage. *Frontiers in immunology* 2018;**9**: 1635.
de Albuquerque JAT, Banerjee PP, Castoldi A *et al.* The Role of AIRE in the Immunity Against Candida Albicans in a Model of Human Macrophages. *Frontiers in immunology* 2018;**9**: 567.
Deepe GS, Jr., Buesing WR. Deciphering the pathways of death of Histoplasma capsulatum-infected macrophages: implications for the immunopathogenesis of early infection. *Journal of immunology (Baltimore, Md : 1950)* 2012;**188**: 334-44.
Dix A, Hunniger K, Weber M *et al.* Biomarker-based classification of bacterial and fungal whole-blood infections in a genome-wide expression study. *Frontiers in microbiology* 2015;**6**: 171.
Dominguez-Andres J, Arts RJW, Ter Horst R *et al.* Rewiring monocyte glucose metabolism via C-type lectin signaling protects against disseminated candidiasis. *PLoS pathogens* 2017;**13**: e1006632.
Drummond RA, Collar AL, Swamydas M *et al.* CARD9-Dependent Neutrophil Recruitment Protects against Fungal Invasion of the Central Nervous System. *PLoS pathogens* 2015;**11**: e1005293.
Drummond RA, Swamydas M, Oikonomou V *et al.* CARD9(+) microglia promote antifungal immunity via IL-1β- and CXCL1-mediated neutrophil recruitment. *Nat Immunol* 2019;**20**: 559-70.
Duggan S, Essig F, Hunniger K *et al.* Neutrophil activation by Candida glabrata but not Candida albicans promotes fungal uptake by monocytes. *Cellular microbiology* 2015;**17**: 1259-76.
Duvenage L, Walker LA, Bojarczuk A *et al.* Inhibition of Classical and Alternative Modes of Respiration in Candida albicans Leads to Cell Wall Remodeling and Increased Macrophage Recognition. *mBio* 2019;**10**.
Eberl C, Speth C, Jacobsen ID *et al.* Candida: Platelet Interaction and Platelet Activity in vitro. *J Innate Immun* 2019;**11**: 52-62.

Ellett F, Jorgensen J, Frydman GH *et al.* Neutrophil Interactions Stimulate Evasive Hyphal Branching by Aspergillus fumigatus. *PLoS pathogens* 2017;**13**: e1006154.

Essig F, Hunniger K, Dietrich S *et al.* Human neutrophils dump Candida glabrata after intracellular killing. *Fungal genetics and biology : FG & B* 2015;**84**: 37-40.

Estrada-Mata E, Navarro-Arias MJ, Pérez-García LA *et al.* Members of the Candida parapsilosis Complex and Candida albicans are Differentially Recognized by Human Peripheral Blood Mononuclear Cells. *Frontiers in microbiology* 2015;**6**: 1527.

Fliesser M, Wallstein M, Kurzai O *et al.* Hypoxia attenuates anti-Aspergillus fumigatus immune responses initiated by human dendritic cells. *Mycoses* 2016;**59**: 503-8.

Fradin C, De Groot P, MacCallum D *et al.* Granulocytes govern the transcriptional response, morphology and proliferation of Candida albicans in human blood. *Molecular microbiology* 2005;**56**: 397-415.

Fréalle E, Gosset P, Leroy S *et al.* In vitro coagulation triggers anti-Aspergillus fumigatus neutrophil response. *Future microbiology* 2018;**13**: 659-69.

Friedrich D, Zapf D, Lohse B *et al.* The HIF-1alpha/LC3-II Axis Impacts Fungal Immunity in Human Macrophages. *Infection and immunity* 2019;**87**.

Garfoot AL, Shen Q, Wuthrich M *et al.* The Eng1 beta-Glucanase Enhances Histoplasma Virulence by Reducing beta-Glucan Exposure. *mBio* 2016;**7**: e01388-15.

Gazendam RP, van Hamme JL, Tool AT *et al.* Human Neutrophils Use Different Mechanisms To Kill Aspergillus fumigatus Conidia and Hyphae: Evidence from Phagocyte Defects. *Journal of immunology (Baltimore, Md : 1950)* 2016;**196**: 1272-83.

Gildea LA, Morris RE, Newman SL. Histoplasma capsulatum yeasts are phagocytosed via very late antigen-5, killed, and processed for antigen presentation by human dendritic cells. *Journal of immunology (Baltimore, Md : 1950)* 2001;**166**: 1049-56.

Gonçalves SM, Duarte-Oliveira C, Campos CF *et al.* Phagosomal removal of fungal melanin reprograms macrophage metabolism to promote antifungal immunity. *Nature communications* 2020;**11**: 2282.

Gresnigt MS, Becker KL, Leenders F *et al.* Differential Kinetics of Aspergillus nidulans and Aspergillus fumigatus Phagocytosis. *J Innate Immun* 2018a;**10**: 145-60.

Gresnigt MS, Cunha C, Jaeger M *et al.* Genetic deficiency of NOD2 confers resistance to invasive aspergillosis. *Nature communications* 2018b;**9**: 2636.

Grimm MJ, D'Auria AC, Segal BH. Assessing anti-fungal activity of isolated alveolar macrophages by confocal microscopy. *Journal of visualized experiments : JoVE* 2014, DOI 10.3791/51678.

Grondman I, Arts RJW, Koch RM *et al.* Frontline Science: Endotoxin-induced immunotolerance is associated with loss of monocyte metabolic plasticity and reduction of oxidative burst. *Journal of leukocyte biology* 2019;**106**: 11-25.

Guiducci E, Lemberg C, Kung N *et al.* Candida albicans-Induced NETosis Is Independent of Peptidylarginine Deiminase 4. *Frontiers in immunology* 2018;**9**: 1573.

Guimaraes AJ, de Cerqueira MD, Zamith-Miranda D *et al.* Host membrane glycosphingolipids and lipid microdomains facilitate Histoplasma capsulatum internalisation by macrophages. *Cellular microbiology* 2019;**21**: e12976.

Haider M, Dambuza IM, Asamaphan P *et al.* The pattern recognition receptors dectin-2, mincle, and FcRγ impact the dynamics of phagocytosis of Candida, Saccharomyces, Malassezia, and Mucor species. *PloS one* 2019;**14**: e0220867.

Halder LD, Abdelfatah MA, Jo EA *et al.* Factor H Binds to Extracellular DNA Traps Released from Human Blood Monocytes in Response to Candida albicans. *Frontiers in immunology* 2016;**7**: 671.

Hansakon A, Mutthakalin P, Ngamskulrungroj P *et al.* Cryptococcus neoformans and Cryptococcus gattii clinical isolates from Thailand display diverse phenotypic interactions with macrophages. *Virulence* 2019;**10**: 26-36.

Hefter M, Lother J, Weiss E *et al.* Human primary myeloid dendritic cells interact with the opportunistic fungal pathogen Aspergillus fumigatus via the C-type lectin receptor Dectin-1. *Medical mycology* 2017;**55**: 573-8.

Hellwig D, Voigt J, Bouzani M *et al.* Candida albicans Induces Metabolic Reprogramming in Human NK Cells and Responds to Perforin with a Zinc Depletion Response. *Frontiers in microbiology* 2016;**7**: 750.

Hopke A, Scherer A, Kreuzburg S *et al.* Neutrophil swarming delays the growth of clusters of pathogenic fungi. *Nature communications* 2020;**11**: 2031.

Hunniger K, Lehnert T, Bieber K *et al.* A virtual infection model quantifies innate effector mechanisms and Candida albicans immune escape in human blood. *PLoS computational biology* 2014;**10**: e1003479.

Ifrim DC, Quintin J, Courjol F *et al.* The Role of Dectin-2 for Host Defense Against Disseminated Candidiasis. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research* 2016;**36**: 267-76.

Jiang HH, Zhang YJ, Sun YZ *et al.* Cell wall mannoprotein of Candida albicans polarizes macrophages and affects proliferation and apoptosis through activation of the Akt signal pathway. *International immunopharmacology* 2019;**72**: 308-21.

Johnson CJ, Kernien JF, Hoyer AR *et al.* Mechanisms involved in the triggering of neutrophil extracellular traps (NETs) by Candida glabrata during planktonic and biofilm growth. *Scientific reports* 2017;**7**: 13065.

Jones CN, Ellett F, Robertson AL *et al.* Bifunctional Small Molecules Enhance Neutrophil Activities Against Aspergillus fumigatus in vivo and in vitro. *Frontiers in immunology* 2019;**10**: 644.

Kämmer P, McNamara S, Wolf T *et al.* Survival Strategies of Pathogenic Candida Species in Human Blood Show Independent and Specific Adaptations. *mBio* 2020;**11**.

Kasper L, Konig A, Koenig PA *et al.* The fungal peptide toxin Candidalysin activates the NLRP3 inflammasome and causes cytolysis in mononuclear phagocytes. *Nature communications* 2018;**9**: 4260.

Kaya EG, Ozbilge H, Ustundag MB *et al.* The effects on immune response of levamisole treatment following infection of U-937 macrophages with Candida albicans. *Acta Microbiol Immunol Hung* 2011;**58**: 279-88.

Kelly RM, Chen J, Yauch LE *et al.* Opsonic requirements for dendritic cell-mediated responses to Cryptococcus neoformans. *Infection and immunity* 2005;**73**: 592-8.

Kuhn DM, Vyas VK. The Candida glabrata adhesin Epa1p causes adhesion, phagocytosis, and cytokine secretion by innate immune cells. *FEMS yeast research* 2012;**12**: 398-414.

Kyrmizi I, Ferreira H, Carvalho A *et al.* Calcium sequestration by fungal melanin inhibits calcium-calmodulin signalling to prevent LC3-associated phagocytosis. *Nature microbiology* 2018;**3**: 791-803.
Leonhardt J, Grosse S, Marx C *et al.* Candida albicans beta-Glucan Differentiates Human Monocytes Into a Specific Subset of Macrophages. *Frontiers in immunology* 2018;**9**: 2818.
Li SS, Ogbomo H, Mansour MK *et al.* Identification of the fungal ligand triggering cytotoxic PRR-mediated NK cell killing of Cryptococcus and Candida. *Nature communications* 2018;**9**: 751.
Li Y, Oosting M, Deelen P *et al.* Inter-individual variability and genetic influences on cytokine responses to bacteria and fungi. *Nat Med* 2016;**22**: 952-60.
Liu NN, Uppuluri P, Broggi A *et al.* Intersection of phosphate transport, oxidative stress and TOR signalling in Candida albicans virulence. *PLoS pathogens* 2018;**14**: e1007076.
Liu Y, Ou Y, Sun L *et al.* Alcohol dehydrogenase of Candida albicans triggers differentiation of THP-1 cells into macrophages. *Journal of advanced research* 2019;**18**: 137-45.
Lopez CM, Wallich R, Riesbeck K *et al.* Candida albicans uses the surface protein Gpm1 to attach to human endothelial cells and to keratinocytes via the adhesive protein vitronectin. *PloS one* 2014;**9**: e90796.
Lother J, Breitschopf T, Krappmann S *et al.* Human dendritic cell subsets display distinct interactions with the pathogenic mould Aspergillus fumigatus. *International journal of medical microbiology : IJMM* 2014;**304**: 1160-8.
Loureiro A, Pais C, Sampaio P. Relevance of Macrophage Extracellular Traps in C. albicans Killing. *Frontiers in immunology* 2019;**10**: 2767.
Ma LL, Wang CL, Neely GG *et al.* NK cells use perforin rather than granulysin for anticryptococcal activity. *Journal of immunology (Baltimore, Md : 1950)* 2004;**173**: 3357-65.
Maher CO, Dunne K, Comerford R *et al.* Candida albicans stimulates IL-23 release by human dendritic cells and downstream IL-17 secretion by Vdelta1 T cells. *Journal of immunology (Baltimore, Md : 1950)* 2015;**194**: 5953-60.
Marischen L, Englert A, Schmitt AL *et al.* Human NK cells adapt their immune response towards increasing multiplicities of infection of Aspergillus fumigatus. *BMC immunology* 2018;**19**: 39.
Marolda A, Hünniger K, Böttcher S *et al.* Candida species-dependent release of IL-12 by dendritic cells induces different levels of NK cell stimulation. *The Journal of infectious diseases* 2020, DOI 10.1093/infdis/jiaa035.
Mattern DJ, Schoeler H, Weber J *et al.* Identification of the antiphagocytic trypacidin gene cluster in the human-pathogenic fungus Aspergillus fumigatus. *Applied microbiology and biotechnology* 2015;**99**: 10151-61.
Miramón P, Dunker C, Windecker H *et al.* Cellular responses of Candida albicans to phagocytosis and the extracellular activities of neutrophils are critical to counteract carbohydrate starvation, oxidative and nitrosative stress. *PloS one* 2012;**7**: e52850.
Mora DJ, Ferreira-Paim K, Andrade-Silva LE *et al.* Cytokine patterns in a prospective cohort of HIV-infected patients with cryptococcal meningitis following initiation of antifungal and antiretroviral therapy. *PloS one* 2017;**12**: e0176304.
Munawara U, Small AG, Quach A *et al.* Cytokines regulate complement receptor immunoglobulin expression and phagocytosis of Candida albicans in human macrophages: A control point in anti-microbial immunity. *Scientific reports* 2017;**7**: 4050.

Muñoz JF, Delorey T, Ford CB *et al.* Coordinated host-pathogen transcriptional dynamics revealed using sorted subpopulations and single macrophages infected with Candida albicans. *Nature communications* 2019;**10**: 1607.
Negoro PE, Xu S, Dagher Z *et al.* Spleen Tyrosine Kinase Is a Critical Regulator of Neutrophil Responses to Candida Species. *mBio* 2020;**11**.
Nguyen TNY, Matangkasombut O, Ritprajak P. Differential dendritic cell responses to cell wall mannan of Candida albicans, Candida parapsilosis, and Candida dubliniensis. *Journal of oral science* 2018;**60**: 557-66.
Niemiec MJ, Grumaz C, Ermert D *et al.* Dual transcriptome of the immediate neutrophil and Candida albicans interplay. *BMC genomics* 2017;**18**: 696.
O'Meara TR, Duah K, Guo CX *et al.* High-Throughput Screening Identifies Genes Required for Candida albicans Induction of Macrophage Pyroptosis. *mBio* 2018;**9**.
Oesterreicher Z, Eberl S, Zeitlinger M. Impact of different antimycotics on cytokine levels in an in vitro aspergillosis model in human whole blood. *Infection* 2019, DOI 10.1007/s15010-019-01346-x.
Okai B, Lyall N, Gow NA *et al.* Rab14 regulates maturation of macrophage phagosomes containing the fungal pathogen Candida albicans and outcome of the host-pathogen interaction. *Infection and immunity* 2015;**83**: 1523-35.
Oya E, Solhaug A, Bolling AK *et al.* Pro-inflammatory responses induced by A. fumigatus and A. versicolor in various human macrophage models. *Journal of toxicology and environmental health Part A* 2019;**82**: 483-501.
Page L, Weis P, Muller T *et al.* Evaluation of Aspergillus and Mucorales specific T-cells and peripheral blood mononuclear cell cytokine signatures as biomarkers of environmental mold exposure. *International journal of medical microbiology : IJMM* 2018;**308**: 1018-26.
Pereira PAT, Assis PA, Prado MKB *et al.* Prostaglandins D2 and E2 have opposite effects on alveolar macrophages infected with Histoplasma capsulatum. *Journal of lipid research* 2018;**59**: 195-206.
Pietrella D, Corbucci C, Perito S *et al.* Mannoproteins from Cryptococcus neoformans promote dendritic cell maturation and activation. *Infection and immunity* 2005;**73**: 820-7.
Rieber N, Gazendam RP, Freeman AF *et al.* Extrapulmonary Aspergillus infection in patients with CARD9 deficiency. *JCI Insight* 2016;**1**: e89890.
Rocha JD, Nascimento MT, Decote-Ricardo D *et al.* Capsular polysaccharides from Cryptococcus neoformans modulate production of neutrophil extracellular traps (NETs) by human neutrophils. *Scientific reports* 2015;**5**: 8008.
Röhm M, Grimm MJ, D'Auria AC *et al.* NADPH oxidase promotes neutrophil extracellular trap formation in pulmonary aspergillosis. *Infection and immunity* 2014;**82**: 1766-77.
Roudbary M, Roudbar Mohammadi S, Bozorgmehr M *et al.* The effects of Candida albicans cell wall protein fraction on dendritic cell maturation. *Iran J Immunol* 2009;**6**: 67-74.
Rubino I, Coste A, Le Roy D *et al.* Species-specific recognition of Aspergillus fumigatus by Toll-like receptor 1 and Toll-like receptor 6. *The Journal of infectious diseases* 2012;**205**: 944-54.
Rudkin FM, Raziunaite I, Workman H *et al.* Single human B cell-derived monoclonal anti-Candida antibodies enhance phagocytosis and protect against disseminated candidiasis. *Nature communications* 2018;**9**: 5288.

Salvatori O, Pathirana RU, Kay JG *et al.* Candida albicans Ras1 Inactivation Increases Resistance to Phagosomal Killing by Human Neutrophils. *Infection and immunity* 2018;**86**.

Santiago V, Rezvani K, Sekine T *et al.* Human NK Cells Develop an Exhaustion Phenotype During Polar Degranulation at the Aspergillus fumigatus Hyphal Synapse. *Frontiers in immunology* 2018;**9**: 2344.

Schmidt H, Vlaic S, Kruger T *et al.* Proteomics of Aspergillus fumigatus Conidia-containing Phagolysosomes Identifies Processes Governing Immune Evasion. *Molecular & cellular proteomics : MCP* 2018;**17**: 1084-96.

Scott AJ, Woods JP. Monitoring internalization of Histoplasma capsulatum by mammalian cell lines using a fluorometric microplate assay. *Medical mycology* 2000;**38**: 15-22.

Shen Q, Beucler MJ, Ray SC *et al.* Macrophage activation by IFN-gamma triggers restriction of phagosomal copper from intracellular pathogens. *PLoS pathogens* 2018;**14**: e1007444.

Shimamura S, Miyazaki T, Tashiro M *et al.* Autophagy-Inducing Factor Atg1 Is Required for Virulence in the Pathogenic Fungus Candida glabrata. *Frontiers in microbiology* 2019;**10**: 27.

Siddiqui AA, Shattock RJ, Harrison TS. Role of capsule and interleukin-6 in long-term immune control of Cryptococcus neoformans infection by specifically activated human peripheral blood mononuclear cells. *Infection and immunity* 2006;**74**: 5302-10.

Silva JC, Rodrigues NC, Thompson-Souza GA *et al.* Mac-1 triggers neutrophil DNA extracellular trap formation to Aspergillus fumigatus independently of PAD4 histone citrullination. *Journal of leukocyte biology* 2019, DOI 10.1002/jlb.4a0119-009rr.

Smith LM, Dixon EF, May RC. The fungal pathogen Cryptococcus neoformans manipulates macrophage phagosome maturation. *Cellular microbiology* 2015;**17**: 702-13.

Srivastava M, Bencurova E, Gupta SK *et al.* Aspergillus fumigatus Challenged by Human Dendritic Cells: Metabolic and Regulatory Pathway Responses Testify a Tight Battle. *Frontiers in cellular and infection microbiology* 2019;**9**: 168.

Stephen-Victor E, Karnam A, Fontaine T *et al.* Aspergillus fumigatus Cell Wall α-(1,3)-Glucan Stimulates Regulatory T-Cell Polarization by Inducing PD-L1 Expression on Human Dendritic Cells. *The Journal of infectious diseases* 2017;**216**: 1281-94.

Stukes S, Casadevall A. Visualizing non-lytic exocytosis of Cryptococcus neoformans from macrophages using digital light microscopy. *Journal of visualized experiments : JoVE* 2014, DOI 10.3791/52084: e52084.

Sun D, Zhang M, Liu G *et al.* Real-Time Imaging of Interactions of Neutrophils with Cryptococcus neoformans Demonstrates a Crucial Role of Complement C5a-C5aR Signaling. *Infection and immunity* 2016;**84**: 216-29.

Sun H, Xu XY, Tian XL *et al.* Activation of NF-κB and respiratory burst following Aspergillus fumigatus stimulation of macrophages. *Immunobiology* 2014;**219**: 25-36.

Tagliari L, Toledo MS, Lacerda TG *et al.* Membrane microdomain components of Histoplasma capsulatum yeast forms, and their role in alveolar macrophage infectivity. *Biochimica et biophysica acta* 2012;**1818**: 458-66.

Thompson-Souza GA, Santos GMP, Silva JC *et al.* Histoplasma capsulatum-induced extracellular DNA trap release in human neutrophils. *Cellular microbiology* 2020, DOI 10.1111/cmi.13195: e13195.

Thompson A, Griffiths JS, Walker L *et al.* Dependence on Dectin-1 Varies With Multiple Candida Species. *Frontiers in microbiology* 2019;**10**: 1800.

Urban CF, Reichard U, Brinkmann V *et al.* Neutrophil extracellular traps capture and kill Candida albicans yeast and hyphal forms. *Cellular microbiology* 2006;**8**: 668-76.

van der Does AM, Joosten SA, Vroomans E *et al.* The antimicrobial peptide hLF1-11 drives monocyte-dendritic cell differentiation toward dendritic cells that promote antifungal responses and enhance Th17 polarization. *J Innate Immun* 2012;**4**: 284-92.

Van Prooyen N, Henderson CA, Hocking Murray D *et al.* CD103+ Conventional Dendritic Cells Are Critical for TLR7/9-Dependent Host Defense against Histoplasma capsulatum, an Endemic Fungal Pathogen of Humans. *PLoS pathogens* 2016;**12**: e1005749.

