Impact of the fungal quorum sensing molecule farnesol on human innate immune cells

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Summary

Microorganisms are able to perceive and respond to changing microbial population density with the help of small signaling molecules. This cell density-dependent mechanism is called quorum sensing (QS) and affects microbial metabolism and gene expression. In fungi, the QS phenomenon has first been described for the opportunistic pathogen *C. albicans*, which produces farnesol as a QS molecule to regulate virulence determinants. Aside from controlling morphogenesis, the impact of farnesol throughout the infection process has been only partially elucidated. The aim of this study was to unravel the impact of farnesol on innate immune cells important for fungal clearance and protective immune response. Therefore, neutrophils, monocytes and dendritic cells (DC) were investigated concerning phenotype and effector mechanisms. The results of this study reveal a novel function of farnesol as an immunomodulatory compound. It activated neutrophils and monocytes, reflected in an enhanced expression of activation markers on the surface of both immune cells and in an increased release of proteolytic enzymes by neutrophils and pro-inflammatory cytokines by monocytes. This low-grade activation was not sufficient to influence fungicidal activity of neutrophils and monocytes towards *C. albicans*. The greatest impact of farnesol was found on the differentiation process of human monocytes into mature DC. Substantial changes were observed to the differentiation phenotype characterized by reduced expression of markers important for maturation and antigen presentation (CD1a, HLA-DR, CD83, CD86 and CD80). Furthermore, farnesol influenced the release of a variety of inflammatory cytokines and chemokines, wherein the secretion of Th1-inducing cytokines such as IL-12 were markedly reduced. Limitations in mobility and a reduced T cell stimulatory capacity of DC generated in the presence of farnesol were ascertained and suggest an immunomodulatory function of farnesol. Supporting transcriptomic analyses showed large-scale alterations and a shift in DC effector molecule expression that revealed potential mechanisms addressed by farnesol.

Altogether, this study promotes the ability of farnesol to act as a virulence factor of *C. albicans* by influencing innate immune cells to promote inflammation and dampening the Th1 response which is essential for fungal clearance.
Zusammenfassung


1 Introduction

1.1 Quorum sensing

Over the past decades it has become clear that microorganisms are capable of communicating with each other by using small molecules. This communication has been termed quorum sensing (QS) and was found to be fundamental for coordinated behavior in a microbial population. QS was first reported in the 1970s in *Vibrio fischeri*, a symbiotic bioluminescent bacterium that colonizes the light organs of marine fish and squid. Nealson and co-workers examined the phenomenon of bioluminescence in these marine bacteria and found a cell-dependent regulation of the genes for bacterial luciferase (1, 2). *V. fischeri* produces freely diffusible signaling molecules, so called autoinducer (AI), later identified as acylated homoserine lactone that accumulate in the environment, reach a “quorum” and subsequently lead to an alteration in gene expression (1, 3-5). Thus, *V. fischeri*, when powered by the host with nutrients, exceeds a certain population cell density followed by induction of the luciferase complex. The produced light is utilized by the host for its own survival tactics (2). Since then, QS has been described in numerous Gram-positive and Gram-negative bacterial species and QS-regulated behavior is extremely diverse (6). Each organism has specific signaling molecules associated with distinct signal synthase and binding proteins. Processes that are regulated by cell density include bioluminescence, virulence, antibiotic production, sporulation and biofilm development (6-10). In general, most of the Gram-negative bacteria synthesize primarily N-acyl homoserine lactones (AHL) (6). So far, one of the best characterized QS systems in Gram-negative bacteria belongs to *Pseudomonas aeruginosa*, an opportunistic human pathogen notable as the main contributor to cystic fibrosis related bacterial lung infections and a frequent cause of nosocomial infections (11). *P. aeruginosa* possesses two separate QS signaling systems las and rhl. The las system consists of the LasR transcription regulator protein and its AI signal molecule N-(3-oxododecanoyl) homoserine lactone (HSL). The second QS circuit is composed of the RhlR transcription regulator and the signal N-butyryl homoserine lactone. These two signaling circuits are arranged in tandem and control the expression of genes involved in virulence and biofilm production (6). A third intercellular signal, the *Pseudomonas* quinolone signal (PQS), also regulates numerous virulence factors and is considered as a regulatory link between the two systems (12-14).
In Gram-positive bacteria cell-cell communication is regulated differently via a two-component signal transduction system (6). In contrast to Gram-negative bacteria QS is mediated by processed oligopeptides (Figure 1). The auto-inducing peptides (AIP) are detected by a two-component adaptive response kinase. Mechanistically, a phosphorylation/dephosphorylation cascade results in activation of response regulators, which in turn alter the expression of a large set of target genes. A well-studied example of peptide-mediated quorum sensing exists in Staphylococcus aureus, a human commensal, which can cause superficial or invasive infections (16, 17). This opportunistic pathogen regulates a variety of virulence factors by peptide QS, e.g. synthesis of hemolysins (18, 19). The system consists of an auto-inducing peptide (AIP) and a two component sensor kinase-response regulator pair AgrC and AgrA. The production of all these factors is controlled by the regulatory locus agr (18, 20).