Vaz C, Reales-Calderon JA, Pitarch A *et al.* Enrichment of ATP Binding Proteins Unveils Proteomic Alterations in Human Macrophage Cell Death, Inflammatory Response, and Protein Synthesis after Interaction with Candida albicans. *Journal of proteome research* 2019;**18**: 2139-59.

Veloso Júnior PHH, Simon KS, de Castro RJA *et al.* Peptides ToAP3 and ToAP4 decrease release of inflammatory cytokines through TLR-4 blocking. *Biomed Pharmacother* 2019;**118**: 109152.

Vivas W, Leonhardt I, Hünniger K *et al.* Multiple Signaling Pathways Involved in Human Dendritic Cell Maturation Are Affected by the Fungal Quorum-Sensing Molecule Farnesol. *J Immunol* 2019;**203**: 2959-69.

Voigt J, Hunniger K, Bouzani M *et al.* Human natural killer cells acting as phagocytes against Candida albicans and mounting an inflammatory response that modulates neutrophil antifungal activity. *The Journal of infectious diseases* 2014;**209**: 616-26.

Walsh NM, Wuthrich M, Wang H *et al.* Characterization of C-type lectins reveals an unexpectedly limited interaction between Cryptococcus neoformans spores and Dectin-1. *PloS one* 2017;**12**: e0173866.

Wang W, Deng Z, Wu H *et al.* A small secreted protein triggers a TLR2/4-dependent inflammatory response during invasive Candida albicans infection. *Nature communications* 2019;**10**: 1015.

Weiss E, Ziegler S, Fliesser M *et al.* First Insights in NK-DC Cross-Talk and the Importance of Soluble Factors During Infection With Aspergillus fumigatus. *Frontiers in cellular and infection microbiology* 2018;**8**: 288.

Wellington M, Dolan K, Krysan DJ. Live Candida albicans suppresses production of reactive oxygen species in phagocytes. *Infection and immunity* 2009;**77**: 405-13.

Wiseman JC, Ma LL, Marr KJ *et al.* Perforin-dependent cryptococcal microbicidal activity in NK cells requires PI3K-dependent ERK1/2 signaling. *Journal of immunology (Baltimore, Md : 1950)* 2007;**178**: 6456-64.

Wolf JE, Kerchberger V, Kobayashi GS *et al.* Modulation of the macrophage oxidative burst by Histoplasma capsulatum. *J Immunol* 1987;**138**: 582-6.

Wu SY, Weng CL, Jheng MJ *et al.* Candida albicans triggers NADPH oxidase-independent neutrophil extracellular traps through dectin-2. *PLoS pathogens* 2019a;**15**: e1008096.

Wu Y, Du S, Johnson JL *et al.* Microglia and amyloid precursor protein coordinate control of transient Candida cerebritis with memory deficits. *Nature communications* 2019b;**10**: 58.

Wu Y, Xu H, Li L *et al.* Susceptibility to Aspergillus Infections in Rats with Chronic Obstructive Pulmonary Disease via Deficiency Function of Alveolar Macrophages and Impaired Activation of TLR2. *Inflammation* 2016;**39**: 1310-8.
Youseff BH, Holbrook ED, Smolnycki KA *et al.* Extracellular superoxide dismutase protects Histoplasma yeast cells from host-derived oxidative stress. *PLoS pathogens* 2012;**8**: e1002713.
Zawrotniak M, Wojtalik K, Rapala-Kozik M. Farnesol, a Quorum-Sensing Molecule of Candida Albicans Triggers the Release of Neutrophil Extracellular Traps. *Cells* 2019;**8**.
Zhang P, Xin X, Fang L *et al.* HMGB1 mediates Aspergillus fumigatus-induced inflammatory response in alveolar macrophages of COPD mice via activating MyD88/NF-kappaB and syk/PI3K signalings. *International immunopharmacology* 2017;**53**: 125-32.

Table S2: Overview about the different lung *in vitro* models, their structure, possible read outs and applications for the different fungi

Model	Structure	Readout	Reference
Alveolar epithelial cells A549 (Human cell line)	Monoculture	Adhesion	*A. fumigatus*: (Gravelat *et al.* 2010; Xu *et al.* 2012) *C. neoformans*: (Choo *et al.* 2015; Ganendren *et al.* 2006; Teixeira *et al.* 2014) *H. capsulatum*: (Pitangui *et al.* 2012)
		Endocytosis	*A. fumigatus:* (Amin *et al.* 2014; Liu *et al.* 2016; Xu *et al.* 2012)
		Damage	*A. fumigatus:* (Bertuzzi *et al.* 2014; Dasari *et al.* 2019; Ejzykowicz *et al.* 2010)
		Cell Detachment	*A. fumigatus:* (Bertuzzi *et al.* 2014; Kogan *et al.* 2004)
		Transcriptomics	*A. fumigatus:* (Jepsen *et al.* 2018; Takahashi-Nakaguchi *et al.* 2018; Watkins *et al.* 2018)
		Proteomics	*A. fumigatus:* (Margalit *et al.* 2020; Voltersen *et al.* 2018)
		Metabolomics	*C. neoformans*: (Liew *et al.* 2016)
		Cytokine release	*C. neoformans:* (Barbosa *et al.* 2007) *H. capsulatum*: (Alcantara *et al.* 2020; Maza and Suzuki 2016)
	Monoculture	Cytokine release	*A. fumigatus*: (Sun *et al.* 2012)

Human bronchial epithelial or small airway epithelial Cells HBE, SAE (Human primary cells)			*C. neoformans:* (Guillot *et al.* 2008)
	Monoculture differentiated in an ALI	Transcriptomics Proteomics	*A. fumigatus*: (Toor *et al.* 2018)
A549 (Human cell line) **+ DCs** (Human primary differentiated cells)	Co-culture on Transwell (top: A549 + DCs)	Damage Membrane integrity Cytokine release	*A. fumigatus*: (Morton *et al.* 2018)
Human pulmonary artery endothelial cells HPAECs (Human cell line) **+ A549** (Human cell line)	Co-culture on Transwell (top: A549 bottom: HPAECs)	Microscopy Kinetics	*A. fumigatus*: (Hope *et al.* 2007)
		Cytokine release	*A. fumigatus*: (Belic *et al.* 2018)
HBE, SAE (Human primary cells) **+ DCs** (Human primary differentiated cells) **+ Macrophages** (Human primary cells)	Co-culture differentiated in an ALI	Cytokine release	*A. fumigatus*: (Chandorkar *et al.* 2017)
HPAECs (Human cell line) **+ A549** (Human cell line) **+ monocyte-derived DCs and myeloid DCs** (Human primary differentiated cells)	Co-culture on Transwell (top: A549 + DCs bottom: HPAECs)	Transcriptomics	*A. fumigatus*: (Morton *et al.* 2014)
Lung-on-chip model alveolar epithelium **+**	Bilayer with ALI on one side and flow on the other side	Model establishment	(Deinhardt-Emmer et al. 2020)

microvascular endothelium			

References Tab. S2:

Alcantara C, Almeida BR, Barros B *et al.* Histoplasma capsulatum chemotypes I and II induce IL-8 secretion in lung epithelial cells in distinct manners. *Medical mycology* 2020, DOI 10.1093/mmy/myaa006.

Amin S, Thywissen A, Heinekamp T *et al.* Melanin dependent survival of Apergillus fumigatus conidia in lung epithelial cells. *International journal of medical microbiology : IJMM* 2014;**304**: 626-36.

Barbosa FM, Fonseca FL, Figueiredo RT *et al.* Binding of glucuronoxylomannan to the CD14 receptor in human A549 alveolar cells induces interleukin-8 production. *Clinical and vaccine immunology : CVI* 2007;**14**: 94-8.

Belic S, Page L, Lazariotou M *et al.* Comparative Analysis of Inflammatory Cytokine Release and Alveolar Epithelial Barrier Invasion in a Transwell((R)) Bilayer Model of Mucormycosis. *Frontiers in microbiology* 2018;**9**: 3204.

Bertuzzi M, Schrettl M, Alcazar-Fuoli L *et al.* The pH-responsive PacC transcription factor of Aspergillus fumigatus governs epithelial entry and tissue invasion during pulmonary aspergillosis. *PLoS pathogens* 2014;**10**: e1004413.

Chandorkar P, Posch W, Zaderer V *et al.* Fast-track development of an in vitro 3D lung/immune cell model to study Aspergillus infections. *Scientific reports* 2017;**7**: 11644.

Choo KK, Chong PP, Ho AS *et al.* The role of host microfilaments and microtubules during opsonin-independent interactions of Cryptococcus neoformans with mammalian lung cells. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2015;**34**: 2421-7.

Dasari P, Koleci N, Shopova IA *et al.* Enolase From Aspergillus fumigatus Is a Moonlighting Protein That Binds the Human Plasma Complement Proteins Factor H, FHL-1, C4BP, and Plasminogen. *Frontiers in immunology* 2019;**10**: 2573.

Deinhardt-Emmer S, Rennert K, Schicke E *et al.* Co-infection with Staphylococcus aureus after primary influenza virus infection leads to damage of the endothelium in a human alveolus-on-a-chip model. *Biofabrication* 2020;**12**: 025012.

Ejzykowicz DE, Solis NV, Gravelat FN *et al.* Role of Aspergillus fumigatus DvrA in host cell interactions and virulence. *Eukaryotic cell* 2010;**9**: 1432-40.

Ganendren R, Carter E, Sorrell T *et al.* Phospholipase B activity enhances adhesion of Cryptococcus neoformans to a human lung epithelial cell line. *Microbes and infection* 2006;**8**: 1006-15.

Gravelat FN, Ejzykowicz DE, Chiang LY *et al.* Aspergillus fumigatus MedA governs adherence, host cell interactions and virulence. *Cellular microbiology* 2010;**12**: 473-88.

Guillot L, Carroll SF, Badawy M *et al.* Cryptococcus neoformans induces IL-8 secretion and CXCL1 expression by human bronchial epithelial cells. *Respir Res* 2008;**9**: 9.

Hope WW, Kruhlak MJ, Lyman CA *et al.* Pathogenesis of Aspergillus fumigatus and the kinetics of galactomannan in an in vitro model of early invasive pulmonary

aspergillosis: implications for antifungal therapy. *The Journal of infectious diseases* 2007;**195**: 455-66.

Jepsen CS, Dubey LK, Colmorten KB *et al.* FIBCD1 Binds Aspergillus fumigatus and Regulates Lung Epithelial Response to Cell Wall Components. *Frontiers in immunology* 2018;**9**: 1967.

Kogan TV, Jadoun J, Mittelman L *et al.* Involvement of secreted Aspergillus fumigatus proteases in disruption of the actin fiber cytoskeleton and loss of focal adhesion sites in infected A549 lung pneumocytes. *The Journal of infectious diseases* 2004;**189**: 1965-73.

Liew KL, Jee JM, Yap I *et al.* In Vitro Analysis of Metabolites Secreted during Infection of Lung Epithelial Cells by Cryptococcus neoformans. *PloS one* 2016;**11**: e0153356.

Liu H, Lee MJ, Solis NV *et al.* Aspergillus fumigatus CalA binds to integrin alpha5beta1 and mediates host cell invasion. *Nature microbiology* 2016;**2**: 16211.

Margalit A, Kavanagh K, Carolan JC. Characterization of the Proteomic Response of A549 Cells Following Sequential Exposure to Aspergillus fumigatus and Pseudomonas aeruginosa. *Journal of proteome research* 2020;**19**: 279-91.

Maza PK, Suzuki E. Histoplasma capsulatum-Induced Cytokine Secretion in Lung Epithelial Cells Is Dependent on Host Integrins, Src-Family Kinase Activation, and Membrane Raft Recruitment. *Frontiers in microbiology* 2016;**7**: 580.

Morton CO, Fliesser M, Dittrich M *et al.* Gene expression profiles of human dendritic cells interacting with Aspergillus fumigatus in a bilayer model of the alveolar epithelium/endothelium interface. *PloS one* 2014;**9**: e98279.

Morton CO, Wurster S, Fliesser M *et al.* Validation of a simplified in vitro Transwell((R)) model of the alveolar surface to assess host immunity induced by different morphotypes of Aspergillus fumigatus. *International journal of medical microbiology : IJMM* 2018;**308**: 1009-17.

Pitangui NS, Sardi JC, Silva JF *et al.* Adhesion of Histoplasma capsulatum to pneumocytes and biofilm formation on an abiotic surface. *Biofouling* 2012;**28**: 711-8.

Sun WK, Lu X, Li X *et al.* Dectin-1 is inducible and plays a crucial role in Aspergillus-induced innate immune responses in human bronchial epithelial cells. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2012;**31**: 2755-64.

Takahashi-Nakaguchi A, Sakai K, Takahashi H *et al.* Aspergillus fumigatus adhesion factors in dormant conidia revealed through comparative phenotypic and transcriptomic analyses. *Cellular microbiology* 2018;**20**.

Teixeira PA, Penha LL, Mendonca-Previato L *et al.* Mannoprotein MP84 mediates the adhesion of Cryptococcus neoformans to epithelial lung cells. *Frontiers in cellular and infection microbiology* 2014;**4**: 106.

Toor A, Culibrk L, Singhera GK *et al.* Transcriptomic and proteomic host response to Aspergillus fumigatus conidia in an air-liquid interface model of human bronchial epithelium. *PloS one* 2018;**13**: e0209652.

Voltersen V, Blango MG, Herrmann S *et al.* Proteome Analysis Reveals the Conidial Surface Protein CcpA Essential for Virulence of the Pathogenic Fungus Aspergillus fumigatus. *mBio* 2018;**9**.

Watkins TN, Liu H, Chung M *et al.* Comparative transcriptomics of Aspergillus fumigatus strains upon exposure to human airway epithelial cells. *Microbial genomics* 2018;**4**.

Xu XY, Shi Y, Zhang PP *et al.* E-cadherin mediates adhesion and endocytosis of Aspergillus fumigatus blastospores in human epithelial cells. *Chinese medical journal* 2012;**125**: 617-21.

Table S3: Overview about the different *in vitro* models for the gastrointestinal tract and vaginal tract, their structure, possible read outs and applications for *Candida* spp.

Model	Structure	Readout	Reference
A) Oral cavity			
Keratinocyte cells TR146 (Human cell line)	Monoculture	Invasion	*C. albicans:* (Puri *et al.* 2019)
		Damage	*C. albicans:* (Wilson *et al.* 2014)
		Transcriptomics	*C. albicans:* (McCall *et al.* 2018; Schaller *et al.* 1998)
		Cytokine release	*C. albicans:* (Ho *et al.* 2019; Verma *et al.* 2017; Verma *et al.* 2018)
Tongue cells SCC15 (Human cell line)	Monoculture	Invasion	*C. albicans:* (Villar et al. 2007)
		Damage	*C. albicans:* (Kumar et al. 2015)
		Cytokine release	*C. albicans:* (Dongari-Bagtzoglou and Kashleva 2003)
Immortalized oral mucosal cells OKF6/TERT-2 (Human cell line)	Monoculture	Invasion	*C. albicans:* (Solis et al. 2017; Swidergall et al. 2018; Zhu et al. 2012)
		Damage	*C. albicans:* (Liu et al. 2014; Solis et al. 2018)
		Transcriptomics	*C. albicans:* (Liu *et al.* 2015)
		Live cell imaging	*C. albicans:* (Wollert *et al.* 2012)
Human palate epithelial cells HPECs (Human primary cells)	Monoculture	Apoptosis	*C. albicans:* (Casaroto *et al.* 2019)
		Host gene expression	*C. albicans:* (Offenbacher *et al.* 2019)
TR146 (Human cell line) **+ Fibroblasts** (Human primary cells)	Co-culture	Adhesion Gene expression	*C. albicans:* (Morse *et al.* 2018)

Reconstituted human oral epithelium RHOE (cell line based)	3D structure Multiculture model with differentiated cells	Damage	*C. albicans* / *C. glabrata:* (Silva *et al.* 2011) *C. albicans:* (Cavalcanti *et al.* 2015; Mailander-Sanchez *et al.* 2017)
		Transcriptomics	*C. albicans:* (Nailis *et al.* 2010; Spiering *et al.* 2010)
		Cytokine release	*C. albicans:* (Wagener *et al.* 2012)
RHOE + Fibroblasts (Human primary cells)	3D structure Multiculture model with differentiated cells	Bacterial interactions	*C. albicans:* (Bertolini *et al.* 2019; de Carvalho Dias *et al.* 2018; Diaz *et al.* 2012)
		Biofilm formation	*C. albicans:* (Sobue *et al.* 2018)
Oral mucosa-on-chip Keratinocytes Gie-No3B11 (Human cell line) **+ gingival fibroblasts** (Human cell line)	Collagen embedded fibroblast and Keratinocytes on a porous membrane under flow	Model establishment	(Rahimi *et al.* 2018)
B) Intestinal tract			
Colorectal adenocarnima cells Caco-2 (Human cell line)	Monoculture	Damage	*C. albicans:* (Wachtler *et al.* 2011a)
		Invasion	*C. albicans:* (Goyer *et al.* 2016; Wachtler *et al.* 2011a)
		Interactions antagonistic yeasts	*C. albicans*: (Lohith and Anu-Appaiah 2018; Murzyn *et al.* 2010) *C. glabrata*: (Kunyeit *et al.* 2019)
		Cytokine release	*C. albicans:* (Gacser *et al.* 2014; Mao *et al.* 2019; Schirbel *et al.* 2018)
Colorectal adenocarnimo cells HT-29 (Human cell line)	Monoculture	Adhesion Invasion Damage	*C. albicans:* (Deng *et al.* 2015; Garcia *et al.* 2018)
Mucus secreting goblet cells HT-29-MTX (Human cell line)	Monoculture	Gene expression	*C. albicans:* (Kavanaugh *et al.* 2014)
		Translocation	*C. albicans:* (Allert *et al.* 2018)

Subclone of Caco-2 C2BBe1 (Human cell line)	Monoculture in transwell	Transcriptomics	*C. albicans:* (Bohringer *et al.* 2016)
Caco-2 (Human cell line) **+ Raji B cells** (Human cell line)	Co-culture	Adhesion Invasion	*C. albicans:* (Albac *et al.* 2016)
C2BBe1 (Human cell line) **+ HT-29-MTX** (Human cell line)	Co-culture	Adhesion Damage	*C. albicans:* (Graf *et al.* 2019)
Intestine-on-Chip C2BBe1 (Human cell line) **+ HUVECs** (Human primary cells) **+ Monocytes** (Human primary cells)	Bilayer under flow (top: C2BBe1 Bottom: HUVECs + Monocytes)	Damage Translocation	*C. albicans:* (Maurer *et al.* 2019)
C) Vaginal tract			
Human immortalized vaginal mucosal cells VK2/E6E7 (Human cell line)	Monoculture	Adhesion	*C. albicans:* (Luan *et al.* 2020; Mikamo *et al.* 2018)
		Bacterial interactions	*C. albicans:* (Pidwill *et al.* 2018)
		Autophagy	*C. albicans:* (Shroff and Reddy 2018)
		Cytokine release	*C. albicans:* (Li *et al.* 2017)
Vaginal epidermoid carcinoma cells A431 (Human cell line)	Monoculture	Damage	*C. albicans:* (Wachtler *et al.* 2011b)
		Cytokine release	*C. albicans:* (Richardson *et al.* 2018)
Reconstituted vaginal epithelium RHVE (cell line based)	3D structure Multiculture model with differentiated cells	Invasion Damage	*C. albicans:* (Alves *et al.* 2014)
		Transcriptomics	*C. glabrata:* (Bernardo *et al.* 2017)
		Cytokine release	*C. albicans:* (Schaller *et al.* 2005)

References Tab. S3:

Albac S, Schmitz A, Lopez-Alayon C *et al.* Candida albicans is able to use M cells as a portal of entry across the intestinal barrier in vitro. *Cell Microbiol* 2016;**18**: 195-210.

Allert S, Forster TM, Svensson CM *et al.* Candida albicans-Induced Epithelial Damage Mediates Translocation through Intestinal Barriers. *MBio* 2018;**9**.

Alves CT, Wei XQ, Silva S *et al.* Candida albicans promotes invasion and colonisation of Candida glabrata in a reconstituted human vaginal epithelium. *The Journal of infection* 2014;**69**: 396-407.

Bernardo RT, Cunha DV, Wang C *et al.* The CgHaa1-Regulon Mediates Response and Tolerance to Acetic Acid Stress in the Human Pathogen Candida glabrata. *G3 (Bethesda, Md)* 2017;**7**: 1-18.

Bertolini M, Ranjan A, Thompson A *et al.* Candida albicans induces mucosal bacterial dysbiosis that promotes invasive infection. *PLoS Pathog* 2019;**15**: e1007717.

Bohringer M, Pohlers S, Schulze S *et al.* Candida albicans infection leads to barrier breakdown and a MAPK/NF-kappaB mediated stress response in the intestinal epithelial cell line C2BBe1. *Cellular microbiology* 2016;**18**: 889-904.

Casaroto AR, da Silva RA, Salmeron S *et al.* Candida albicans-Cell Interactions Activate Innate Immune Defense in Human Palate Epithelial Primary Cells via Nitric Oxide (NO) and beta-Defensin 2 (hBD-2). *Cells* 2019;**8**.

Cavalcanti YW, Morse DJ, da Silva WJ *et al.* Virulence and pathogenicity of Candida albicans is enhanced in biofilms containing oral bacteria. *Biofouling* 2015;**31**: 27-38.

de Carvalho Dias K, de Sousa DL, Barbugli PA *et al.* Development and characterization of a 3D oral mucosa model as a tool for host-pathogen interactions. *Journal of microbiological methods* 2018;**152**: 52-60.

Deng K, Chen T, Wu Q *et al.* In vitro and in vivo examination of anticolonization of pathogens by Lactobacillus paracasei FJ861111.1. *Journal of dairy science* 2015;**98**: 6759-66.

Diaz PI, Xie Z, Sobue T *et al.* Synergistic interaction between Candida albicans and commensal oral streptococci in a novel in vitro mucosal model. *Infection and immunity* 2012;**80**: 620-32.

Dongari-Bagtzoglou A, Kashleva H. Candida albicans triggers interleukin-8 secretion by oral epithelial cells. *Microbial pathogenesis* 2003;**34**: 169-77.

Gacser A, Tiszlavicz Z, Nemeth T *et al.* Induction of human defensins by intestinal Caco-2 cells after interactions with opportunistic Candida species. *Microbes and infection* 2014;**16**: 80-5.

Garcia C, Burgain A, Chaillot J *et al.* A phenotypic small-molecule screen identifies halogenated salicylanilides as inhibitors of fungal morphogenesis, biofilm formation and host cell invasion. *Scientific reports* 2018;**8**: 11559.

Goyer M, Loiselet A, Bon F *et al.* Intestinal Cell Tight Junctions Limit Invasion of Candida albicans through Active Penetration and Endocytosis in the Early Stages of the Interaction of the Fungus with the Intestinal Barrier. *PloS one* 2016;**11**: e0149159.

Graf K, Last A, Gratz R *et al.* Keeping Candida commensal - How lactobacilli antagonize pathogenicity of Candida albicans in an in vitro gut model. *Disease models & mechanisms* 2019, DOI 10.1242/dmm.039719.

Ho J, Yang X, Nikou SA *et al.* Candidalysin activates innate epithelial immune responses via epidermal growth factor receptor. *Nature communications* 2019;**10**: 2297.

Kavanaugh NL, Zhang AQ, Nobile CJ *et al.* Mucins suppress virulence traits of Candida albicans. *mBio* 2014;**5**: e01911.

Kumar R, Saraswat D, Tati S *et al.* Novel Aggregation Properties of Candida albicans Secreted Aspartyl Proteinase Sap6 Mediate Virulence in Oral Candidiasis. *Infection and immunity* 2015;**83**: 2614-26.

Kunyeit L, Kurrey NK, Anu-Appaiah KA *et al.* Probiotic Yeasts Inhibit Virulence of Non-albicans Candida Species. *mBio* 2019;**10**.

Li T, Niu X, Zhang X *et al.* Recombinant Human IFNalpha-2b Response Promotes Vaginal Epithelial Cells Defense against Candida albicans. *Frontiers in microbiology* 2017;**8**: 697.

Liu Y, Shetty AC, Schwartz JA *et al.* New signaling pathways govern the host response to C. albicans infection in various niches. *Genome research* 2015;**25**: 679-89.

Liu Y, Solis NV, Heilmann CJ *et al.* Role of retrograde trafficking in stress response, host cell interactions, and virulence of Candida albicans. *Eukaryot Cell* 2014;**13**: 279-87.

Lohith K, Anu-Appaiah KA. Antagonistic effect of Saccharomyces cerevisiae KTP and Issatchenkia occidentalis ApC on hyphal development and adhesion of Candida albicans. *Medical mycology* 2018;**56**: 1023-32.

Luan T, Liu X, Mao P *et al.* The Role of 17β-Estrogen in Candida albicans Adhesion on Human Vaginal Epithelial Cells via FAK Phosphorylation. *Mycopathologia* 2020, DOI 10.1007/s11046-020-00440-1.

Mailander-Sanchez D, Braunsdorf C, Grumaz C *et al.* Antifungal defense of probiotic Lactobacillus rhamnosus GG is mediated by blocking adhesion and nutrient depletion. *PloS one* 2017;**12**: e0184438.

Mao X, Qiu X, Jiao C *et al.* Candida albicans SC5314 inhibits NLRP3/NLRP6 inflammasome expression and dampens human intestinal barrier activity in Caco-2 cell monolayer model. *Cytokine* 2019;**126**: 154882.

Maurer M, Gresnigt MS, Last A *et al.* A three-dimensional immunocompetent intestine-on-chip model as in vitro platform for functional and microbial interaction studies. *Biomaterials* 2019;**220**: 119396.

McCall AD, Kumar R, Edgerton M. Candida albicans Sfl1/Sfl2 regulatory network drives the formation of pathogenic microcolonies. *PLoS pathogens* 2018;**14**: e1007316-e.

Mikamo H, Yamagishi Y, Sugiyama H *et al.* High glucose-mediated overexpression of ICAM-1 in human vaginal epithelial cells increases adhesion of Candida albicans. *Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology* 2018;**38**: 226-30.

Morse DJ, Wilson MJ, Wei X *et al.* Denture-associated biofilm infection in three-dimensional oral mucosal tissue models. *Journal of medical microbiology* 2018;**67**: 364-75.

Murzyn A, Krasowska A, Augustyniak D *et al.* The effect of Saccharomyces boulardii on Candida albicans-infected human intestinal cell lines Caco-2 and Intestin 407. *FEMS microbiology letters* 2010;**310**: 17-23.

Nailis H, Kucharikova S, Ricicova M *et al.* Real-time PCR expression profiling of genes encoding potential virulence factors in Candida albicans biofilms: identification of model-dependent and -independent gene expression. *BMC microbiology* 2010;**10**: 114.

Offenbacher S, Barros SP, Bencharit S *et al.* Differential Mucosal Gene Expression Patterns in Candida-Associated, Chronic Oral Denture Stomatitis. *Journal of prosthodontics : official journal of the American College of Prosthodontists* 2019;**28**: 202-8.

Pidwill GR, Rego S, Jenkinson HF *et al.* Coassociation between Group B Streptococcus and Candida albicans Promotes Interactions with Vaginal Epithelium. *Infection and immunity* 2018;**86**.
Puri S, Kumar R, Rojas IG *et al.* Iron Chelator Deferasirox Reduces Candida albicans Invasion of Oral Epithelial Cells and Infection Levels in Murine Oropharyngeal Candidiasis. *Antimicrobial agents and chemotherapy* 2019;**63**.
Rahimi C, Rahimi B, Padova D *et al.* Oral mucosa-on-a-chip to assess layer-specific responses to bacteria and dental materials. *Biomicrofluidics* 2018;**12**: 054106.
Richardson JP, Willems HME, Moyes DL *et al.* Candidalysin Drives Epithelial Signaling, Neutrophil Recruitment, and Immunopathology at the Vaginal Mucosa. *Infection and immunity* 2018;**86**.
Schaller M, Korting HC, Borelli C *et al.* Candida albicans-secreted aspartic proteinases modify the epithelial cytokine response in an in vitro model of vaginal candidiasis. *Infection and immunity* 2005;**73**: 2758-65.
Schaller M, Schafer W, Korting HC *et al.* Differential expression of secreted aspartyl proteinases in a model of human oral candidosis and in patient samples from the oral cavity. *Molecular microbiology* 1998;**29**: 605-15.
Schirbel A, Shouval DS, Hebecker B *et al.* Intestinal epithelial cells and T cells differentially recognize and respond to Candida albicans yeast and hypha. *European journal of immunology* 2018;**48**: 1826-37.
Shroff A, Reddy KVR. Autophagy gene ATG5 knockdown upregulates apoptotic cell death during Candida albicans infection in human vaginal epithelial cells. *American journal of reproductive immunology (New York, NY : 1989)* 2018;**80**: e13056.
Silva S, Henriques M, Hayes A *et al.* Candida glabrata and Candida albicans co-infection of an in vitro oral epithelium. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology* 2011;**40**: 421-7.
Sobue T, Bertolini M, Thompson A *et al.* Chemotherapy-induced oral mucositis and associated infections in a novel organotypic model. *Molecular oral microbiology* 2018;**33**: 212-23.
Solis NV, Park YN, Swidergall M *et al.* Candida albicans White-Opaque Switching Influences Virulence but Not Mating during Oropharyngeal Candidiasis. *Infection and immunity* 2018;**86**.
Solis NV, Swidergall M, Bruno VM *et al.* The Aryl Hydrocarbon Receptor Governs Epithelial Cell Invasion during Oropharyngeal Candidiasis. *mBio* 2017;**8**.
Spiering MJ, Moran GP, Chauvel M *et al.* Comparative transcript profiling of Candida albicans and Candida dubliniensis identifies SFL2, a C. albicans gene required for virulence in a reconstituted epithelial infection model. *Eukaryotic cell* 2010;**9**: 251-65.
Swidergall M, Solis NV, Lionakis MS *et al.* EphA2 is an epithelial cell pattern recognition receptor for fungal β-glucans. *Nature microbiology* 2018;**3**: 53-61.
Verma AH, Richardson JP, Zhou C *et al.* Oral epithelial cells orchestrate innate type 17 responses to Candida albicans through the virulence factor candidalysin. *Science immunology* 2017;**2**.
Verma AH, Zafar H, Ponde NO *et al.* IL-36 and IL-1/IL-17 Drive Immunity to Oral Candidiasis via Parallel Mechanisms. *J Immunol* 2018;**201**: 627-34.
Villar CC, Kashleva H, Nobile CJ *et al.* Mucosal tissue invasion by Candida albicans is associated with E-cadherin degradation, mediated by transcription factor Rim101p and protease Sap5p. *Infection and immunity* 2007;**75**: 2126-35.

Wachtler B, Wilson D, Haedicke K *et al.* From attachment to damage: defined genes of Candida albicans mediate adhesion, invasion and damage during interaction with oral epithelial cells. *PloS one* 2011a;**6**: e17046.
Wachtler B, Wilson D, Hube B. Candida albicans adhesion to and invasion and damage of vaginal epithelial cells: stage-specific inhibition by clotrimazole and bifonazole. *Antimicrobial agents and chemotherapy* 2011b;**55**: 4436-9.
Wagener J, Mailander-Sanchez D, Schaller M. Immune responses to Candida albicans in models of in vitro reconstituted human oral epithelium. *Methods Mol Biol* 2012;**845**: 333-44.
Wilson D, Mayer FL, Miramon P *et al.* Distinct roles of Candida albicans-specific genes in host-pathogen interactions. *Eukaryot Cell* 2014;**13**: 977-89.
Wollert T, Rollenhagen C, Langford GM *et al.* Human oral keratinocytes: a model system to analyze host-pathogen interactions. *Methods in molecular biology (Clifton, NJ)* 2012;**845**: 289-302.
Zhu W, Phan QT, Boontheung P *et al.* EGFR and HER2 receptor kinase signaling mediate epithelial cell invasion by Candida albicans during oropharyngeal infection. *Proceedings of the National Academy of Sciences of the United States of America* 2012;**109**: 14194-9.

Table S4: Overview about the different endothelial and blood-brain barrier *in vitro* models, their structure, possible read outs and applications for the different fungi

Model	Structure	Readout	Reference
Human umbilical vein endothelial cells HUVECs (Human primary cells)	Monoculture	Adhesion	*C. albicans:* (Citiulo *et al.* 2012; Lopez *et al.* 2014; Phan *et al.* 2013) *C. neoformans*: (Ibrahim *et al.* 1995)
		Invasion	*A. fumigatus:* (Liu et al. 2016) *C. albicans:* (Phan *et al.* 2013; Phan *et al.* 2005; Phan *et al.* 2007)
		Damage	*A. fumigatus:* (Ejzykowicz et al. 2010) *C. albicans:* (Rotrosen et al. 1985; Sanchez et al. 2004)
		Proteomics	*A. fumigatus:* (Neves *et al.* 2016; Zhang *et al.* 2017b) *C. neoformans*: (Wang *et al.* 2011)
		Transcriptomics	*C. albicans:* (Barker et al. 2008) *C. neoformans*: (Coenjaerts *et al.* 2006)
		Cytokine release	*A.fumigatus:* (Kamai *et al.* 2009; Neves *et al.* 2017) *C. albicans*: (Orozco *et al.* 2000)
	Monoculture under flow	Adhesion	*C. albicans:* (Wilson and Hube 2010)
HUVECs	Co-culture	Damage	*C. albicans:* (Edwards et al. 1987)

(Human primary cells) + **Neutrophils** (Human primary cells)			
Human brain microvascular endothelial cells HMBEC HCMEC/D3 (Human cell line)	Monoculture on Transwell	Adhesion Invasion	*C. albicans*: (Liu *et al.* 2011)
		TEER Permeability assay	*A. fumigatus*: (Patel *et al.* 2018) *C. neoformans:* (Stie and Fox 2012)
		Traversal	*C. neoformans:* (Aaron *et al.* 2018; Chang *et al.* 2004; Huang *et al.* 2011; Kim *et al.* 2012; Na Pombejra *et al.* 2018; Na Pombejra *et al.* 2017; Vu *et al.* 2013; Vu *et al.* 2009; Zhu *et al.* 2017)
		TEM	*C. neoformans*: (Chen *et al.* 2003)
		Transcriptomics	*C. neoformans*: (Lahiri *et al.* 2019)
HMBEC HCMEC/D3 (Human cell line) **+ THP-1** (Human cell line) **or Monocytes** (Human primary cells)	Co-culture on transwell	Model development	*C. neoformans*: (Santiago-Tirado *et al.* 2019)
		Traversal	*C. neoformans*: (He *et al.* 2016; Santiago-Tirado *et al.* 2017; Sorrell *et al.* 2016)
BBB -on-Chip HUVECs (Human primary cells)	Monoculture under flow	Model establishment	(Yeon *et al.* 2012)
BBB -on-Chip HCMEC/D3 (Human cell line)	Monoculture under flow	Model establishment	(Griep *et al.* 2013)
BBB -on-Chip HCMEC/D3 + astrocytes (Human primary cells) **+ pericytes**	Multiculture under flow	Model establishment	(Lee *et al.* 2020)

(Human primary cells) **+ fibrobasts** (Human primary cells)			

References Tab. S4:

Aaron PA, Jamklang M, Uhrig JP *et al.* The blood-brain barrier internalises Cryptococcus neoformans via the EphA2-tyrosine kinase receptor. *Cellular microbiology* 2018;**20**.

Barker KS, Park H, Phan QT *et al.* Transcriptome profile of the vascular endothelial cell response to Candida albicans. *The Journal of infectious diseases* 2008;**198**: 193-202.

Chang YC, Stins MF, McCaffery MJ *et al.* Cryptococcal yeast cells invade the central nervous system via transcellular penetration of the blood-brain barrier. *Infection and immunity* 2004;**72**: 4985-95.

Chen SHM, Stins MF, Huang SH *et al.* Cryptococcus neoformans induces alterations in the cytoskeleton of human brain microvascular endothelial cells. *Journal of medical microbiology* 2003;**52**: 961-70.

Citiulo F, Jacobsen ID, Miramon P *et al.* Candida albicans scavenges host zinc via Pra1 during endothelial invasion. *PLoS Pathog* 2012;**8**: e1002777.

Coenjaerts FE, Hoepelman AI, Scharringa J *et al.* The Skn7 response regulator of Cryptococcus neoformans is involved in oxidative stress signalling and augments intracellular survival in endothelium. *FEMS yeast research* 2006;**6**: 652-61.

Edwards JE, Jr., Rotrosen D, Fontaine JW *et al.* Neutrophil-mediated protection of cultured human vascular endothelial cells from damage by growing Candida albicans hyphae. *Blood* 1987;**69**: 1450-7.

Ejzykowicz DE, Solis NV, Gravelat FN *et al.* Role of Aspergillus fumigatus DvrA in host cell interactions and virulence. *Eukaryot Cell* 2010;**9**: 1432-40.

Griep LM, Wolbers F, de Wagenaar B *et al.* BBB on chip: microfluidic platform to mechanically and biochemically modulate blood-brain barrier function. *Biomed Microdevices* 2013;**15**: 145-50.

He X, Shi X, Puthiyakunnon S *et al.* CD44-mediated monocyte transmigration across Cryptococcus neoformans-infected brain microvascular endothelial cells is enhanced by HIV-1 gp41-I90 ectodomain. *J Biomed Sci* 2016;**23**: 28.

Huang SH, Long M, Wu CH *et al.* Invasion of Cryptococcus neoformans into human brain microvascular endothelial cells is mediated through the lipid rafts-endocytic pathway via the dual specificity tyrosine phosphorylation-regulated kinase 3 (DYRK3). *J Biol Chem* 2011;**286**: 34761-9.

Ibrahim AS, Filler SG, Alcouloumre MS *et al.* Adherence to and damage of endothelial cells by Cryptococcus neoformans in vitro: role of the capsule. *Infection and immunity* 1995;**63**: 4368-74.

Kamai Y, Lossinsky AS, Liu H *et al.* Polarized response of endothelial cells to invasion by Aspergillus fumigatus. *Cellular microbiology* 2009;**11**: 170-82.

Kim JC, Crary B, Chang YC *et al.* Cryptococcus neoformans activates RhoGTPase proteins followed by protein kinase C, focal adhesion kinase, and ezrin to promote traversal across the blood-brain barrier. *J Biol Chem* 2012;**287**: 36147-57.

Lahiri S, Banerjee A, Bhutda S *et al.* In vitro expression of vital virulent genes of clinical and environmental isolates of Cryptococcus neoformans/gattii in endothelial cells of human blood-brain barrier. *J Mycol Med* 2019;**29**: 239-44.
Lee S, Chung M, Lee SR *et al.* 3D brain angiogenesis model to reconstitute functional human blood-brain barrier in vitro. *Biotechnol Bioeng* 2020;**117**: 748-62.
Liu H, Lee MJ, Solis NV *et al.* Aspergillus fumigatus CalA binds to integrin alpha5beta1 and mediates host cell invasion. *Nature microbiology* 2016;**2**: 16211.
Liu Y, Mittal R, Solis NV *et al.* Mechanisms of Candida albicans trafficking to the brain. *PLoS pathogens* 2011;**7**: e1002305.
Lopez CM, Wallich R, Riesbeck K *et al.* Candida albicans uses the surface protein Gpm1 to attach to human endothelial cells and to keratinocytes via the adhesive protein vitronectin. *PloS one* 2014;**9**: e90796.
Na Pombejra S, Jamklang M, Uhrig JP *et al.* The structure-function analysis of the Mpr1 metalloprotease determinants of activity during migration of fungal cells across the blood-brain barrier. *PloS one* 2018;**13**: e0203020.
Na Pombejra S, Salemi M, Phinney BS *et al.* The Metalloprotease, Mpr1, Engages AnnexinA2 to Promote the Transcytosis of Fungal Cells across the Blood-Brain Barrier. *Frontiers in cellular and infection microbiology* 2017;**7**: 296.
Neves GW, Curty N, Kubitschek-Barreira PH *et al.* Dataset of differentially regulated proteins in HUVECs challenged with wild type and UGM1 mutant Aspergillus fumigatus strains. *Data in brief* 2016;**9**: 24-31.
Neves GW, Curty NA, Kubitschek-Barreira PH *et al.* Modifications to the composition of the hyphal outer layer of Aspergillus fumigatus modulates HUVEC proteins related to inflammatory and stress responses. *Journal of proteomics* 2017;**151**: 83-96.
Orozco AS, Zhou X, Filler SG. Mechanisms of the proinflammatory response of endothelial cells to Candida albicans infection. *Infection and immunity* 2000;**68**: 1134-41.
Patel R, Hossain MA, German N *et al.* Gliotoxin penetrates and impairs the integrity of the human blood-brain barrier in vitro. *Mycotoxin research* 2018;**34**: 257-68.
Phan QT, Eng DK, Mostowy S *et al.* Role of endothelial cell septin 7 in the endocytosis of Candida albicans. *mBio* 2013;**4**: e00542-13.
Phan QT, Fratti RA, Prasadarao NV *et al.* N-cadherin mediates endocytosis of Candida albicans by endothelial cells. *J Biol Chem* 2005;**280**: 10455-61.
Phan QT, Myers CL, Fu Y *et al.* Als3 is a Candida albicans invasin that binds to cadherins and induces endocytosis by host cells. *PLoS Biol* 2007;**5**: e64.
Rotrosen D, Edwards JE, Jr., Gibson TR *et al.* Adherence of Candida to cultured vascular endothelial cells: mechanisms of attachment and endothelial cell penetration. *The Journal of infectious diseases* 1985;**152**: 1264-74.
Sanchez AA, Johnston DA, Myers C *et al.* Relationship between Candida albicans virulence during experimental hematogenously disseminated infection and endothelial cell damage in vitro. *Infection and immunity* 2004;**72**: 598-601.
Santiago-Tirado FH, Klein RS, Doering TL. An In Vitro Brain Endothelial Model for Studies of Cryptococcal Transmigration into the Central Nervous System. *Current protocols in microbiology* 2019;**53**: e78.
Santiago-Tirado FH, Onken MD, Cooper JA *et al.* Trojan Horse Transit Contributes to Blood-Brain Barrier Crossing of a Eukaryotic Pathogen. *mBio* 2017;**8**.
Sorrell TC, Juillard PG, Djordjevic JT *et al.* Cryptococcal transmigration across a model brain blood-barrier: evidence of the Trojan horse mechanism and

differences between Cryptococcus neoformans var. grubii strain H99 and Cryptococcus gattii strain R265. *Microbes and infection* 2016;**18**: 57-67.

Stie J, Fox D. Blood-brain barrier invasion by Cryptococcus neoformans is enhanced by functional interactions with plasmin. *Microbiology (Reading, England)* 2012;**158**: 240-58.