Figure 1 QS system of Gram-negative and Gram-positive bacteria.
(A) The Gram-negative QS system uses two regulator proteins that have homologs in other QS systems, LuxI and LuxR. LuxI synthesizes the N-acyl homoserine lactones (AHL) signal, so-called autoinducer (AI) that then diffuses out of the cell. With increasing cell density AHL concentration reaches a threshold concentration and binds to LuxR. LuxR then induces expression of a set of genes. (B) In contrast, the system of Gram-positive bacteria is mainly based on a two-component signal transduction system, using an auto-inducing peptide (AIP) as signal. While AHL can diffuse easily into and out of the cell, AIP molecules need to be released via a transporter protein. AIP molecules are not directly interfering with a transcription activator. They bind to a histidine kinase receptor, which leads to phosphorylation of the response regulator. The response regulator functions as a DNA-binding transcription factor and modulates gene expression. Figure modified after (15).
1.2 *Candida albicans* and quorum sensing

Until the discovery of farnesol (FOH) as a quorum sensing molecule (QSM) of *C. albicans*, QS was thought to be confined to bacteria (21). *C. albicans* is an opportunistic fungal pathogen and a commensal of the gastrointestinal tract, the vaginal mucosa and the oral cavity (22). To colonize these niches, the fungus needs to adapt to multiple environmental conditions e.g. different pH, oxygen levels or microbial flora. Furthermore, during infection, expression of virulence factors including adhesins, biofilm formation, phenotypic switching, polymorphism or the production of secreted proteases is regulated in response to environmental conditions (23). In healthy immunocompetent hosts proliferation and invasion of the fungus is prevented by mechanical barriers, humoral and cellular immunity as well as by other microorganisms of the microbial flora. In contrast, in an immunocompromised host and in a variety of other permissive circumstances, e.g. imbalance of the microflora, *C. albicans* can cause superficial and invasive opportunistic infections (24, 25). The ability to adhere to surfaces is the most important prerequisite for *C. albicans* to survive as a commensal and as a pathogen in the host. Specific proteins on the cell surface of *C. albicans*, so-called adhesins, enable the fungus to adhere and invade epithelial and endothelial cells via induced endocytosis or active penetration (26). Furthermore, effective adhesion allows biofilm formation on artificial surfaces such as implanted medical devices e.g. shunts or catheters, which plays a role especially in nosocomial *Candida* infections (27-29). Important adhesins are Als3 and Hwp1, both expressed on the surface of *C. albicans* (30). Als3 belongs to the Als (agglutin-like sequence) family, binds to cadherins, induces endocytosis by the host cells and enables the supply of iron during infection (31-33). Together with the hyphae-associated GPI (glycophosphatidylinositol) linked protein Hwp1, it allows complementary binding during biofilm growth and contributes to the virulence of the fungus (34). Another important virulence factor of *C. albicans* is the release of various hydrolytic enzymes, such as phospholipases and proteases that can destroy host tissue (23, 35). *C. albicans* has at least ten secretory aspartic proteases (SAPs), which are expressed under various growth conditions (36, 37). They degrade host cell proteins for nutrient acquisition or to protect the fungus from complement factors (38-40). In general, the high extracellular proteolytic activity of *C. albicans* is associated with virulence (41). After adhesion, the ability of *C. albicans* to form filaments is critical for the invasiveness of the fungus and crucial to establish a systemic infection (42). Therefore, the morphological plasticity belongs to the major virulence traits of
C. albicans and has been extensively investigated. C. albicans is able to grow in four distinct morphological forms, as yeast, pseudohyphae, true hyphae and rarely as a chlamydospore, the first three types most likely play an important role in the process of infection (43). The yeast form is associated with colonization and dissemination, whereas the filamentous forms (pseudohyphae/hyphae) play a role in adhesion, penetration and invasion of tissues (44). Growth as a thick-walled round chlamydospore has not been reported as important for C. albicans pathogenicity. The transition from yeast to filamentous forms (pseudohyphae/hyphae) is associated with the expression of a set of virulence associated genes that mediate adhesion, invasion and acquisition of micronutrients and are important for the organism to survive in the host (45, 46). However, many genes and other factors, which play a role in morphogenesis, have not been identified yet. QS is one contributing phenomenon that has been described to control morphogenesis. C. albicans produces QSMs to auto-regulate filamentous growth. Aside farnesol other QSMs described in C. albicans are farnesoic acid and tyrosol (47). Like farnesol farnesoic acid blocks the switch from yeast to filamentous form but has only 3% of the activity compared to farnesol and is so far only secreted by a strain of C. albicans (ATCC 10231), that is not producing farnesol (48, 49). Tyrosol stimulates the switch from yeast to filamentous form by shorten the lag-time that is seen between this shift (50). Tyrosol is considered to play a minor role in regulation of morphogenesis, because its influence on C. albicans can only be seen when farnesol is limited or absent from the environment (51). Farnesol as a blocking molecule might consequently play a role in virulence of the fungus.