Vu K, Eigenheer RA, Phinney BS *et al.* Cryptococcus neoformans promotes its transmigration into the central nervous system by inducing molecular and cellular changes in brain endothelial cells. *Infection and immunity* 2013;**81**: 3139-47.

Vu K, Weksler B, Romero I *et al.* Immortalized human brain endothelial cell line HCMEC/D3 as a model of the blood-brain barrier facilitates in vitro studies of central nervous system infection by Cryptococcus neoformans. *Eukaryotic cell* 2009;**8**: 1803-7.

Wang XJ, Zhu YJ, Cui JG *et al.* Proteomic analysis of human umbilical vein endothelial cells incubated with Cryptococcus neoformans var. neoformans. *Mycoses* 2011;**54**: e336-43.

Wilson D, Hube B. Hgc1 mediates dynamic Candida albicans-endothelium adhesion events during circulation. *Eukaryot Cell* 2010;**9**: 278-87.

Yeon JH, Na D, Choi K *et al.* Reliable permeability assay system in a microfluidic device mimicking cerebral vasculatures. *Biomed Microdevices* 2012;**14**: 1141-8.

Zhang PP, Xin XF, Xu XY *et al.* Toll-like receptor 2 and dectin-1 function as promising biomarker for Aspergillus fumigatus infection. *Experimental and therapeutic medicine* 2017;**14**: 3836-40.

Zhu L, Maruvada R, Sapirstein A *et al.* Cysteinyl leukotrienes as novel host factors facilitating Cryptococcus neoformans penetration into the brain. *Cellular microbiology* 2017;**19**.

4. Unpublished data – manuscript in preparation

4.1 Establishment of T cell perfusion in the intestine-on-chip model

Before T cells were isolated and added to the endothelial cells medium, the luminal compartment was stimulated with 100 ng/ml LPS to trigger T cells infiltration of the endothelial layer and epithelial layer. T cells sufficiently adhered to the endothelial layer without disturbing endothelial barrier integrity, since endothelial cells expressed VE-cadherin and formed a confluent layer (Fig. 3A). T cells also sufficiently invaded the epithelial layer, as $CD3^+$ cells were found within the layer. Some of these $CD3^+$ T cells also expressed CD103 (Fig. 3B). Furthermore, $CD103^+$ DCs were detected adjacent to $CD3^+$ T cells (Fig. 3C).

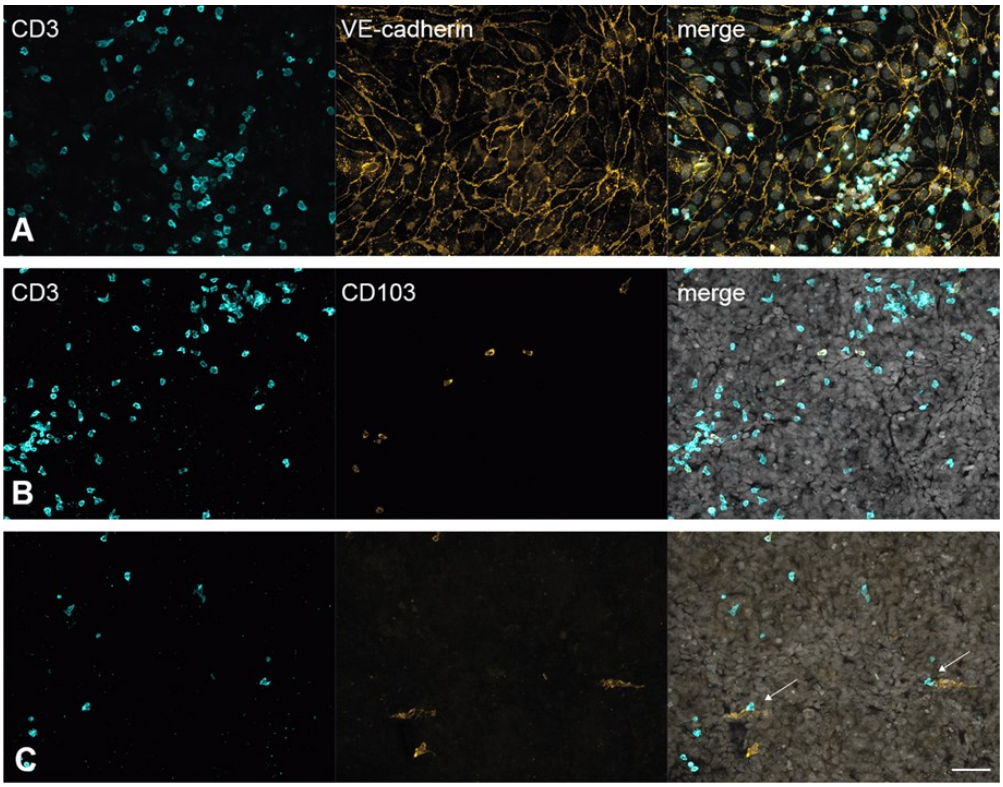


Fig. 3: Establishment of T cell perfusion. (A) T cells adherence in the endothelial layer. **(B)** CD103 expression in T cells in the epithelial layer. **(C)** T cells and DCs in the epithelial layer. Arrows indicate DCs and T cells found in close proximity. Blue: $CD3^+$ T cells; orange: $CD103^+$ T cells and DCs; white: DAPI; n=3; scale bar=50 µm

4.2 Phagocytosis assay using microspheres

4.2.1 Preliminary test: Uptake of microspheres by macrophages in static cell culture

Inflammation in the GI tract is not only associated with the microbiota but can also be triggered by xenobiotic substances. Everyday products commonly contain microplastic particles and their accumulation in the human body may represent a health threat (Galloway 2015). Therefore, a phagocytosis assay was carried out to evaluate the ability of both macrophages and DCs to take up microparticles made of polystyrene. Before microspheres were added to the luminal side of the intestine-on-chip mode, the general capacity of macrophages to take up microspheres was analyzed, and the appropriate number of particles was determined under static conditions. Monocytes were cultured in 24 well plates on coverslips for seven days to allow them to fully differentiate into macrophages. Microspheres in three different quantities were added to the cell culture medium on day seven and incubated for 24 h. Increasing numbers of particles added the medium resulted in increased accumulation in macrophages (Fig. 4). The highest concentration of microspheres was chosen for further experiments.

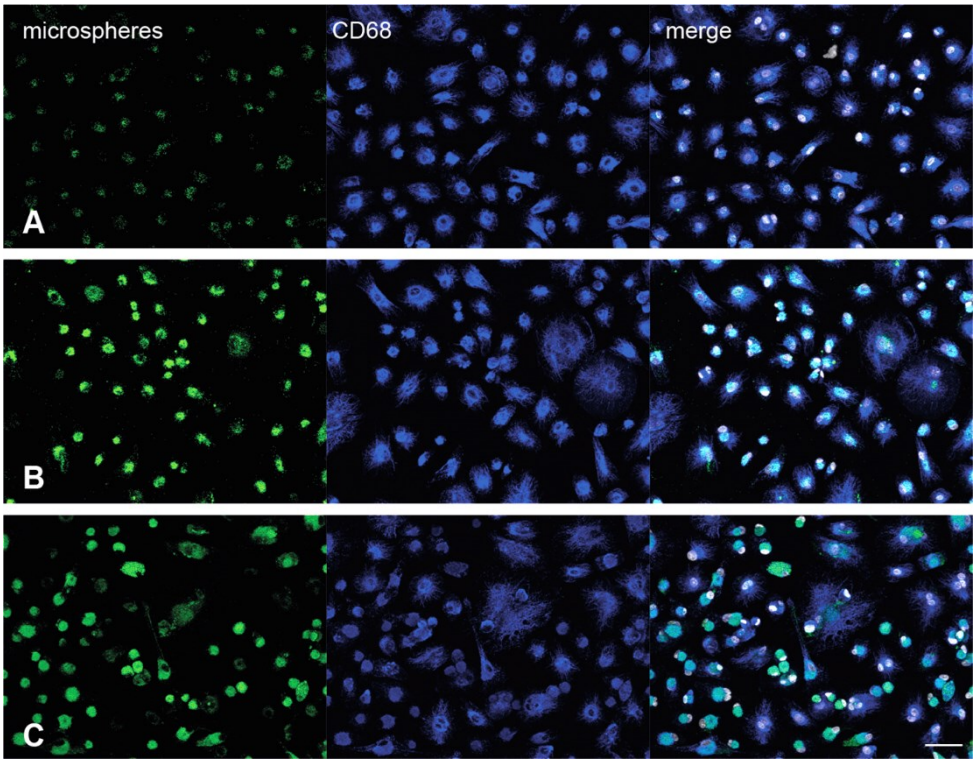


Fig. 4: Microsphere uptake of macrophages in monoculture. (A) $2x10^7$ particles/ml, **(B)** $2x10^8$ particles and **(C)** $2x10^9$ particles/ml. Green: microspheres; blue: CD68; white: DAPI; n=1; scale bar=50 µm

4.2.2 Administration of microspheres at the luminal side of the intestine-on-chip model

In the presence of T cells, microspheres were administered at the luminal side and were detected in both layers after 24 h of perfusion. Macrophages in the endothelial layer were elongated (Fig. 5A) Microspheres were detectable in both $CD68^+$ macrophages and $CD103^+$ DCs (Fig. 5B+C).

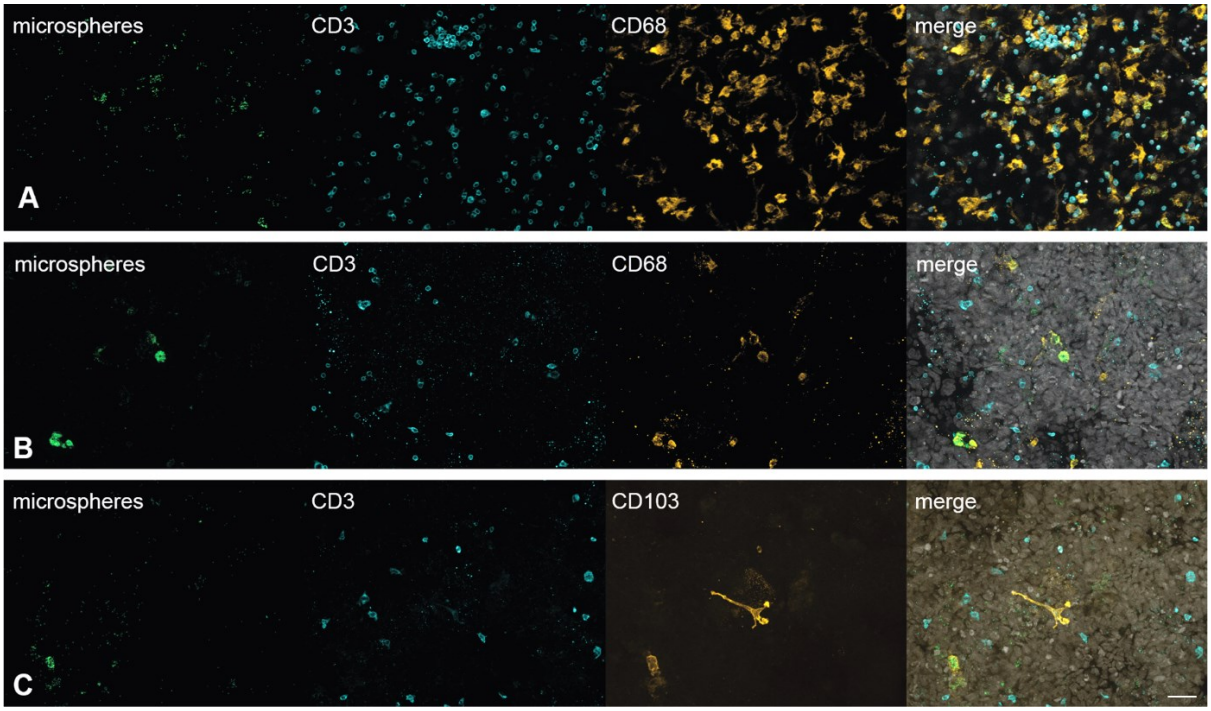


Fig. 5: Microsphere uptake in the intestine-on-chip model. (A) Microsphere uptake by $CD68^+$ macrophages in the endothelial layer. **(B)** Microsphere uptake by $CD68^+$ macrophages in the epithelial layer. **(C)** Microsphere uptake by $CD103^+$ DCs in the epithelial layer. Green: microspheres; blue: $CD3^+$ T cells; orange: $CD103^+$ T cells and DCs; white: DAPI; n=5; scale bar=50 µm

4.2.3 Influence of microspheres on T cell numbers in the epithelial layer

Previous studies have shown that polystyrene particles can evoke proinflammatory responses in the intestinal tissue (Hwang et al. 2020). An increased number of T cells in the epithelial layer can direct towards inflammation in response to microsphere uptake by macrophages and DCs. The number of T cells in the epithelial layer lacking xenobiotic particles was compared with the number of T cells in the presence of microspheres (Fig. 6). The number of T cells was slightly increased when the microspheres were present.

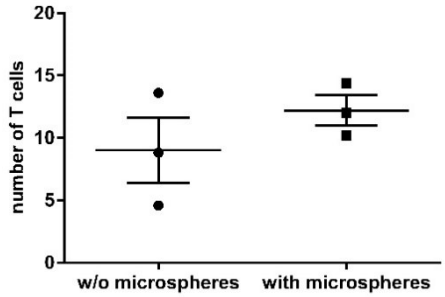


Fig. 6: Number of T cells in the epithelial layer without and with microspheres. n=3

4.3 *S. typhimurium* infection model

4.3.1 Establishment of infection model

In order to infect the intestine-on-chip model with the enteric pathogen *S. typhimurium*, the optimal optical density (OD) was determined. An optimal OD was defined as the OD that damages the tissue to a low degree. The experimental setup is shown in Fig. 7. To assess the damage caused by *S. typhimurium*, the endothelial layer was stained for VE-cadherin and the epithelial layer for E-cadherin (Fig. 8). The largest decline in expression of VE-cadherin and E-cadherin was detected at an OD of 0.01 while the smallest drop was observed at an OD of 0.008 which was chosen for the following experiment (Fig. 8C).

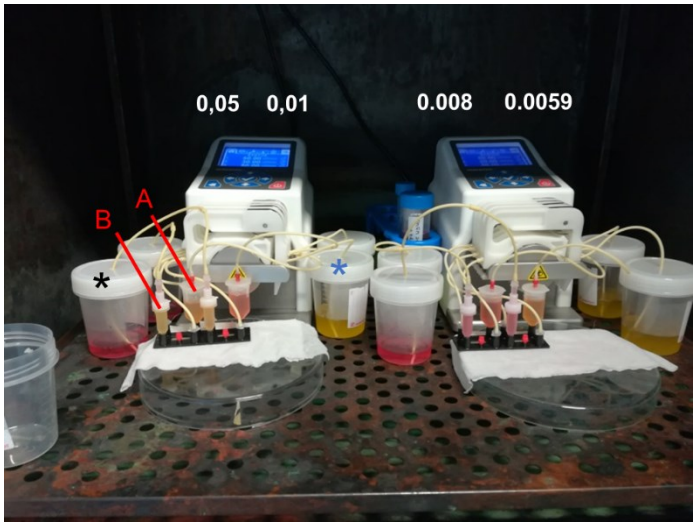


Fig. 7: Experimental set up to determine optimal OD of *S. typhimurium* for infection models. Numbers indicate OD. Black asterisk: medium reservoir for luminal compartment; blue asterisk: waste of luminal compartment; A: medium reservoir of vascular compartment; B: connecting reservoir of luminal compartment.

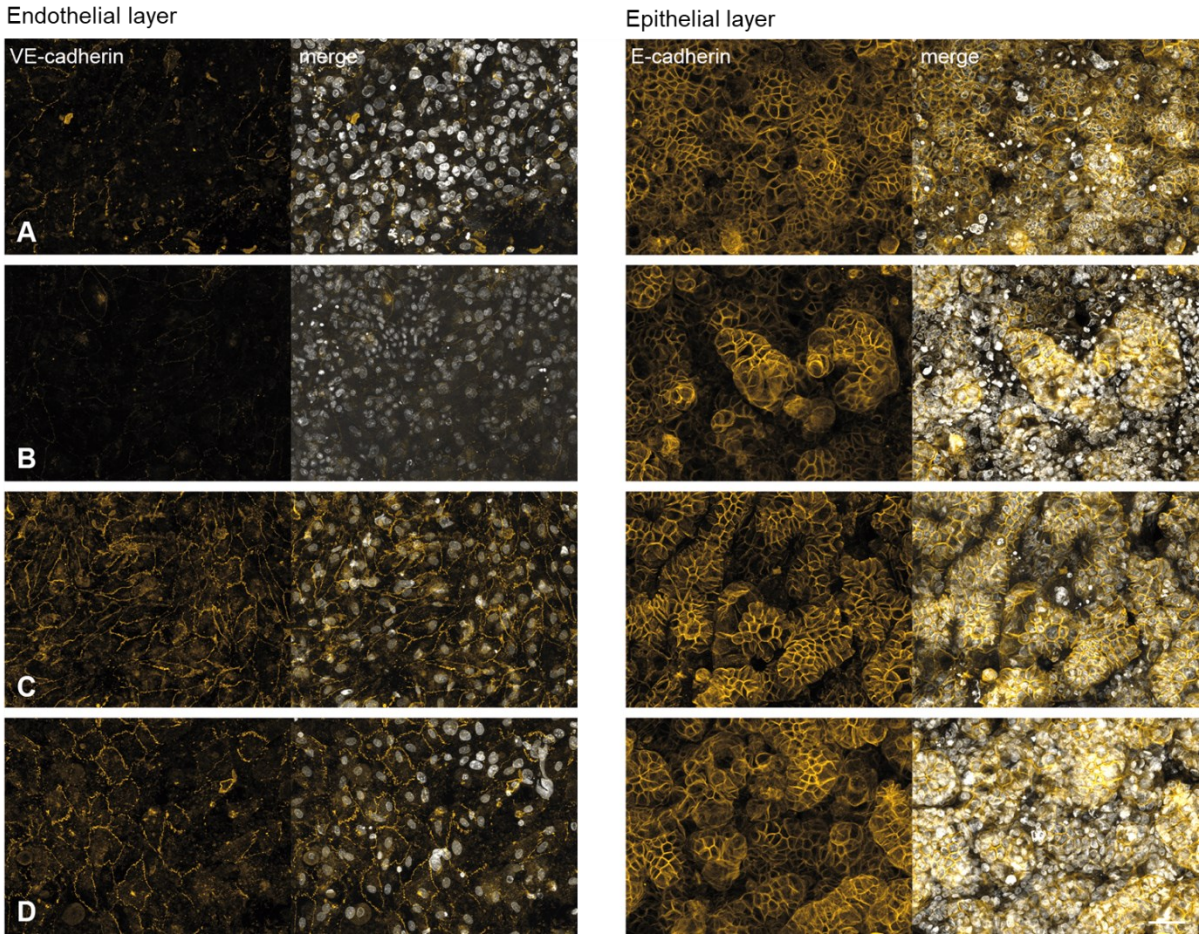


Fig. 8: *S. typhimurium*-induced damage to endothelial and epithelial layer. (A) OD=0.05. **(B)** OD=0.01. **(C)** OD=0.008. **(D)** OD=0.0059. Left side: endothelial layer, VE-cadherin in orange; right side: epithelial layer, E-cadherin in orange; white: DAPI; n=1; scale bar=50 µm

4.3.2 *S. typhimurium* infection in the presence and absence of T cells

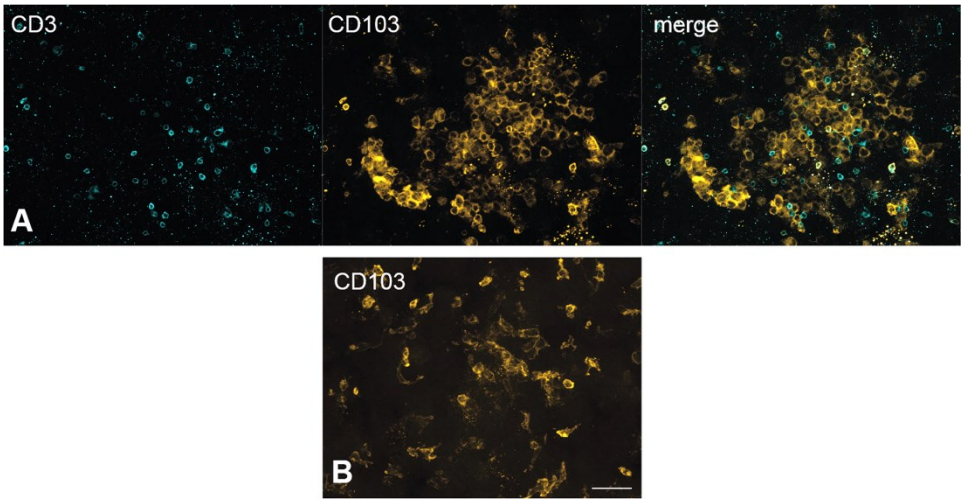


Fig. 9: Effect *of S. typhimurium* infection on DC distribution in the epithelial layer. (A) $CD103^+$ DCs in the presence of $CD3^+$ T cells. **(B)** $CD103^+$ DCs in the absence of $CD3^+$ T cells. Blue: $CD3^+$ T cells; orange: $CD103^+$ T cells and DCs; n=1; scale bar=50 µm

Finally, the distribution of DCs in the epithelial layer during an *S. typhimurium* infection in the presence and absence of T cells was analyzed (Fig. 9). In the presence of T cells, DCs and T cells accumulated in some areas of the epithelial layer, while DCs are more evenly distributed in the absence of T cells.

4.4 Methods

Intestine-on-chip models were assembled as described in Manuscript I. On day eight, LPS (100 ng/ml) was added to the luminal compartment, 3 h before T cells were added to the vascular compartment.

4.4.1 T cell perfusion

For T cell isolation, PBMCs were isolated from blood monovettes from healthy human donors. Subsequently, $CD3^+$ cells were isolated using a human Pan T cell Isolation Kit (Miltenyi Biotech, Bergisch Gladbach, Germany). Briefly, PBMCs were incubated with a biotinylated antibody cocktail for 10 min at 4°C, followed by incubation with an anti-biotin microbeads cocktail, containing antibodies for all cell types except CD3 for T cells, for 5 min at 4°C. Using magnetic-activated cell sorting (MACS) $CD3^-$ cells were separated from the $CD3^+$ fraction. The endothelial layer was perfused with 1.5 ml medium comprising $2x10^6$ $CD3^+$ T cells for 1 h. Afterward, the medium was removed and replaced by fresh medium. T cells were stained with rabbit anti-human CD3 (abcam, Cambridge, UK).

4.4.2 Microsphere perfusion

The intestine-on-chip model was assembled as described in Manuscript I. Fluoresbrite® YG Microspheres, Calibration Grade 0.50 µm (Polysciences, Hirschberg an der Bergstraße, Germany) were added to the luminal compartment on day eleven and perfused for 24 h.

4.4.3 *Salmonella typhimurium* infection model

Salmonella enterica serovar Typhimurium was kindly provided by PD Dr. Lorena Tuchscherr de Hauschopp from the Institute for Medical Microbiology, University Hospital Jena. The bacterial cells were grown on lysogeny broth (LB) agar plates (Carl

Roth, Karlsruhe, Germany). For use in experiments, cells were grown at 37°C at 160 ppm for 24 h in LB broth (Carl Roth, Karlsruhe, Germany). Prior to experiments, *S. typhimurium* was collected by centrifugation, washed twice in PBS, and diluted to an OD_{600nm} of 0.05, 0.01, 0.08, 0.0059 in DMEM. Chips were infected with bacterial cells for 30 min at 37°C. Subsequently, non-adherent cells were washed out and the luminal compartment was perfused linearly for 24 h.

5. Discussion

The human GI tract is a diverse ecosystem shaped by microorganisms such as bacteria and fungi. The microbiota plays an important role in sustaining our health but is also involved in disease progression. The intestinal immune system is constantly exposed to foreign antigens. In order to maintain intestinal homeostasis, macrophages and DCs tolerate commensal microbes, while quickly eliciting proinflammatory responses when pathogens are taking over. An alteration of the microbial community known as dysbiosis is associated with a dysregulated immune response and chronic inflammation. To shed light on underlying mechanisms, sophisticated models are needed that provide a human genetic background and an organ-specific environment resembling human physiology. We have established a microfluidic intestine-on-chip model comprising of epithelial and endothelial cells as well as macrophages and DCs that we further characterized and used to analyze microbiota-host interactions.

5.1 Characterization of mononuclear phagocytes in the intestine-on-chip model

Macrophages and DCs are crucial players in maintaining intestinal homeostasis, inflammation and resolution. The phenotypic and functional distinction between the cell types is an ongoing debate and was hampered in the past by the lack of appropriate markers, as well as differences between mice and humans (Cerovic et al. 2014; Mann et al. 2013). In the present study, we used antibodies against CD68, the fractalkine receptor (CX3CR1), and integrin αEβ7 (CD103) for phenotypic characterization of mononuclear phagocytes in the intestine-on-chip model. Based on the expression of the markers, we found macrophage-like cells expressing high levels of CD68 and CX3CR1 and DC-like cells expressing high levels of CD103 and low levels of CD68. The macrophages within the model are derived from monocytes, differentiated in the presence of M-CSF and GM-CSF. When $CD14^+$ monocytes enter the mucosa in a CCR2-dependent manner, the surrounding environment conditions allows them to adapt a tolerogenic phenotype. In its soluble form, Fractalkine (CX3CL1) functions as a chemoattractant, whereas the membrane-bound form mediates adhesion and is expressed by intestinal epithelial and endothelial cells (Pachot et al. 2008). CX3CL1 mediates tissue infiltration of a range of immune cells such as mononuclear intra-

epithelial lymphocytes (IELs), NK cells, and mononuclear phagocytes, which express CX3CR1 in response (Muehlhoefer et al. 2000). In our intestinal model, CX3CR1 expression was found upregulated in macrophages residing in the epithelial layer in both LPS conditions. This is in accordance with findings from Brand and colleagues, showing that intestinal epithelial cells express CX3CL1 to enable adhesion of immune cells to the tissue (Brand et al. 2002). Presumably, Caco-2 cells in the model similarly upregulated CX3CL1 and tissue-resident macrophages upregulated CX3CR1 in response. In the small intestine as well as in the colon, four subsets of macrophages have been identified. Subset 1 and 2 ($CD14^+$ $CD11c^+$ $HLA\text{-}DR^{int}$ and $CD14^+$ $CD11c^+$ $HLA\text{-}DR^{hi}$) showed a monocyte-like phenotype, and subset 3 and 4 ($CD14^+$ $CD11c^-$ $CD11b^-$ and $CD14^{high}$ $CD11c^-$ $CD11b^+$), resembled mature macrophages (Bujko et al. 2018; Ogino et al. 2013). Subset 2 is believed to represent a transitional state between 1 and the more mature subsets 3 and 4. Subset 1 secreted low amounts of cytokines and induced a strong response upon TLR stimulation, while subset 3 and 4 remained unresponsive. Applying the classification to our model, macrophages in the endothelial layer may be assigned to subset 1 as they displayed little cytokine secretion in control experiments and a strong proinflammatory response when stimulated with LPS. Macrophages of the mature subsets 3 and 4 differentially located within intestinal tissue with subset 3 macrophages forming a network within the villi, whereas macrophages of subset 4 rather locate deep within the tissue, adjacent to the crypts and in the submucosa (Bujko et al. 2018; Ogino et al. 2013). In our model, macrophages in the epithelial layer were only detected adjacent to the crypts, just above the membrane, and were largely unresponsive to luminal LPS treatment. It is therefore likely that macrophages differentiated into subset 4 as they were absent from the villi. The microenvironment may not support the differentiation towards subset 3 macrophages, which locate within villi. In 2005, Niess and colleagues found $CX3CR1^+$ cells that formed transepithelial dendrites (TEDs) in the terminal ileum of the murine GI tract, however, TEDs were absent from other sections of the small intestine from the same mice (Niess et al. 2005). Despite the scarce evidence for TED-forming $CX3CR1^+$ macrophages, macrophages are frequently attributed in the literature as phagocytic cells that directly sample antigens from the lumen (Bain and Mowat 2014; Farache et al. 2013). Macrophages in our model did not show TED formation, and whether human macrophages are generally able to form TEDs is unknown (Kuhl et al. 2015). Niess stated in 2010 that $CX3CR1^+$ cells in mice are a very heterogeneous population and

might also be classified as DCs (Niess 2010). In their study from 2005, Niess and colleagues also claimed that TED formation was CX3CR1-dependent. This could neither be confirmed in another study using a mouse model nor by us, as $CX3CR1^{-}$ $CD103^{+}$ DCs were able to form TEDs (Schulz et al. 2009). Unlike $CX3CR1^{+}$ macrophages, $CD103^{+}$ DCs have the capacity to migrate to lymphoid sites, present antigens to T cells, and induce their differentiation (Annacker et al. 2005). The exact function of CD103 is ambiguous. It seems to be involved in the binding of DCs to the epithelium since the ligand of CD103 is E-cadherin, but it does not seem to be a vital mediator. It locates within endosomal compartments and relocates to the surface upon binding to E-cadherin expressed by epithelial cells (Swain et al. 2018). Differentiation of monocytes into moDCs *in vitro* is usually achieved by culture in media supplemented with IL-4 and GM-CSF (Romani et al. 1994; Sallusto and Lanzavecchia 1994). In our model, GM-CSF was present, however, no IL-4 was added to the medium and there were no IL-4 producing cells such as T cells, basophils, eosinophils present (Junttila 2018). Therefore, it was assumed that the microenvironment promoted differentiation. In mice, Bain and colleagues showed that TGF-β drives CD103 expression on DCs (Bain et al. 2017). In addition, it has been demonstrated that epithelial cells are involved in human moDC differentiation towards a tolerogenic phenotype via secretion of TGF-β and RA (Iliev et al. 2009). Therefore, it is likely that the epithelial cells condition moDCs via TGF-β-signaling in the intestine-on-chip model. The role of moDCs *in vivo* is often not taken into account since some researchers do not consider them “naturally occurring” DCs (Bernardo et al. 2018b). However, a study by Randolph and colleagues in 1999 showed that a small percentage of monocytes differentiated into DCs and migrated to draining lymph nodes in mice (Randolph et al. 1999). In fact, there are substantial indications that moDCs occur *in vivo* and act as an “emergency back-up” during acute infections (Qu et al. 2014). Three subsets of circulating monocytes have been identified: (i) the classical $CD14^{++}$ $CD16^{-}$, (ii) the intermediate $CD14^{++}$ $CD16^{+}$ and (iii) the nonclassical $CD14^{+}$ $CD16^{++}$ subset (Gren and Grip 2016). It has been shown that the intermediate and nonclassical subsets are more likely to give rise to moDCs, however, classical monocytes might represent a less mature version of the other subsets (Randolph et al. 2002). In mice, monocytes differentiate into fully functional moDCs upon microbial stimulation in a TLR-dependent manner. They exhibit DC morphology, effectively capture antigens, and migrate to trigger T cell responses just like cDCs (Cheong et al. 2010). Similarly, human moDCs mature and develop the

capacity to stimulate T cells in response to infection with Influenza A virus (Qu et al. 2003). We did not evaluate the ability of moDCs in the intestinal model to induce T cell responses, however, T cells were implemented into the model, by isolating them from PBMCs and perfusion of the vascular compartment. They adhered to the endothelium and invaded the epithelial layer (Unpublished data, Fig. 3). DCs and T cells were found in close proximity, indicating DC-T cell communication and antigen presentation. T cells were identified via expression of CD3, however, some cells co-expressed CD103. CD103 is not only found on DCs but also on IELs residing between enterocytes and providing a first-line defense against microbial invasion (Ma et al. 2019; Roosenboom et al. 2019). Integration of T cells into the system laid the foundation to elucidate the development and adaption of T cells to intestinal tissue in conjunction with the analysis of adaptive immune responses towards normal microbiota, pathogens, and their metabolites in follow-up studies.

5.2 Interaction of *L. rhamnosus* and *C. albicans*

As a proof-of-concept, the immunocompetent intestine-on-chip model was used for functional microbial interaction studies. The colonization with *L. rhamnosus* resulted in a slight increase of IL-1β and IL-6 secretion when endothelial cells were stimulated with LPS (Manuscript I). However, the increased cytokine release did not lead to an impairment of the intestinal barrier. The opposite was the case: as demonstrated by volume measurement of E-cadherin and ZO-1, the presence of the commensals led to an enhanced volume of the junctional proteins and therefore enhanced barrier integrity. These observations are in accordance with *in vitro* and *in vivo* studies, showing that colonization with *L. rhamnosus* restores barrier function in TNF-treated Caco-2 cells and in mice treated with dextran sodium sulfate (DSS) (Miyauchi et al. 2009). Enhancement of barrier integrity is mediated via TLR signaling in epithelial cells (Burgueño and Abreu 2020; Cario et al. 2004). TLR2 is the main receptor for the recognition of lipoproteins and LTA of Gram-positive bacteria and has been reported to be activated by *L. rhamnosus* (Oliveira-Nascimento et al. 2012; Vizoso Pinto et al. 2009). Although not analyzed in the present study, the upregulation of barrier proteins may result at least partially from *L. rhamnosus*-induced TLR2 signaling. The pre-colonization of the intestine-on-chip model with *L. rhamnosus* led to reduced growth of *C. albicans* (Manuscript I). The growth-reducing capacity of *L. rhamnosus* has been previously demonstrated by other groups (Allonsius et al. 2017; Hasslöf et al. 2010).

Even though the fungi were still able to form hyphae, cross the epithelial layer, and reach the endothelium, the presence of *L. rhamnosus* resulted in reduced translocation. Investigations of how exactly *L. rhamnosus* conveys protection against pathogenic forms of *C. albicans* are currently ongoing and a range of mechanisms have been proposed. Allonsius and colleagues claimed that exopolysaccharides (EPS) mediate the protective effects of *L. rhamnosus* during *C. candida* infection by inhibiting hyphal formation (Allonsius et al. 2017). Inhibition of hyphal formation is also seen in other Lactobacilli species, and it was assumed that the inhibitory effect is caused by the complex polymer structure containing galactose. Contradictory to their results, other studies identified lactic acid as the main driver of growth inhibition (Kohler et al. 2012). In addition, using a static culture model, Graf and colleagues demonstrated that adhesion of *C. albicans* to epithelial cells was unaffected by *L. rhamnosus*. However, the bacteria suppressed filamentation and mediated damaged protection, which involves shedding of fungal cells (Graf et al. 2019). In this study, neither ESP nor lactic acid played a role in conveying protection.

Our intestinal model can also be used to examine how enteric pathogens interact with commensals and host immune cells. Following the establishment of an *S. typhimurium* infection model (Unpublished data, Fig. 7 and 8), the distribution of DCs was analyzed in the presence and absence of T cells. A preliminary experiment revealed a local accumulation of DCs in the presence of T cells 24 h post-infection (Unpublished data, Fig. 9). Since this experiment has only been carried out once further investigation is required to confirm the observation.

5.3 Microparticle uptake in the intestine-on-chip model

The accumulation of microplastic particles in the human body is recognized as a potential health threat as it might, for example, trigger inflammation in the GI tract (Galloway 2015). Therefore, a phagocytosis assay using fluorescent microparticles made of polystyrene was carried out. In an initial test, macrophages were cultured in a monoculture to determine an appropriate number of particles to be added to the luminal compartment of the intestinal model (Unpublished data, Fig. 4). It was observed that microparticles were taken up by macrophages, showing a round-shaped morphology that potentially indicates a non-activated state. As shown in Fig. 5B+C (Unpublished data), both $CD68^+$ and $CD103^+$ cells were able to take up microspheres. However, unlike macrophages in monoculture, monocytes or macrophages residing in the

endothelial layer had an elongated shape, which might indicate their activation (Unpublished data, Fig. 5A). Furthermore, a tendency of T cell accumulation in the epithelial layer in the presence of microspheres was observed (Unpublished data, Fig. 6). The results need to be confirmed by image analysis of larger areas of the membrane. The observation that immune cells might have been activated in the endothelial layer in the intestinal model but not in monoculture are in accordance with the assumption made in 5.1. Macrophages residing in the endothelial layer may rather resemble monocytes than fully differentiated macrophages and may therefore be triggered by the microparticles to release proinflammatory cytokines, which needs to be confirmed in follow-up studies. The findings are supported by a previous report demonstrating activation of the immune system upon the accumulation of polystyrene-based microparticles in monocytes and neutrophils (Hwang et al. 2020). In this study, phagocytes released TNF and IL-6 after phagocytosis of particles smaller than 1 µm. Taken together, the results indicate that microparticles can represent a potential health risk due to the activation of the innate immune system.

5.4 Intestine-on-chip models for host-microbiota research

5.4.1 How valuable are Caco-2 cells for host-microbiota research?

The human colon adenocarcinoma cell line Caco-2 is the most frequently used cell line for *in vitro* modeling of the human intestine. Since the cell line had been isolated in 1977, it has served as a useful tool for studies analyzing drug transport, metabolism, and permeability (Fogh et al. 1977). This is due to the fact that a large proportion of enzymes and transporters expressed in the human intestine are expressed in Caco-2 cell monolayers (Sun et al. 2008). Whilst interest in microbiota research has grown during the past decades, Caco-2 cells have been increasingly used for microbiota studies (Bahrami et al. 2011; Chen et al. 2017; Sadaghian Sadabad et al. 2015). The majority of microfluidic intestine-on-chip models employ the cell line as well (Tab. 1) since they are easy to obtain and maintain. When the cells are cultured as a monolayer it takes about 20 days until they are differentiated (Vachon and Beaulieu 1992). As demonstrated by Kim and colleagues Caco-2 cells have the unique capacity to grow out and form villi-like structures under dynamic growth conditions (Kim et al. 2012). We, as well as others, have shown that perfusion accelerates differentiation and induces the expression of α-Defensin and Mucin-2, which are both required to study the microbiota under physiologically relevant conditions. α-Defensin has bactericidal

activity acting against Gram-positive and Gram-negative bacteria but also fungi and viruses, which is presumably based on the destruction of the barrier integrity (Salzman et al. 2007). However, α-Defensin functions in a selective manner, leaving commensals unaffected while most pathogens are eliminated (Nakamura et al. 2016). Mucin-2 is the major component of the mucus layer covering the epithelium and therefore forms the barrier between the microbiota and the epithelium. The mucus layer serves as an attachment site for microbes and in some cases provides a source of nutrition (Sicard et al. 2017). In addition, DSS-induced pathophysiology observed in murine models was sufficiently recapitulated using a Caco-2 cell-based intestine-on-chip model (Shin and Kim 2018). DSS treatment led to an impairment of the mucosal barrier, proinflammatory responses, and reduction of goblet cells. It was demonstrated that pre-colonization with probiotics significantly reduced damage evoked by DSS treatment. Besides these advantages of Caco-2 cells in this regard, there are disadvantages that need to be considered for the application in microbiota research. Although the cells originate from the colon, they display characteristics of the small intestine, for example, the expression of hydrolase enzymes and formation of microvilli which are absent from the colon (Sambuy et al. 2005). In 1998, it was already shown that the cells express colonocyte markers, which decrease during culture after reaching confluence (Engle et al. 1998). At the same time, the expression of enterocyte markers is induced, however, the cells rather display properties of fetal ileal epithelial cells than characteristics of mature enterocytes. The observation is explained by either the persistence of a tumor-derived phenotype or by an insufficient shift from a crypt to a villous phenotype. The colonic origin and the small intestine-characteristics of Caco-2 cells are problematic for modeling a specific section of the intestine. The majority of the microbiota does not reside in the small intestine but in the colon. It is therefore questionable, whether a cell line having small intestinal traits can accurately mimic a microenvironment that is suitable for the culture of colon-inhabiting microorganisms. The presence of villi and absence of a thick inner mucus layer, characteristic for the small intestine, might impact the growth conditions. In addition, as Caco-2 cells are tumor-derived, it is questionable whether they can fully mimic healthy tissue. If not, it might have a considerable impact on host-microbiota crosstalk.

Tab. 1: Overview of microfluidic Organ-on-chip models of the human intestine. Models are grouped by cell source of the epithelial cells.