1.3 Farnesol – synthesis, properties and functions

Farnesol is an acyclic sesquiterpene (isoprenoid/ 1-hydroxy-3, 7, 11-trimethyl-2, 6, 10-dodecatrien). It was first discovered and isolated from plants and named after the Farnese acacia tree (Acacia famesiana). The isoprenoid can be extracted from oils of plants such as citronella, neroli, cyclamen, and tuberose or produced synthetically. Farnesol has long been used in cosmetics industry for skin care products, in perfumes to make the scent more intense and uniform, and in deodorants due to its anti-bacterial activity (52). Furthermore, farnesol is produced in mammalian cells as a precursor of FPP (farnesylpyrophosphate), a key intermediate in the mevalonate pathway. The mevalonate pathway allows the biosynthesis of molecules, such as cholesterol, coenzyme Q, dolichol or prenyl groups necessary for protein isoprenylation (53, 54). As
isoprenoids are important for diverse cellular processes, farnesol is involved in differentiation of cells, synthesis of lipids and proteins, as well as in apoptosis (55-59). *C. albicans* synthesizes farnesol as a precursor of ergosterol which is essential for the membrane integrity of the fungus. Of four existing isomers only the E, E-isomer of farnesol shows QS activity (49). Therefore, concentrations of 1.2 µM are sufficient to block filamentation and to reduce germ tube formation to 50% in *C. albicans* (51). In addition to the influence of the structure, the activity of farnesol is dependent on the composition of the growth media. The concentration of serum influences the concentration of farnesol needed to block filamentation. With increasing serum levels more farnesol is necessary to be functional (60). The production of farnesol is not specific for *C. albicans*. Weber and co-workers compared the farnesol production levels of eight different *Candida* species (*C. albicans*, *C. glabrata*, *C. krusei*, *C. dubliniensis*, *C. parapsilosis*, *C. tropicalis*, *C. kefyr* and *C. guilliermondii*) and observed that farnesol is predominantly secreted by *C. albicans* and *C. dubliniensis* (61). The main effects and characteristics of farnesol are well known for *C. albicans*. For the non–*albicans* *Candida* spp. a temporary overall response to farnesol is described but needs to be further elucidated.

**1.3.1 Response to farnesol in *C. albicans***

The first described effect of farnesol on *C. albicans* was on the regulation of morphology (21). Farnesol is able to reduce germ tube formation from budding yeasts, but does not block the elongation of cells that were already committed to filamentation (60). Furthermore, farnesol is involved in biofilm formation. Microbial biofilms are surface associated microbial communities embedded in a self-made extracellular matrix. The growth in biofilms is important for both superficial and systemic candidiasis because cells of the biofilm exhibit increased resistance to anti-fungal drugs, thus making anti-fungal therapy more difficult (62). Ramage and colleagues showed that farnesol can effectively block *C. albicans* biofilm formation (63). At early stages of development, increasing farnesol inhibited yeast attachment to the surface. Therefore, the extent of biofilm inhibition depends on how much attachment was proceeding before farnesol was added. Additionally, it was observed that mature biofilm stops hyphal formation and continues with yeast growth after exposure to high farnesol concentrations (~300 µM) (63). This might have an influence on biofilm dispersal as newly formed yeast cells are released and able to colonize new surfaces. Due to the biofilm lifestyle it is anticipated, that farnesol, secreted by *C. albicans* can locally reach
very high levels (≥ 1mM) (64). For planktonic cultures farnesol is secreted continuously by *C. albicans* up to 55 µM, in FBS containing media (64, 65). Another role described for farnesol is its protection from oxidative burst (66). *Candida* is frequently confronted with high levels of reactive oxygen species (ROS) during its opportunistic lifestyle. One major source of exogenous ROS are phagocytes of the human immune system (67). It has been shown that on the one hand farnesol contributes to oxidative stress resistance of *C. albicans* by induction of catalase expression and interference with the Ras1-Cyr1-PKA signaling pathway (68) and on the other hand farnesol evokes ROS production, which may participate in cell-cell signaling and adapt cells to oxidative stress (69, 70). In addition, farnesol has an impact on multidrug resistance of *C. albicans* by acting as a specific modulator of drug efflux pump proteins (71). Sharma and Prasad showed that farnesol can reverse the extrusion of specific compounds mediated by the ABC drug transporter without being a substrate for efflux pump proteins. Moreover, farnesol induces up-regulation of drug resistance genes (72).

As described above, farnesol shows multiple interactions with *C. albicans* and therefore influences the fungal physiology. The underlying molecular mechanisms are still under investigation but several regulating pathways for the impact of farnesol on *C. albicans* are suggested and partially evidenced (Figure 2). Langford and colleagues intended that possibly multiple targets for farnesol in the cell may exist (73). The Ras-cyclic AMP-protein kinase (cAMP/PKA) signaling pathway was identified as one of the first potential involved pathways, because of its implication in the regulation of filamentation in *C. albicans* (74). Ras proteins are GTPases localized to the plasma membrane via posttranslational modifications like farnesylation. Furthermore, it has been shown that farnesylated membrane bound Ras1 is much more efficient than the cytoplasmic form in activating adenyl cyclase, an enzyme essential for hyphal growth in *C. albicans* (75, 76). Therefore, several ways how farnesol might interfere with Ras1 were suggested, e.g. blocking the farnesylation of Ras1, releasing farnesylated Ras1 from the membrane or direct binding to Ras1 or its adenyl cyclase (73). Hall and colleagues could confirm direct inhibition of the catalytic activity of the adenyl cyclase Cyr1p by farnesol (77). The membrane localization of Ras1 during *C. albicans* hyphal growth and farnesol response was investigated by Piispanen and co-workers. Only at high concentrations of farnesol (300 µM) mislocalization of Ras1 was observed (78). More factors and pathways beside the cAMP/PKA signaling pathway are involved in the response to farnesol, e.g. the Hog1-mediated mitogen-activated protein kinase (MAPK) pathway, the Cek1-MAPK pathway and factors like Tup1/Nrg1 or the
C. albicans histidine kinase (Chk1) (68, 77, 79-82). A primary role for the cAMP-PKA signaling pathway was further confirmed by investigation of the transcriptional regulators Efg1 and Czf1 that strongly contribute to the morphological response to farnesol (83).

Figure 2 Farnesol influence on pathways important for the yeast-to-hyphae switch. Farnesol interferes with the Ras1/MAP kinase pathway, the Ras1 and Cyr1 in the cAMP/PKA pathway, the global transcriptional regulator Tup1 and Chk1. Dashed arrows indicate unknown pathways. Figure modified after (84).

1.3.2 Farnesol and interspecies communication

As microorganisms are frequently found in mixed populations it is apparent that cell to cell communication plays a role not only within but also between species. Farnesol exerts diverse effects on many microbes in bacteria as well as in other fungi. Most importantly, farnesol modulates growth and behavior of bacterial species that are commonly found in similar ecological niches as C. albicans. The two opportunistic pathogens C. albicans and P. aeruginosa interact in various polymicrobial infections in human and are typically found on medical devices, e.g. in a mixed biofilm on catheters.
Their coexistence is influenced by cross-kingdom signaling, in which the QS molecule HSL produced by *P. aeruginosa* prevents the switch from yeast to filamentous form in *C. albicans*, without inhibiting fungal growth (87). In addition, *P. aeruginosa* is able to destroy filamentous but not yeast forms of *C. albicans* (88). On the other hand farnesol secreted by *C. albicans* leads to down-regulation of the *P. aeruginosa* quinolone signal (PQS) (86). Furthermore, farnesol increases the susceptibility of *Escherichia coli* and *S. aureus* towards antibiotics, interferes with membrane integrity and inhibits biofilm formation of *S. aureus* (89, 90). Farnesol triggers apoptosis in *Aspergillus nidulans* and in *A. flavus* (91, 92). In *A. fumigatus*, farnesol induced cell wall stress by inhibiting the cell wall integrity and leading to misplacement of tip-localized Rho GTPases that are important for hyphal morphology (93).