Caco-2-based models			
	additional host cell types	**bacteria**	**hypoxia**
Gijzen et al. 2020	acute monocytic leukemia cell line THP-1, acute myelomonocytic leukemia cell line MUTZ-3	-	-
Grassart et al. 2019	-	*Shigella flexneri*	-
De Gregorio et al. 2020	subepithelial myofibroblast (ISEMFs)	-	-
Gumuscu et al. 2017	-	*E. coli*	-
Jalili-Firoozinezhad et al. 2018	HUVECs		
Jalili et al. 2019	HUVECs	*B. fragilis*, 11 different genera of bacteria from human stool samples	0,3%
Kim et al. 2012	-	*L. rhamnosus* GG	-
Kim and Ingber 2013	-	-	-
Kim et al. 2015	HUVECs, lymphatic microvascular endothelial cells, PBMCs	VSL#3 probiotics, *E. coli*, enteroinvasive *E.coli* (EIEC)	-
Mazorati et al. 2014	-	Bacteroidetes, Firmicutes, Bifidobacteria, Lactobacilli	-
Pocock et al. 2017	-	-	-
Ramadan et al. 2013	monocytic cell line U937 differentiated into macrophages	-	-
Shah et al. 2016	$CD4^+$ T cells	*L. rhamnosus* GG, *B. caccae*	0,1%
Greenhalgh et al. 2019	-	*L. rhamnosus* GG	0,1%
Shin and Kim 2018	PBMCs	VSL#3 probiotics, *E. coli*	-
Shim et al. 2017	-	-	-
Primary cells-based models			
	source and additional host cell types	**bacteria**	**hypoxia**
Dawson et al. 2016	sections from small and large intestine	-	-
Jalili et al. 2019	epithelial cells from terminal ileum	*B. fragilis*, 11 different genera of bacteria from human stool samples	0,3%
Kasandra et al. 2018	epithelial cells from duodenum biopsies expanded as organoids, human intestinal microvascular endothelial cells (HIMECs)	-	-
Zhang et al. 2020	colon cells derived from colon organoids	*E. rectale, B. thetaiotaomicron, Faecalibacterium prausnitzii*	3%
iPSC-based models			
	differentiated cells	**bacteria**	**hypoxia**
Naumovska et al. 2020	intestinal progenitor cells expressing epithelial and stem cell markers	-	-
Workman et al. 2018	epithelium expressing epithelial and stem cell markers	-	-

5.4.2 Alternative cell sources for microphysiological intestine models

Due to its limitations, the relevance of Caco-2 cell line for modeling the intestine has been questioned and other cell sources are used for *in vitro* models (Bein et al. 2018). Primary cells have been incorporated into intestinal MPS as well (Tab. 1). Obtained from patient biopsies they are enzymatically broken up into individual cell types such as epithelium and stem cells, which are subsequently expanded and seeded onto organ-on-chip platforms. Although primary cells recapitulate the *in vivo* physiology to a high degree, they are generally more difficult to access, less robust than Caco-2 cells, and quickly lose their phenotype after several passages. Another way to use primary cells is to culture them as organoids, miniature stem cell-derived organs (Kim et al. 2020). Human intestinal organoids (HIOs) derive from adult stem cells (ASCs) extracted from intestine biopsies and form an enclosed luminal space. HIOs show villi and crypt-like structures similarly to Caco-2 cells. Sato and colleagues were the first to culture ASC-derived organoids in presence of growth factors and Matrigel® as a supporting matrix (Sato et al. 2011b). However, these organoids solely consist of epithelial cells; additional cell types are lacking. Organoids can also arise from human induced pluripotent stem cells (hiPSCs). In 2007, human dermal fibroblasts were reprogrammed via transfection with a retrovirus overexpressing transcription factors, which are necessary to induce a pluripotent state (Takahashi et al. 2007). The great advantage over Caco-2 cells is the capability of hiPSCs to self-renew and theoretically differentiate in all cell types arising from the germ layers ectoderm, mesoderm, and endoderm. hiPSC differentiation into HIOs requires the preceding differentiation into definitive endoderm, followed by differentiation into mid-hindgut cells and finally epithelial cells (Spence et al. 2011). In contrast to ASC-derived HIOs, a small fraction of hiPSCs differentiates into mesenchymal cells, allowing epithelial-mesenchymal interactions. In addition, HIOs containing neurons and glial cells have been established, however, other cell types such as immune cells, which are highly relevant for a physiological environment, have not been incorporated yet (Workman et al. 2017). HIOs have also been used to study host-microbiota or host-pathogen interactions. Therefore, bacteria are injected into the lumen of HIOs. In the case of a nonpathogenic *E. coli* strain, the HIOs and bacteria formed a stable co-culture, leading to enhanced barrier integrity, similarly to our model (Hill et al. 2017). In an HIO derived from mouse crypt cells, injection of *S. typhimurium* resulted in a decline of stem cell markers, barrier leakage, and a proinflammatory response comparable to *in vivo* infections (Zhang et

al. 2014). A range of other pathogens have been injected into HIOs as well, however, culture conditions need to be improved to reflect intestinal physiology more closely (Min et al. 2020). HIOs are commonly embedded in Matrigel®, which does not resemble tissue-specific matrices, and the lack of perfusion potentially leads to the accumulation of waste products and bacterial overgrowth. The use of cells from different human donors in one system can be avoided by the implementation of hiPSCs. However, the cells are more difficult to handle and increase time requirements as wells as costs for a single experiment compared to models that use Caco-2 cells. Furthermore, differentiation of hiPSCs into all epithelial cell types, macrophages, DCs, T cells and other cell types is an enormous challenge. Optimization and standardization of differentiation protocols also remain major issues. Regarding the application of hiPSC-culture in OoC platforms, attention must be drawn to differentiation under static and dynamic conditions which might lead to different outcomes. Nevertheless, hiPSCs hold great potential for future models of the intestine. The combination of hiPSCs with advanced OoC and microfluidic technologies can substantially contribute to the development of more physiologically relevant models. As a long-term goal researchers aim at establishing multi-organ-on-chip models (MOCs), integrating several organotypic models on one platform in order to investigate systemic effects (Luni et al. 2014). This is especially relevant in host-microbiota research since it is known that the microbiota is part of a bidirectional connection of the intestine with other organs such as the liver and the brain.

5.4.3 Improvements for accurately mimicking the microenvironment for host-microbiota interactions

Our intestine-on-chip model consists of epithelial cells displaying Paneth and goblet cell markers, macrophages, and DCs, allows T cell perfusion, colonization with commensal microbiota, and infection with pathogenic bacteria. Still, the model lacks important features to accurately mimic the colonic environment. First, the oxygen levels range from 0.1-1% between the mucus layer and the anaerobic luminal zone and from 80-100% in the crypt region (Espey 2013). This oxygen gradient is crucial to maintain the normal function of the intestinal ecosystem (Zheng et al. 2015). For example, the hypoxia-inducible factor-1α (HIF-1α) globally regulates oxygen homeostasis, is involved in the maintenance of barrier integrity and many oxygen-responsive signaling pathways, such as the Wnt/β-Catenin signaling pathway. The microbiota is adapted to

the low oxygen tension and is therefore dominated by obligate anaerobes. The imitation of the gradient enables the culture of important commensal bacteria in the intestine-on-chip model and can be achieved, for example, by perfusion of the vascular compartment with oxygenated cell culture medium, while the chip model is placed in a hypoxic incubator. Currently, the intestine-on-chip model can only be analyzed by end-point analysis. The implementation of sensors for the measurement of parameters, such as oxygen, pH, and glucose is another important aspect that needs to be considered. *In situ* measurement of these parameters in real-time can provide pivotal insights into growth dynamics within the chip. Implementation of additional analytical methods such as mass spectrometry has been suggested for OoC systems, which can be useful to identify microbiota-derived metabolites exerting effects on host cells (Ashammakhi et al. 2020; Oedit et al. 2015). Advanced image analysis is needed to appropriately study immune cells residing in villi-like structures, as the epithelial layer can be up to 70 µm thick. The signal of labeled antibodies against cells locating to the crypt-like areas appears weak, making visualization of cells difficult.

Furthermore, as discussed in 5.4.1, Caco-2 cells do not fully recapitulate healthy intestinal tissue. Epithelial cells, endothelial cells, and immune cells all derive from different human donors, which might lead to an unintended immune reaction. Using hiPSCs, this issue can be eliminated. Moreover, patient-specific intestine-on-chip models can be developed from hiPSCs. This approach allows the investigation of diseases such as IBD with cells that already carry a predisposing genetic background. Personalized medicine holds great potential for elucidating the pathogenesis of numerous diseases (Chun et al. 2011).

Finally, the intestine-on-chip model may include a range of cell types, still, other cell types such as B cells, eosinophils, mast cells, fibroblasts, muscle cells, and neurons are lacking. However, one should bear in mind that the increasing complexity of the model comes with increasing difficulties to handle the model as a trade-off. Multiple strains of microorganisms must be cultured in parallel before colonization or infection of the model. Therefore, the complexity and easy handling of intestine-on-chip models studying microbiota-host interactions should be balanced.

6. CONCLUSION

We have established a microfluidic intestine-on-chip model comprising an epithelial and endothelial layer, and monocyte-derived macrophages and DCs (Manuscript I). The characterization revealed that monocytes differentiated into $CD68^{++}$ $CX3CR1^{+}$ macrophages and $CD68^{+}$ $CD103^{+}$ dendritic cells, with a distinct location within the intestinal tissue and presumably, different functions. Macrophages and DCs demonstrated to be largely unresponsive to luminal LPS administration. Furthermore, the intestinal model allowed stable colonization with the probiotic strain *L. rhamnosus*, which is also tolerated by macrophages and DCs, as well as by recruited PBMCs from the vascular compartment. This immune tolerance is a necessary feature of intestinal immune cells to avoid chronic inflammation in response to commensal microbes and their metabolic products. It can therefore be concluded that the microenvironment created by the intestinal tissue supports the differentiation of monocytes into tolerogenic macrophages and DCs. In contrast, immune cells residing in the endothelial layer retain their responsiveness towards microbial products. LPS treatment leads to a proinflammatory response, which is evident by increased secretion of the cytokines IL-1β, IL-6, IL-8, and TNF, and a compromised endothelial lining. Therefore, endothelial cells might keep monocytes in a rather undifferentiated, responsive state. These findings undermine the importance of implementing relevant cell types cultured under physiological conditions into organotypic models.

Moreover, the intestinal model facilitated microbial interaction studies with the opportunistic pathogen *C. albicans.* The presence of *L. rhamnosus* limited the growth of the fungi, reduced tissue damage, and translocation from the luminal into the vascular compartment. It is known that *L. rhamnosus* also conveys a protective effect for infections with the enteric pathogen *S. typhimurium*. An *S. typhimurium* infection protocol was established, as well as a perfusion protocol for T cells. Thus, the intestine-on-chip model enables a systematic examination of a co-culture comprising of *L. rhamnosus* and *S. typhimurium* and may reveal novel mechanisms employed by the pathogen to act against commensals and host cells. In addition, DCs and T cells were found to be located adjacent to each other indicating antigen presentation and priming of T cells by DCs. Following antigen presentation *in vivo*, naïve T cells differentiate into Th17 or Treg cells, for example. Future studies are needed to determine whether DCs in the intestinal model present antigens to T cells and if so, identify the subtype into

which T cells differentiate in the presence of *L. rhamnosus* and *S. typhimurium*. Moreover, cytokine secretion by T cells during homeostasis and infection can provide more information about their role during these conditions and how the pathogens evade elimination.

However, to enhance the physiological relevance the model requires further improvement. The integration of hypoxic conditions will enable the culture of anaerobic bacteria and is required for the activation of specific signaling pathways involved in maintaining intestinal homeostasis. As another optimization, instead of using Caco-2 cells and primary cell from different human donors, hiPSCs are the cell source of choice for future models. This also holds true for *in vitro* modeling of other tissue structures such as the respiratory tract and blood-brain barrier that can be overcome by pathogenic fungal species (Manuscript II). Bacteria, fungi, and viruses do not only impact host cells locally but also systemically by releasing metabolites that are taken up by epithelial cells and released into the bloodstream. Consequently, the interconnection of OoC models, for example, the intestine with the liver would allow an analysis of the impact of microbial metabolites on liver function.

In summary, despite having its limitations the intestine-on-chip model proved to be a suitable platform to dissect microbial interactions and further, provides the opportunity to unravel antagonistic mechanisms employed by probiotic strains and adaption of pathogens to survive and evade the immune system. Advanced MPS can play a major role in speeding up drug discovery in the near future.

A REFERENCES

Alakomi, H. L., Skyttä, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K., and Helander, I. M. (2000), 'Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane', *Appl Environ Microbiol,* 66 (5), 2001-5.

Albac, S., Schmitz, A., Lopez-Alayon, C., d'Enfert, C., Sautour, M., Ducreux, A., Labruere-Chazal, C., Laue, M., Holland, G., Bonnin, A., and Dalle, F. (2016), 'Candida albicans is able to use M cells as a portal of entry across the intestinal barrier in vitro', *Cell Microbiol,* 18 (2), 195-210.

Allert, S., Förster, T. M., Svensson, C. M., Richardson, J. P., Pawlik, T., Hebecker, B., Rudolphi, S., Juraschitz, M., Schaller, M., Blagojevic, M., Morschhäuser, J., Figge, M. T., Jacobsen, I. D., Naglik, J. R., Kasper, L., Mogavero, S., and Hube, B. (2018), 'Candida albicans-Induced Epithelial Damage Mediates Translocation through Intestinal Barriers', *mBio,* 9 (3), e00915-18.

Allonsius, C. N., van den Broek, M. F. L., De Boeck, I., Kiekens, S., Oerlemans, E. F. M., Kiekens, F., Foubert, K., Vandenheuvel, D., Cos, P., Delputte, P., and Lebeer, S. (2017), 'Interplay between Lactobacillus rhamnosus GG and Candida and the involvement of exopolysaccharides', *Microb Biotechnol,* 10 (6), 1753-63.

Annacker, O., Coombes, J. L., Malmstrom, V., Uhlig, H. H., Bourne, T., Johansson-Lindbom, B., Agace, W. W., Parker, C. M., and Powrie, F. (2005), 'Essential role for CD103 in the T cell-mediated regulation of experimental colitis', *J Exp Med,* 202 (8), 1051-61.

Arnold, J. W., Roach, J., and Azcarate-Peril, M. A. (2016), 'Emerging Technologies for Gut Microbiome Research', *Trends Microbiol,* 24 (11), 887-901.

Artis, D. (2008), 'Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut', *Nat Rev Immunol,* 8 (6), 411-20.

Ashammakhi, N., Nasiri, R., Barros, N. R., Tebon, P., Thakor, J., Goudie, M., Shamloo, A., Martin, M. G., and Khademhosseini, A. (2020), 'Gut-on-a-chip: Current progress and future opportunities', *Biomaterials,* 255, 120196.

Bahrami, B., Child, M. W., Macfarlane, S., and Macfarlane, G. T. (2011), 'Adherence and cytokine induction in Caco-2 cells by bacterial populations from a three-stage continuous-culture model of the large intestine', *Appl Environ Microbiol,* 77 (9), 2934-42.

Bain, C. C. and Mowat, A. M. (2014), 'Macrophages in intestinal homeostasis and inflammation', *Immunol Rev,* 260 (1), 102-17.

Bain, C. C., Montgomery, J., Scott, C. L., Kel, J. M., Girard-Madoux, M. J. H., Martens, L., Zangerle-Murray, T. F. P., Ober-Blobaum, J., Lindenbergh-Kortleve, D., Samsom, J. N., Henri, S., Lawrence, T., Saeys, Y., Malissen, B., Dalod, M., Clausen, B. E., and Mowat, A. M. (2017), 'TGFbetaR signalling controls CD103(+)CD11b(+) dendritic cell development in the intestine', *Nat Commun,* 8 (1), 620.

Banchereau, J. and Steinman, R. M. (1998), 'Dendritic cells and the control of immunity', *Nature,* 392 (6673), 245-52.

Basson, M. D. (2003), 'Paradigms for mechanical signal transduction in the intestinal epithelium. Category: molecular, cell, and developmental biology', *Digestion,* 68 (4), 217-25.

Bein, A., Shin, W., Jalili-Firoozinezhad, S., Park, M. H., Sontheimer-Phelps, A., Tovaglieri, A., Chalkiadaki, A., Kim, H. J., and Ingber, D. E. (2018), 'Microfluidic Organ-on-a-Chip Models of Human Intestine', *Cell Mol Gastroenterol Hepatol,* 5 (4), 659-68.

Bernardo, D., Chaparro, M., and Gisbert, J. P. (2018a), 'Human Intestinal Dendritic Cells in Inflammatory Bowel Diseases', *Mol Nutr Food Res,* 62 (7), e1700931.

Bernardo, D., Marin, A. C., Fernandez-Tome, S., Montalban-Arques, A., Carrasco, A., Tristan, E., Ortega-Moreno, L., Mora-Gutierrez, I., Diaz-Guerra, A., Caminero-Fernandez, R., Miranda, P., Casals, F., Caldas, M., Jimenez, M., Casabona, S., De la Morena, F., Esteve, M., Santander, C., Chaparro, M., and Gisbert, J. P. (2018b), 'Human intestinal pro-inflammatory CD11c(high)CCR2(+)CX3CR1(+) macrophages, but not their tolerogenic CD11c(-)CCR2(-)CX3CR1(-) counterparts, are expanded in inflammatory bowel disease', *Mucosal Immunol,* 11 (4), 1114-26.

Bougnoux, M. E., Diogo, D., Francois, N., Sendid, B., Veirmeire, S., Colombel, J. F., Bouchier, C., Van Kruiningen, H., d'Enfert, C., and Poulain, D. (2006), 'Multilocus sequence typing reveals intrafamilial transmission and microevolutions of Candida albicans isolates from the human digestive tract', *J Clin Microbiol,* 44 (5), 1810-20.

Brand, S., Sakaguchi, T., Gu, X., Colgan, S. P., and Reinecker, H. C. (2002), 'Fractalkine-mediated signals regulate cell-survival and immune-modulatory responses in intestinal epithelial cells', *Gastroenterology,* 122 (1), 166-77.

Brown, G. D., Denning, D. W., Gow, N. A., Levitz, S. M., Netea, M. G., and White, T. C. (2012), 'Hidden killers: human fungal infections', *Sci Transl Med,* 4 (165), 165rv13.

Broz, P., Ohlson, M. B., and Monack, D. M. (2012), 'Innate immune response to Salmonella typhimurium, a model enteric pathogen', *Gut Microbes,* 3 (2), 62-70.

Bujko, A., Atlasy, N., Landsverk, O. J. B., Richter, L., Yaqub, S., Horneland, R., Oyen, O., Aandahl, E. M., Aabakken, L., Stunnenberg, H. G., Baekkevold, E. S., and Jahnsen, F. L. (2018), 'Transcriptional and functional profiling defines human small intestinal macrophage subsets', *J Exp Med,* 215 (2), 441-58.

Burgueño, Juan F. and Abreu, Maria T. (2020), 'Epithelial Toll-like receptors and their role in gut homeostasis and disease', *Nat Rev Gastroenterol Hepat,* 17 (5), 263-78.

Carabotti, M., Scirocco, A., Maselli, M. A., and Severi, C. (2015), 'The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems', *Ann Gastroenterol,* 28 (2), 203-09.

Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M., and Owen, L. J. (2015), 'Dysbiosis of the gut microbiota in disease', *Microb Ecol Health Dis,* 26, 26191.

Cario, E., Gerken, G., and Podolsky, D. K. (2004), 'Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase C', *Gastroenterology,* 127 (1), 224-38.

Cebula, A., Seweryn, M., Rempala, G. A., Pabla, S. S., McIndoe, R. A., Denning, T. L., Bry, L., Kraj, P., Kisielow, P., and Ignatowicz, L. (2013), 'Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota', *Nature,* 497 (7448), 258-62.

Cerovic, V., Bain, C. C., Mowat, A. M., and Milling, S. W. (2014), 'Intestinal macrophages and dendritic cells: what's the difference?', *Trends Immunol,* 35 (6), 270-7.

Chen, T., Kim, C. Y., Kaur, A., Lamothe, L., Shaikh, M., Keshavarzian, A., and Hamaker, B. R. (2017), 'Dietary fibre-based SCFA mixtures promote both protection and repair of intestinal epithelial barrier function in a Caco-2 cell model', *Food Funct,* 8 (3), 1166-73.

Cheong, C., Matos, I., Choi, J. H., Dandamudi, D. B., Shrestha, E., Longhi, M. P., Jeffrey, K. L., Anthony, R. M., Kluger, C., Nchinda, G., Koh, H., Rodriguez, A., Idoyaga, J., Pack, M., Velinzon, K., Park, C. G., and Steinman, R. M. (2010), 'Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas', *Cell,* 143 (3), 416-29.

Chun, Y. S., Byun, K., and Lee, B. (2011), 'Induced pluripotent stem cells and personalized medicine: current progress and future perspectives', *Anat Cell Biol,* 44 (4), 245-55.

Claes, I. J., Segers, M. E., Verhoeven, T. L., Dusselier, M., Sels, B. F., De Keersmaecker, S. C., Vanderleyden, J., and Lebeer, S. (2012), 'Lipoteichoic acid is an important microbe-associated molecular pattern of Lactobacillus rhamnosus GG', *Microb Cell Fact,* 11, 161.

Clevers, H. C. and Bevins, C. L. (2013), 'Paneth cells: maestros of the small intestinal crypts', *Annu Rev Physiol,* 75, 289-311.

Cong, Yingzi, Feng, Ting, Fujihashi, Kohtaro, Schoeb, Trenton R., and Elson, Charles O. (2009), 'A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota', *Proc Natl Acad Sci USA,* 106 (46), 19256-61.

Coombes, J. L., Siddiqui, K. R., Arancibia-Carcamo, C. V., Hall, J., Sun, C. M., Belkaid, Y., and Powrie, F. (2007), 'A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism', *J Exp Med,* 204 (8), 1757-64.

De Keersmaecker, S. C., Verhoeven, T. L., Desair, J., Marchal, K., Vanderleyden, J., and Nagy, I. (2006), 'Strong antimicrobial activity of Lactobacillus rhamnosus GG against Salmonella typhimurium is due to accumulation of lactic acid', *FEMS Microbiol Lett,* 259 (1), 89-96.

Enaud, R., Vandenborght, L. E., Coron, N., Bazin, T., Prevel, R., Schaeverbeke, T., Berger, P., Fayon, M., Lamireau, T., and Delhaes, L. (2018), 'The Mycobiome: A Neglected Component in the Microbiota-Gut-Brain Axis', *Microorganisms,* 6 (1), 22.

Engle, M. J., Goetz, G. S., and Alpers, D. H. (1998), 'Caco-2 cells express a combination of colonocyte and enterocyte phenotypes', *J Cell Physiol,* 174 (3), 362-9.

Espey, M. G. (2013), 'Role of oxygen gradients in shaping redox relationships between the human intestine and its microbiota', *Free Radic Biol Med,* 55, 130-40.

Faith, J. J., Rey, F. E., O'Donnell, D., Karlsson, M., McNulty, N. P., Kallstrom, G., Goodman, A. L., and Gordon, J. I. (2010), 'Creating and characterizing communities of human gut microbes in gnotobiotic mice', *ISME J,* 4 (9), 1094-8.

Farache, J., Zigmond, E., Shakhar, G., and Jung, S. (2013), 'Contributions of dendritic cells and macrophages to intestinal homeostasis and immune defense', *Immunol Cell Biol,* 91 (3), 232-9.

Filler, S. G. and Sheppard, D. C. (2006), 'Fungal invasion of normally non-phagocytic host cells', *PLoS Pathog,* 2 (12), e129.

Fodor, A. A., DeSantis, T. Z., Wylie, K. M., Badger, J. H., Ye, Y., Hepburn, T., Hu, P., Sodergren, E., Liolios, K., Huot-Creasy, H., Birren, B. W., and Earl, A. M. (2012), 'The "most wanted" taxa from the human microbiome for whole genome sequencing', *PLoS One,* 7 (7), e41294.

Fogh, J., Fogh, J. M., and Orfeo, T. (1977), 'One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice', *J Natl Cancer Inst,* 59 (1), 221-6.

Fong, F. L. Y., Kirjavainen, P. V., and El-Nezami, H. (2016), 'Immunomodulation of Lactobacillus rhamnosus GG (LGG)-derived soluble factors on antigen-presenting cells of healthy blood donors', *Sci Rep,* 6, 22845.

Franklin, C. L. and Ericsson, A. C. (2017), 'Microbiota and reproducibility of rodent models', *Lab Anim (NY),* 46 (4), 114-22.

Friswell, M. K., Gika, H., Stratford, I. J., Theodoridis, G., Telfer, B., Wilson, I. D., and McBain, A. J. (2010), 'Site and strain-specific variation in gut microbiota profiles and metabolism in experimental mice', *PLoS One,* 5 (1), e8584.

Galloway, Tamara S. (2015), 'Micro- and Nano-plastics and Human Health', in Melanie Bergmann, Lars Gutow, and Michael Klages (eds.), *Marine Anthropogenic Litter* (Cham: Springer International Publishing), 343-66.

Gart, E. V., Suchodolski, J. S., Welsh, T. H., Jr., Alaniz, R. C., Randel, R. D., and Lawhon, S. D. (2016), 'Salmonella Typhimurium and Multidirectional Communication in the Gut', *Front Microbiol,* 7, 1827.

Gaudino, S. J. and Kumar, P. (2019), 'Cross-Talk Between Antigen Presenting Cells and T Cells Impacts Intestinal Homeostasis, Bacterial Infections, and Tumorigenesis', *Front Immunol,* 10, 360.

Gibbons, D. L. and Spencer, J. (2011), 'Mouse and human intestinal immunity: same ballpark, different players; different rules, same score', *Mucosal Immunol,* 4 (2), 148-57.

Gilbert, J. A., Blaser, M. J., Caporaso, J. G., Jansson, J. K., Lynch, S. V., and Knight, R. (2018), 'Current understanding of the human microbiome', *Nat Med,* 24 (4), 392-400.

Gouba, N. and Drancourt, M. (2015), 'Digestive tract mycobiota: a source of infection', *Med Mal Infect,* 45 (1-2), 9-16.

Graf, K., Last, A., Gratz, R., Allert, S., Linde, S., Westermann, M., Groger, M., Mosig, A. S., Gresnigt, M. S., and Hube, B. (2019), 'Keeping Candida commensal: how lactobacilli antagonize pathogenicity of Candida albicans in an in vitro gut model', *Dis Model Mech,* 12;12 (9).

Greenhalgh, K., Ramiro-Garcia, J., Heinken, A., Ullmann, P., Bintener, T., Pacheco, M. P., Baginska, J., Shah, P., Frachet, A., Halder, R., Fritz, J. V., Sauter, T., Thiele, I., Haan, S., Letellier, E., and Wilmes, P. (2019), 'Integrated In Vitro and In Silico Modeling Delineates the Molecular Effects of a Synbiotic Regimen on Colorectal-Cancer-Derived Cells', *Cell Rep,* 27 (5), 1621-32 e9.

Gren, S. T. and Grip, O. (2016), 'Role of Monocytes and Intestinal Macrophages in Crohn's Disease and Ulcerative Colitis', *Inflamm Bowel Dis,* 22 (8), 1992-8.

Groger, M., Rennert, K., Giszas, B., Weiss, E., Dinger, J., Funke, H., Kiehntopf, M., Peters, F. T., Lupp, A., Bauer, M., Claus, R. A., Huber, O., and Mosig, A. S. (2016), 'Monocyte-induced recovery of inflammation-associated hepatocellular dysfunction in a biochip-based human liver model', *Sci Rep,* 6, 21868.

Guinan, J., Wang, S., Hazbun, T. R., Yadav, H., and Thangamani, S. (2019), 'Antibiotic-induced decreases in the levels of microbial-derived short-chain fatty acids correlate with increased gastrointestinal colonization of Candida albicans', *Sci Rep,* 9 (1), 8872.

Hallen-Adams, H. E. and Suhr, M. J. (2017), 'Fungi in the healthy human gastrointestinal tract', *Virulence,* 8 (3), 352-58.

Hansson, G. C. and Johansson, M. E. (2010), 'The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria', *Gut Microbes,* 1 (1), 51-54.

Hasslöf, P., Hedberg, M., Twetman, S., and Stecksén-Blicks, C. (2010), 'Growth inhibition of oral mutans streptococci and candida by commercial probiotic lactobacilli--an in vitro study', *BMC Oral Health,* 10, 18.

Hegstrand, L. R. and Hine, R. J. (1986), 'Variations of brain histamine levels in germ-free and nephrectomized rats', *Neurochem Res,* 11 (2), 185-91.

Heintz-Buschart, A. and Wilmes, P. (2018), 'Human Gut Microbiome: Function Matters', *Trends Microbiol,* 26 (7), 563-74.

Hemmi, H. and Akira, S. (2005), 'TLR signalling and the function of dendritic cells', *Chem Immunol Allergy,* 86, 120-35.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., and Sanders, M. E. (2014), 'Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic', *Nat Rev Gastroenterol Hepatol,* 11 (8), 506-14.

Hill, D. R., Huang, S., Nagy, M. S., Yadagiri, V. K., Fields, C., Mukherjee, D., Bons, B., Dedhia, P. H., Chin, A. M., Tsai, Y. H., Thodla, S., Schmidt, T. M., Walk, S., Young, V. B., and Spence, J. R. (2017), 'Bacterial colonization stimulates a complex physiological response in the immature human intestinal epithelium', *Elife,* 6:e29132.

Hine, A. M. and Loke, P. (2019), 'Intestinal Macrophages in Resolving Inflammation', *J Immunol,* 203 (3), 593-99.

Holscher, H. D. (2017), 'Dietary fiber and prebiotics and the gastrointestinal microbiota', *Gut Microbes,* 8 (2), 172-84.

Hube, B. (2004), 'From commensal to pathogen: stage- and tissue-specific gene expression of Candida albicans', *Curr Opin Microbiol,* 7 (4), 336-41.

Hugenholtz, F. and de Vos, W. M. (2018), 'Mouse models for human intestinal microbiota research: a critical evaluation', *Cell Mol Life Sci,* 75 (1), 149-60.

Huh, D. D. (2015), 'A human breathing lung-on-a-chip', *Ann Am Thorac Soc,* 12 Suppl 1, S42-4.

Hwang, J., Choi, D., Han, S., Jung, S. Y., Choi, J., and Hong, J. (2020), 'Potential toxicity of polystyrene microplastic particles', *Sci Rep,* 10 (1), 7391.

Iliev, I. D., Spadoni, I., Mileti, E., Matteoli, G., Sonzogni, A., Sampietro, G. M., Foschi, D., Caprioli, F., Viale, G., and Rescigno, M. (2009), 'Human intestinal epithelial cells promote the differentiation of tolerogenic dendritic cells', *Gut,* 58 (11), 1481-9.

Juge, N. (2012), 'Microbial adhesins to gastrointestinal mucus', *Trends Microbiol,* 20 (1), 30-9.

Junttila, I. S. (2018), 'Tuning the Cytokine Responses: An Update on Interleukin (IL)-4 and IL-13 Receptor Complexes', *Front Immunol,* 9, 888.

Kalyan, S. and Kabelitz, D. (2014), 'When neutrophils meet T cells: beginnings of a tumultuous relationship with underappreciated potential', *Eur J Immunol,* 44 (3), 627-33.

Kankainen, M., Paulin, L., Tynkkynen, S., von Ossowski, I., Reunanen, J., Partanen, P., Satokari, R., Vesterlund, S., Hendrickx, A. P., Lebeer, S., De Keersmaecker, S. C., Vanderleyden, J., Hämäläinen, T., Laukkanen, S., Salovuori, N., Ritari, J., Alatalo, E., Korpela, R., Mattila-Sandholm, T., Lassig, A., Hatakka, K., Kinnunen, K. T., Karjalainen, H., Saxelin, M., Laakso, K., Surakka, A., Palva, A., Salusjärvi, T., Auvinen, P., and de Vos, W. M. (2009), 'Comparative genomic analysis of Lactobacillus rhamnosus GG reveals pili containing a human- mucus binding protein', *Proc Natl Acad Sci U S A,* 106 (40), 17193-8.

Kawamoto, S., Maruya, M., Kato, L. M., Suda, W., Atarashi, K., Doi, Y., Tsutsui, Y., Qin, H., Honda, K., Okada, T., Hattori, M., and Fagarasan, S. (2014), 'Foxp3(+) T cells regulate immunoglobulin a selection and facilitate diversification of bacterial species responsible for immune homeostasis', *Immunity,* 41 (1), 152-65.

Kelsall, B. (2008), 'Recent progress in understanding the phenotype and function of intestinal dendritic cells and macrophages', *Mucosal Immunol,* 1 (6), 460-9.

Khan, I., Ullah, N., Zha, L., Bai, Y., Khan, A., Zhao, T., Che, T., and Zhang, C. (2019), 'Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBD): Cause or Consequence? IBD Treatment Targeting the Gut Microbiome', *Pathogens,* 8 (3), 126.

Kim, H. J., Huh, D., Hamilton, G., and Ingber, D. E. (2012), 'Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow', *Lab Chip,* 12 (12), 2165-74.

Kim, J., Koo, B. K., and Knoblich, J. A. (2020), 'Human organoids: model systems for human biology and medicine', *Nat Rev Mol Cell Biol,* 21 (10), 571-84.

Klaassen, C. D. and Cui, J. Y. (2015), 'Review: Mechanisms of How the Intestinal Microbiota Alters the Effects of Drugs and Bile Acids', *Drug Metab Dispos,* 43 (10), 1505-21.

Kohler, G. A., Assefa, S., and Reid, G. (2012), 'Probiotic interference of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 with the opportunistic fungal pathogen Candida albicans', *Infect Dis Obstet Gynecol,* 2012, 636474.

Konturek, P. C., Harsch, I. A., Konturek, K., Schink, M., Konturek, T., Neurath, M. F., and Zopf, Y. (2018), 'Gut(-)Liver Axis: How Do Gut Bacteria Influence the Liver?', *Med Sci (Basel),* 6 (3), 79.

Kornitzer, D. (2019), 'Regulation of Candida albicans Hyphal Morphogenesis by Endogenous Signals', *J Fungi (Basel),* 5 (1), 21.

Kuhl, A. A., Erben, U., Kredel, L. I., and Siegmund, B. (2015), 'Diversity of Intestinal Macrophages in Inflammatory Bowel Diseases', *Front Immunol,* 6, 613.

Lavelle, E. C., Murphy, C., O'Neill, L. A., and Creagh, E. M. (2010), 'The role of TLRs, NLRs, and RLRs in mucosal innate immunity and homeostasis', *Mucosal Immunol,* 3 (1), 17-28.

LeBlanc, J. G., Milani, C., de Giori, G. S., Sesma, F., van Sinderen, D., and Ventura, M. (2013), 'Bacteria as vitamin suppliers to their host: a gut microbiota perspective', *Curr Opin Biotechnol,* 24 (2), 160-8.

Li, D., Gao, C., Zhang, F., Yang, R., Lan, C., Ma, Y., and Wang, J. (2020), 'Seven facts and five initiatives for gut microbiome research', *Protein Cell,* 11 (6), 391-400.

Liang, Y., Dong, T., Chen, M., He, L., Wang, T., Liu, X., Chang, H., Mao, J. H., Hang, B., Snijders, A. M., and Xia, Y. (2020), 'Systematic Analysis of Impact of Sampling Regions and Storage Methods on Fecal Gut Microbiome and Metabolome Profiles', *mSphere,* 5 (1), e00763-19.

Lloyd-Price, J., Abu-Ali, G., and Huttenhower, C. (2016), 'The healthy human microbiome', *Genome Med,* 8 (1), 51.

Lockhart, S. R. and Guarner, J. (2019), 'Emerging and reemerging fungal infections', *Semin Diagn Pathol,* 36 (3), 177-81.

Lu, Y. C., Yeh, W. C., and Ohashi, P. S. (2008), 'LPS/TLR4 signal transduction pathway', *Cytokine,* 42 (2), 145-51.

Luni, C., Serena, E., and Elvassore, N. (2014), 'Human-on-chip for therapy development and fundamental science', *Curr Opin Biotechnol,* 25, 45-50.

Ma, H., Tao, W., and Zhu, S. (2019), 'T lymphocytes in the intestinal mucosa: defense and tolerance', *Cell Mol Immunol,* 16 (3), 216-24.

Mabbott, N. A., Donaldson, D. S., Ohno, H., Williams, I. R., and Mahajan, A. (2013), 'Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium', *Mucosal Immunol,* 6 (4), 666-77.

Makras, L., Triantafyllou, V., Fayol-Messaoudi, D., Adriany, T., Zoumpopoulou, G., Tsakalidou, E., Servin, A., and De Vuyst, L. (2006), 'Kinetic analysis of the antibacterial activity of probiotic lactobacilli towards Salmonella enterica serovar Typhimurium reveals a role for lactic acid and other inhibitory compounds', *Res Microbiol,* 157 (3), 241-7.

Mangiola, F., Ianiro, G., Franceschi, F., Fagiuoli, S., Gasbarrini, G., and Gasbarrini, A. (2016), 'Gut microbiota in autism and mood disorders', *World J Gastroenterol,* 22 (1), 361-8.

Mann, E. R., Landy, J. D., Bernardo, D., Peake, S. T., Hart, A. L., Al-Hassi, H. O., and Knight, S. C. (2013), 'Intestinal dendritic cells: their role in intestinal inflammation, manipulation by the gut microbiota and differences between mice and men', *Immunol Lett,* 150 (1-2), 30-40.

Martin, R., Chamignon, C., Mhedbi-Hajri, N., Chain, F., Derrien, M., Escribano-Vazquez, U., Garault, P., Cotillard, A., Pham, H. P., Chervaux, C., Bermudez-Humaran, L. G., Smokvina, T., and Langella, P. (2019), 'The potential probiotic Lactobacillus rhamnosus CNCM I-3690 strain protects the intestinal barrier by stimulating both mucus production and cytoprotective response', *Sci Rep,* 9 (1), 5398.

Mathur, A., Loskill, P., Shao, K., Huebsch, N., Hong, S., Marcus, S. G., Marks, N., Mandegar, M., Conklin, B. R., Lee, L. P., and Healy, K. E. (2015), 'Human iPSC-based cardiac microphysiological system for drug screening applications', *Sci Rep,* 5, 8883.

Mestas, J. and Hughes, C. C. (2004), 'Of mice and not men: differences between mouse and human immunology', *J Immunol,* 172 (5), 2731-8.

Metidji, A., Omenetti, S., Crotta, S., Li, Y., Nye, E., Ross, E., Li, V., Maradana, M. R., Schiering, C., and Stockinger, B. (2018), 'The Environmental Sensor AHR Protects from Inflammatory Damage by Maintaining Intestinal Stem Cell Homeostasis and Barrier Integrity', *Immunity,* 49 (2), 353-62 e5.

Mileti, E., Matteoli, G., Iliev, I. D., and Rescigno, M. (2009), 'Comparison of the immunomodulatory properties of three probiotic strains of Lactobacilli using complex culture systems: prediction for in vivo efficacy', *PLoS One,* 4 (9), e7056.

Min, S., Kim, S., and Cho, S. W. (2020), 'Gastrointestinal tract modeling using organoids engineered with cellular and microbiota niches', *Exp Mol Med,* 52 (2), 227-37.

Miron, N. and Cristea, V. (2012), 'Enterocytes: active cells in tolerance to food and microbial antigens in the gut', *Clin Exp Immunol,* 167 (3), 405-12.

Miyauchi, E., Morita, H., and Tanabe, S. (2009), 'Lactobacillus rhamnosus alleviates intestinal barrier dysfunction in part by increasing expression of zonula occludens-1 and myosin light-chain kinase in vivo', *J Dairy Sci,* 92 (6), 2400-8.

Mizuno, H., Arce, L., Tomotsune, K., Albarracin, L., Funabashi, R., Vera, D., Islam, M. A., Vizoso-Pinto, M. G., Takahashi, H., Sasaki, Y., Kitazawa, H., and Villena, J. (2020), 'Lipoteichoic Acid Is Involved in the Ability of the Immunobiotic Strain Lactobacillus plantarum CRL1506 to Modulate the Intestinal Antiviral Innate Immunity Triggered by TLR3 Activation', *Front Immunol,* 11, 571.

Monteagudo-Mera, Andrea, Rastall, Robert A., Gibson, Glenn R., Charalampopoulos, Dimitris, and Chatzifragkou, Afroditi (2019), 'Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health', *Appl Microbiol Biotechnol,* 103 (16), 6463-72.

Mosig, Alexander S. (2016), 'Organ-on-chip models: new opportunities for biomedical research', *Future science OA,* 3 (2), FSO130-FSO30.

Mowat, A. M. (2003), 'Anatomical basis of tolerance and immunity to intestinal antigens', *Nat Rev Immunol,* 3 (4), 331-41.

Mowat, A. M. and Agace, W. W. (2014), 'Regional specialization within the intestinal immune system', *Nat Rev Immunol,* 14 (10), 667-85.

Muehlhoefer, A., Saubermann, L. J., Gu, X., Luedtke-Heckenkamp, K., Xavier, R., Blumberg, R. S., Podolsky, D. K., MacDermott, R. P., and Reinecker, H. C. (2000), 'Fractalkine is an epithelial and endothelial cell-derived chemoattractant for intraepithelial lymphocytes in the small intestinal mucosa', *J Immunol,* 164 (6), 3368-76.

Muller, P. A., Koscso, B., Rajani, G. M., Stevanovic, K., Berres, M. L., Hashimoto, D., Mortha, A., Leboeuf, M., Li, X. M., Mucida, D., Stanley, E. R., Dahan, S., Margolis, K. G., Gershon, M. D., Merad, M., and Bogunovic, M. (2014), 'Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility', *Cell,* 158 (2), 300-13.

Nagashima, R., Maeda, K., Imai, Y., and Takahashi, T. (1996), 'Lamina propria macrophages in the human gastrointestinal mucosa: their distribution, immunohistological phenotype, and function', *J Histochem Cytochem,* 44 (7), 721-31.

Nakamura, Kiminori, Sakuragi, Naoya, Takakuwa, Akiko, and Ayabe, Tokiyoshi (2016), 'Paneth cell α-defensins and enteric microbiota in health and disease', *Biosci Microbiota Food Health,* 35 (2), 57-67.

Ni, K. and O'Neill, H. C. (1997), 'The role of dendritic cells in T cell activation', *Immunol Cell Biol,* 75 (3), 223-30.

Niess, J. H. (2010), 'What are CX3CR1+ mononuclear cells in the intestinal mucosa?', *Gut Microbes,* 1 (6), 396-400.

Niess, J. H., Brand, S., Gu, X., Landsman, L., Jung, S., McCormick, B. A., Vyas, J. M., Boes, M., Ploegh, H. L., Fox, J. G., Littman, D. R., and Reinecker, H. C. (2005), 'CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance', *Science,* 307 (5707), 254-8.

Oedit, A., Vulto, P., Ramautar, R., Lindenburg, P. W., and Hankemeier, T. (2015), 'Lab-on-a-Chip hyphenation with mass spectrometry: strategies for bioanalytical applications', *Curr Opin Biotechnol,* 31, 79-85.

Ogino, T., Nishimura, J., Barman, S., Kayama, H., Uematsu, S., Okuzaki, D., Osawa, H., Haraguchi, N., Uemura, M., Hata, T., Takemasa, I., Mizushima, T., Yamamoto, H., Takeda, K., Doki, Y., and Mori, M. (2013), 'Increased Th17-inducing activity of CD14+ CD163 low myeloid cells in intestinal lamina propria of patients with Crohn's disease', *Gastroenterology,* 145 (6), 1380-91 e1.

Okumura, R. and Takeda, K. (2016), 'Maintenance of gut homeostasis by the mucosal immune system', *Proc Jpn Acad Ser B Phys Biol Sci,* 92 (9), 423-35.

Oliveira-Nascimento, L., Massari, P., and Wetzler, L. M. (2012), 'The Role of TLR2 in Infection and Immunity', *Front Immunol,* 3, 79.

Onali, S., Favale, A., and Fantini, M. C. (2019), 'The Resolution of Intestinal Inflammation: The Peace-Keeper's Perspective', *Cells,* 8 (4), 344.

Pachot, A., Cazalis, M. A., Venet, F., Turrel, F., Faudot, C., Voirin, N., Diasparra, J., Bourgoin, N., Poitevin, F., Mougin, B., Lepape, A., and Monneret, G. (2008), 'Decreased expression of the fractalkine receptor CX3CR1 on circulating monocytes as new feature of sepsis-induced immunosuppression', *J Immunol,* 180 (9), 6421-9.

Park, J. H., Kotani, T., Konno, T., Setiawan, J., Kitamura, Y., Imada, S., Usui, Y., Hatano, N., Shinohara, M., Saito, Y., Murata, Y., and Matozaki, T. (2016), 'Promotion of Intestinal Epithelial Cell Turnover by Commensal Bacteria: Role of Short-Chain Fatty Acids', *PLoS One,* 11 (5), e0156334.

Patten, D. A. and Collett, A. (2013), 'Exploring the immunomodulatory potential of microbial-associated molecular patterns derived from the enteric bacterial microbiota', *Microbiology (Reading),* 159 (Pt 8), 1535-44.

Patterson, E., Ryan, P. M., Cryan, J. F., Dinan, T. G., Ross, R. P., Fitzgerald, G. F., and Stanton, C. (2016), 'Gut microbiota, obesity and diabetes', *Postgrad Med J,* 92 (1087), 286-300.

Poretsky, R., Rodriguez, R. Lm, Luo, C., Tsementzi, D., and Konstantinidis, K. T. (2014), 'Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics', *PLoS One,* 9 (4), e93827.

Possemiers, S., Bolca, S., Verstraete, W., and Heyerick, A. (2011), 'The intestinal microbiome: a separate organ inside the body with the metabolic potential to influence the bioactivity of botanicals', *Fitoterapia,* 82 (1), 53-66.

Pull, S. L., Doherty, J. M., Mills, J. C., Gordon, J. I., and Stappenbeck, T. S. (2005), 'Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury', *Proc Natl Acad Sci U S A,* 102 (1), 99-104.

Qu, C., Moran, T. M., and Randolph, G. J. (2003), 'Autocrine type I IFN and contact with endothelium promote the presentation of influenza A virus by monocyte-derived APC', *J Immunol,* 170 (2), 1010-8.

Qu, C., Brinck-Jensen, N. S., Zang, M., and Chen, K. (2014), 'Monocyte-derived dendritic cells: targets as potent antigen-presenting cells for the design of vaccines against infectious diseases', *Int J Infect Dis,* 19, 1-5.

Randolph, G. J., Sanchez-Schmitz, G., Liebman, R. M., and Schakel, K. (2002), 'The CD16(+) (FcgammaRIII(+)) subset of human monocytes preferentially becomes migratory dendritic cells in a model tissue setting', *J Exp Med,* 196 (4), 517-27.

Randolph, G. J., Inaba, K., Robbiani, D. F., Steinman, R. M., and Muller, W. A. (1999), 'Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo', *Immunity,* 11 (6), 753-61.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J. P., and Ricciardi-Castagnoli, P. (2001), 'Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria', *Nat Immunol,* 2 (4), 361-7.

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A. D., Gasbarrini, A., and Mele, M. C. (2019), 'What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases', *Microorganisms,* 7 (1), 14.

Rocha-Ramirez, L. M., Perez-Solano, R. A., Castanon-Alonso, S. L., Moreno Guerrero, S. S., Ramirez Pacheco, A., Garcia Garibay, M., and Eslava, C. (2017), 'Probiotic Lactobacillus Strains Stimulate the Inflammatory Response and Activate Human Macrophages', *J Immunol Res,* 2017, 4607491.

Romani, N., Gruner, S., Brang, D., Kämpgen, E., Lenz, A., Trockenbacher, B., Konwalinka, G., Fritsch, P. O., Steinman, R. M., and Schuler, G. (1994), 'Proliferating dendritic cell progenitors in human blood', *J Exp Med,* 180 (1), 83-93.

Roosenboom, B., Wahab, P. J., Smids, C., Groenen, M. J. M., van Koolwijk, E., van Lochem, E. G., and Horjus Talabur Horje, C. S. (2019), 'Intestinal CD103+CD4+ and CD103+CD8+ T-Cell Subsets in the Gut of Inflammatory Bowel Disease Patients at Diagnosis and During Follow-up', *Inflamm Bowel Dis,* 25 (9), 1497-509.

Rubin, D. C., Shaker, A., and Levin, M. S. (2012), 'Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer', *Front Immunol,* 3, 107.

Sadaghian Sadabad, M., von Martels, J. Z., Khan, M. T., Blokzijl, T., Paglia, G., Dijkstra, G., Harmsen, H. J., and Faber, K. N. (2015), 'A simple coculture system shows mutualism between anaerobic faecalibacteria and epithelial Caco-2 cells', *Sci Rep,* 5, 17906.

Sallusto, F. and Lanzavecchia, A. (1994), 'Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha', *J Exp Med,* 179 (4), 1109-18.

Salzman, N. H., Underwood, M. A., and Bevins, C. L. (2007), 'Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa', *Semin Immunol,* 19 (2), 70-83.

Salzman, N. H., Hung, K., Haribhai, D., Chu, H., Karlsson-Sjoberg, J., Amir, E., Teggatz, P., Barman, M., Hayward, M., Eastwood, D., Stoel, M., Zhou, Y., Sodergren, E., Weinstock, G. M., Bevins, C. L., Williams, C. B., and Bos, N. A. (2010), 'Enteric defensins are essential regulators of intestinal microbial ecology', *Nat Immunol,* 11 (1), 76-83.

Sambuy, Yula, Angelis, Isabella, Ranaldi, Giulia, Scarino, M. L., Stammati, A., and Zucco, F. (2005), 'The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics', *Cell Biol Toxicol,* 21, 1-26.

Sato, T., van Es, J. H., Snippert, H. J., Stange, D. E., Vries, R. G., van den Born, M., Barker, N., Shroyer, N. F., van de Wetering, M., and Clevers, H. (2011a), 'Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts', *Nature,* 469 (7330), 415-8.

Sato, T., Stange, D. E., Ferrante, M., Vries, R. G., Van Es, J. H., Van den Brink, S., Van Houdt, W. J., Pronk, A., Van Gorp, J., Siersema, P. D., and Clevers, H. (2011b), 'Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium', *Gastroenterology,* 141 (5), 1762-72.

Schulz, O., Jaensson, E., Persson, E. K., Liu, X., Worbs, T., Agace, W. W., and Pabst, O. (2009), 'Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions', *J Exp Med,* 206 (13), 3101-14.

Segers, M. E. and Lebeer, S. (2014), 'Towards a better understanding of Lactobacillus rhamnosus GG--host interactions', *Microb Cell Fact,* 13, S7.

Shah, P., Fritz, J. V., Glaab, E., Desai, M. S., Greenhalgh, K., Frachet, A., Niegowska, M., Estes, M., Jager, C., Seguin-Devaux, C., Zenhausern, F., and Wilmes, P. (2016), 'A microfluidics-based in vitro model of the gastrointestinal human-microbe interface', *Nat Commun,* 7, 11535.

Shin, W., Hinojosa, C. D., Ingber, D. E., and Kim, H. J. (2019), 'Human Intestinal Morphogenesis Controlled by Transepithelial Morphogen Gradient and Flow-Dependent Physical Cues in a Microengineered Gut-on-a-Chip', *iScience,* 15, 391-406.

Shin, Woojung and Kim, Hyun Jung (2018), 'Intestinal barrier dysfunction orchestrates the onset of inflammatory host–microbiome cross-talk in a human gut inflammation-on-a-chip', *Proc Natl Acad Sci USA,* 115 (45), E10539-E47.

Sicard, J. F., Le Bihan, G., Vogeleer, P., Jacques, M., and Harel, J. (2017), 'Interactions of Intestinal Bacteria with Components of the Intestinal Mucus', *Front Cell Infect Microbiol,* 7, 387.

Smith, P. D., Smythies, L. E., Mosteller-Barnum, M., Sibley, D. A., Russell, M. W., Merger, M., Sellers, M. T., Orenstein, J. M., Shimada, T., Graham, M. F., and Kubagawa, H. (2001), 'Intestinal macrophages lack CD14 and CD89 and consequently are down-regulated for LPS- and IgA-mediated activities', *J Immunol,* 167 (5), 2651-6.

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly, Y. M., Glickman, J. N., and Garrett, W. S. (2013), 'The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis', *Science,* 341 (6145), 569-73.

Smythies, L. E., Sellers, M., Clements, R. H., Mosteller-Barnum, M., Meng, G., Benjamin, W. H., Orenstein, J. M., and Smith, P. D. (2005), 'Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity', *J Clin Invest,* 115 (1), 66-75.

Song, X., Dai, D., He, X., Zhu, S., Yao, Y., Gao, H., Wang, J., Qu, F., Qiu, J., Wang, H., Li, X., Shen, N., and Qian, Y. (2015), 'Growth Factor FGF2 Cooperates with Interleukin-17 to Repair Intestinal Epithelial Damage', *Immunity,* 43 (3), 488-501.

Sonnenburg, J. L. and Backhed, F. (2016), 'Diet-microbiota interactions as moderators of human metabolism', *Nature,* 535 (7610), 56-64.

Spence, J. R., Mayhew, C. N., Rankin, S. A., Kuhar, M. F., Vallance, J. E., Tolle, K., Hoskins, E. E., Kalinichenko, V. V., Wells, S. I., Zorn, A. M., Shroyer, N. F., and Wells, J. M. (2011), 'Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro', *Nature,* 470 (7332), 105-9.

Spielman, L. J., Gibson, D. L., and Klegeris, A. (2018), 'Unhealthy gut, unhealthy brain: The role of the intestinal microbiota in neurodegenerative diseases', *Neurochem Int,* 120, 149-63.

Steele-Mortimer, O. (2008), 'The Salmonella-containing vacuole: moving with the times', *Curr Opin Microbiol,* 11 (1), 38-45.

Suez, J., Zmora, N., Segal, E., and Elinav, E. (2019), 'The pros, cons, and many unknowns of probiotics', *Nat Med,* 25 (5), 716-29.

Sun, H., Chow, E. C., Liu, S., Du, Y., and Pang, K. S. (2008), 'The Caco-2 cell monolayer: usefulness and limitations', *Expert Opin Drug Metab Toxicol,* 4 (4), 395-411.

Swain, S., Roe, M. M., Sebrell, T. A., Sidar, B., Dankoff, J., VanAusdol, R., Smythies, L. E., Smith, P. D., and Bimczok, D. (2018), 'CD103 (alphaE Integrin) Undergoes Endosomal Trafficking in Human Dendritic Cells, but Does Not Mediate Epithelial Adhesion', *Front Immunol,* 9, 2989.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007), 'Induction of pluripotent stem cells from adult human fibroblasts by defined factors', *Cell,* 131 (5), 861-72.

Tang, D., Kang, R., Coyne, C. B., Zeh, H. J., and Lotze, M. T. (2012), 'PAMPs and DAMPs: signal 0s that spur autophagy and immunity', *Immunol Rev,* 249 (1), 158-75.

Thaiss, C. A., Zmora, N., Levy, M., and Elinav, E. (2016), 'The microbiome and innate immunity', *Nature,* 535 (7610), 65-74.

Tong, Y. and Tang, J. (2017), 'Candida albicans infection and intestinal immunity', *Microbiol Res,* 198, 27-35.

Toscano, M, De Grandi, R, Stronati, L, De Vecchi, E, and Drago, L (2017), 'Effect of Lactobacillus rhamnosus HN001 and Bifidobacterium longum BB536 on the healthy gut microbiota composition at phyla and species level: A preliminary study', *World J Gastroenterol,* 23 (15), 2696-704.

Tso, G. H. W., Reales-Calderon, J. A., Tan, A. S. M., Sem, X., Le, G. T. T., Tan, T. G., Lai, G. C., Srinivasan, K. G., Yurieva, M., Liao, W., Poidinger, M., Zolezzi, F., Rancati, G., and Pavelka, N. (2018), 'Experimental evolution of a fungal pathogen into a gut symbiont', *Science,* 362 (6414), 589-95.

Vachon, P. H. and Beaulieu, J. F. (1992), 'Transient mosaic patterns of morphological and functional differentiation in the Caco-2 cell line', *Gastroenterology,* 103 (2), 414-23.

Vaishnava, S., Behrendt, C. L., Ismail, A. S., Eckmann, L., and Hooper, L. V. (2008), 'Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface', *Proc Natl Acad Sci U S A,* 105 (52), 20858-63.

Valík, L., Medveďová, A., and Liptakova, D. (2008), 'Characterization of the growth of Lactobacillus rhamnosus GG in milk at suboptimal temperatures', *J Food Nutr Res,* 47 (2), 60-67.

Veckman, V., Miettinen, M., Matikainen, S., Lande, R., Giacomini, E., Coccia, E. M., and Julkunen, I. (2003), 'Lactobacilli and streptococci induce inflammatory chemokine production in human macrophages that stimulates Th1 cell chemotaxis', *J Leukoc Biol,* 74 (3), 395-402.

Viola, M. F. and Boeckxstaens, G. (2020), 'Intestinal resident macrophages: Multitaskers of the gut', *Neurogastroenterol Motil,* 32 (8), e13843.

Vizoso Pinto, M. G., Rodriguez Gomez, M., Seifert, S., Watzl, B., Holzapfel, W. H., and Franz, C. M. (2009), 'Lactobacilli stimulate the innate immune response and modulate the TLR expression of HT29 intestinal epithelial cells in vitro', *Int J Food Microbiol,* 133 (1-2), 86-93.

Weiss, G. A. and Hennet, T. (2017), 'Mechanisms and consequences of intestinal dysbiosis', *Cell Mol Life Sci,* 74 (16), 2959-77.

White, Charles R. and Frangos, John A. (2007), 'The shear stress of it all: the cell membrane and mechanochemical transduction', *Philos Trans R Soc Lond B Biol Sci* 362 (1484), 1459-67.

Winter, S. E., Thiennimitr, P., Winter, M. G., Butler, B. P., Huseby, D. L., Crawford, R. W., Russell, J. M., Bevins, C. L., Adams, L. G., Tsolis, R. M., Roth, J. R., and Baumler, A. J. (2010), 'Gut inflammation provides a respiratory electron acceptor for Salmonella', *Nature,* 467 (7314), 426-9.

Wittkopf, N., Neurath, M. F., and Becker, C. (2014), 'Immune-epithelial crosstalk at the intestinal surface', *J Gastroenterol,* 49 (3), 375-87.

Workman, M. J., Mahe, M. M., Trisno, S., Poling, H. M., Watson, C. L., Sundaram, N., Chang, C. F., Schiesser, J., Aubert, P., Stanley, E. G., Elefanty, A. G., Miyaoka, Y., Mandegar, M. A., Conklin, B. R., Neunlist, M., Brugmann, S. A., Helmrath, M. A., and Wells, J. M. (2017), 'Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system', *Nat Med,* 23 (1), 49-59.

Worthington, J. J., Reimann, F., and Gribble, F. M. (2018), 'Enteroendocrine cells-sensory sentinels of the intestinal environment and orchestrators of mucosal immunity', *Mucosal Immunol,* 11 (1), 3-20.

Wright, S. D., Ramos, R. A., Tobias, P. S., Ulevitch, R. J., and Mathison, J. C. (1990), 'CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein', *Science,* 249 (4975), 1431-3.

Zeuthen, L. H., Fink, L. N., and Frokiaer, H. (2008), 'Epithelial cells prime the immune response to an array of gut-derived commensals towards a tolerogenic phenotype through distinct actions of thymic stromal lymphopoietin and transforming growth factor-beta', *Immunology,* 123 (2), 197-208.

Zhang, Y. G., Wu, S., Xia, Y., and Sun, J. (2014), 'Salmonella-infected crypt-derived intestinal organoid culture system for host-bacterial interactions', *Physiol Rep,* 2 (9), e12147.

Zheng, L., Kelly, C. J., Battista, K. D., Schaefer, R., Lanis, J. M., Alexeev, E. E., Wang, R. X., Onyiah, J. C., Kominsky, D. J., and Colgan, S. P. (2017), 'Microbial-Derived Butyrate Promotes Epithelial Barrier Function through IL-10 Receptor-Dependent Repression of Claudin-2', *J Immunol,* 199 (8), 2976-84.

Zheng, Leon, Kelly, Caleb J., and Colgan, Sean P. (2015), 'Physiologic hypoxia and oxygen homeostasis in the healthy intestine. A Review in the Theme: Cellular Responses to Hypoxia', *Am J Physiol Cell Physiol,* 309 (6), C350-C60.

B LIST OF FIGURES

C ACKNOLEDGEMENT

Zuerst möchte ich mich bei PD Dr. Alexander S. Mosig dafür bedanken, meine Master- und Doktorarbeit in seiner Arbeitsgruppe schreiben zu können, die vielen interessanten Diskussionen und die Ermöglichung meines Frankreichaufenthaltes.

Außerdem bedanke ich mich bei meinem Betreuer Prof. Dr. Otmar Huber und meiner Betreuerin PD Dr. Lorena Tuchscherr de Hauschopp für viele hilfreiche Hinweise und die Unterstützung während der Promotion.

Mein Dank geht auch an Prof. Dr. Bernhard Hube und Jun.-Prof. Dr. Peter Loskill für die Begutachtung meiner Arbeit.

Ich danke Antonia Last, Dr. Mark S. Gresnigt und Prof. Dr. Bernhard Hube für die angenehme und erfolgreiche Zusammenarbeit an zwei Publikationen.

Weiterhin bedanke ich mich bei der gesamten Arbeitsgruppe INSPIRE für die gute Arbeitsatmosphäre und das Mensa-Spiel.

Insbesondere will ich mich bei Marko Gröger für das Aushalten meiner Launen nach gescheiterten Versuchen und seine unglaublich gute Betreuung über drei Jahre hinweg bedanken. Genauso wichtig waren aber auch die zahllosen Gespräche über Gott und die Welt mit Melanie Ulrich.

Eine großartige Stütze waren ebenfalls die Jena Jobberknolls, die mir einen perfekten Ausgleich zum Laboralltag boten.

Ich bedanke mich bei meinen Eltern Petra und Rainer, die mich immer meinen eigenen Weg gehen ließen und mich bei all meinen Vorhaben unterstützten.

Ich danke meinen langjährigen Freundinnen und Freunden und jenen, die ich entlang des Weges noch kennen lernen durfte. Ganz besonders danke ich Adrian Saal für die Unterstützung mit Katzenmemes und Linsenchips auf den letzten Metern.

Danke an alle Korrekturleser*innen!

Merci!

D CURRICULUM VITAE

E LIST OF PUBLICATIONS

1. Maurer M, Gresnigt MS, Last A, Wollny T, Berlinghof F, Pospich R, Cseresnyes Z, Medyukhina A, Graf K, Gröger M, Raasch M, Siwczak F, Nietzsche S, Jacobsen ID, Figge MT, Hube B, Huber O, Mosig AS. “A three-dimensional immunocompetent intestine-on-chip model as *in vitro* platform for functional and microbial interaction studies”, *Biomaterials*. **2019**, 220:119396.

2. Last A, Maurer M, Mosig AS, Gresnigt MS, Hube B. “*In vitro* infection models to study fungal-host interactions”, *FEMS Microbiology Reviews*. **2021**, fuab005, 1–22

F AUTHOR CONTRIBUTION STATEMENT

Manuscript I:

Michelle Maurer, Mark S. Gresnigt, Antonia Last, Tony Wollny, Florian Berlinghof, Rebecca Pospich, Zoltan Cseresnyes, Anna Medyukhina, Katja Graf, Marko Gröger, Martin Raasch, Fatina Siwczak, Sandor Nietzsche, Ilse D. Jacobsen, Marc Thilo Figge, Bernhard Hube, Otmar Huber, Alexander S. Mosig. 2019. A three-dimensional immunocompetent intestine-on-chip model as *in vitro* platform for functional and microbial interaction studies. Biomaterials, 220:119396.

Author	**Contribution**
Michelle Maurer	Integration and characterization of immune cells, characterization of endothelial and epithelial barrier function, establishment of *L. rhamnosus* colonization protocol, establishment of PBMC-perfusion, evaluation of image analysis data, cytokine measurement, writing parts of the manuscript
Mark S. Gresnigt	Establishment of the *C. albicans* infection protocol and interaction studies, writing parts of the manuscript
Antonia Last	Establishment of the *C. albicans* infection protocol and interaction studies, writing parts of the manuscript
Tony Wollny	Establishment of epithelial cell layers, performing experimentation for electron microscopy analysis
Florian Berlinghof	Establishment of epithelial cell layers
Rebecca Pospich	Establishment of the *C. albicans* infection protocol
Zoltan Cseresnyes	Image analysis and quantification strategies
Anna Medyukhina	Establishment of image analysis algorithms
Katja Graf	Support of microbiological experimentation
Marko Gröger	Support for establishment of the model
Martin Raasch	Support for establishment of the model
Fatina Siwczak	Support for establishment of the model
Sandor Nietzsche	Electron microscopy images
Ilse D. Jacobsen	Establishment of the *C. albicans* infection protocol
Marc Thilo Figge	Image analysis and quantification strategies
Bernhard Hube	Establishment of the *C. albicans* infection protocol
Otmar Huber	Supervision of the study
Alexander S. Mosig	Design and supervision of the study, writing the manuscript

Manuscript II:

Antonia Last, Michelle Maurer, Alexander S. Mosig, Mark S. Gresnigt, and Bernhard Hube. 2021. *In vitro* infection models to study fungal-host interactions. FEMS Microbiology Reviews, fuab005, 1–22.

Author	**Contribution**
Antonia Last	Conception of the review, writing large parts of the manuscript, preparation of Fig. 1
Michelle Maurer	Writing large parts of the manuscript, preparation of Fig. 1 and Fig. 2
Mark S. Gresnigt	Conception of the review, support of writing the manuscript
Bernhard Hube	Supervision of the review
Alexander S. Mosig	Supervision of the review

G EHRENWÖRTLICHE ERKLÄRUNG

Hiermit erkläre ich, dass mir die Promotionsordnung der Medizinischen Fakultät der Friedrich-Schiller-Universität bekannt ist,

ich die Dissertation selbst angefertigt habe und alle von mir benutzten Hilfsmittel, persönlichen Mitteilungen und Quellen in meiner Arbeit angegeben sind,

mich folgende Personen bei der Auswahl und Auswertung des Materials sowie bei der Herstellung des Manuskripts unterstützt haben: PD Dr. Alexander S. Mosig und Dr. Mark S. Gresnigt,

die Hilfe eines Promotionsberaters nicht in Anspruch genommen wurde und dass Dritte weder unmittelbar noch mittelbar geldwerte Leistungen von mir für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen,

dass ich die Dissertation noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht habe und dass ich die gleiche, eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung nicht bei einer anderen Hochschule als Dissertation eingereicht habe.

Ort, Datum Unterschrift von Michelle Maurer