### 1.3.3 Effects of farnesol on mammalian cells

Aside from triggering apoptosis in other fungal species it has been shown that farnesol exerts pro-apoptotic effects in certain malignant cell lines, e.g. lung carcinoma cells, human oral squamous carcinoma cells or prostate cancer cells in concentrations ranging from 30 µM to 300 µM (94-96). As non-neoplastic cell lines and primary cells showed no cell death under similar conditions, farnesol toxicity might be selective on neoplastically derived cells (58, 97). These farnesol-mediated anti-tumor effects raised the possibility that farnesol might be a useful tool in cancer chemotherapy (98). However, some publications indicate that farnesol also harms non-malignant cells, e.g. human spermatozoa (99). Rennemeier and colleagues observed a loss of membrane integrity in spermatozoa after exposure to 50 µM farnesol for one hour. Furthermore, farnesol cytotoxicity has been seen in murine macrophages in the presence of 90 µM FOH (100). In addition, Abe *et al.* showed suppression of anti-*Candida* activity of macrophages, through reduction of oxidative stress. Ghosh *et al.* reported that farnesol stimulates the expression of IL-6, IL-1β, IL-10 and TNF-α in the murine RAW264.7 macrophage cell line (101). They suggested that farnesol acts as a co-stimulator of pathogen-associated molecular patterns (PAMPs) within the fungal cell wall, as they observed synergistic effects of farnesol and zymosan, a yeast cell wall preparation, in stimulating IL-6 production. Interestingly, in the absence of cell wall PAMPs, farnesol decreased IL-6 levels secreted by the murine cell line (101).

Navarathna and co-workers identified the first gene, *DPP3*, with a role in farnesol production. A knockout mutant produced 6 times less farnesol and was 4 times less
pathogenic in a mouse model of systemic candidiasis (102). Furthermore, orally or i.p. given (exogenous) farnesol enhanced the mortality of *C. albicans* infected mice. This was confirmed later on by Tashiro *et al.* who showed that pravastatin inhibits farnesol production in *C. albicans* and improves survival in a mouse model of systemic candidiasis (103). Curiously, Hisajima and colleagues published in 2008 protective effects of farnesol against oral candidiasis in mice. Farnesol was orally given 3, 24 and 30 hours after oral infection of mice with *C. albicans*. White patches on the tongue surface and bodyweight loss in mice were inhibited by farnesol. Moreover, Hisajima observed no alteration of *Candida* growth in the intestinal tract, kidneys or liver after oral farnesol administration, that might imply that the protective effects of farnesol on oral candidiasis are locally limited (104). A further role of farnesol in oral *Candida* infections was examined by Décanis and colleagues (105). Exogenous farnesol showed synergistic interaction with gingival epithelial cells against *C. albicans* infection *in vitro*, by enhancing TLR2 expression, IL-6 and β-defensin 2 production.

### 1.4 Host immune response to *C. albicans*

Innate immunity, mainly cell-mediated, is the dominant protective mechanism against disseminated candidiasis. Thus, quantity and quality defects of cell-mediated immunity are the main risk factor that predispose to invasive *Candida* infections (106). While neutrophils and monocytes constitute the first line of defense against the fungal pathogen, subsequently the recognition and processing by dendritic cells (DC) represents an important link to specific immunity that enables T cell-mediated fungal clearance (107, 108). The following sections will consider in more detail properties and functions of neutrophils, monocytes and DC important for mounting an anti-fungal immune response.

#### 1.4.1 Neutrophils as first line of defense

Polymorphonuclear neutrophilic granulocytes (PMN) represent the majority (50 - 75%) of circulating blood leukocytes (109). They originate in the bone marrow from a pluripotent stem cell and develop under the influence of growth factors and cytokines into myeloblasts and finally into mature granulocytes (109). As soon as they reach the bloodstream they circulate for only a short live-span of about 6-8 h and undergo apoptosis in absence of a stimulatory signal (110). Neutrophils, activated in case of an injury or inflammation, are highly motile and follow a chemotactic stimulus to the site of
infection. Neutrophil activation contains a variety of processes with diverse effects and outcomes. It starts with neutrophil migration dependent on expression of adhesion molecules, e.g. L-selectin (CD62L), which facilitate interactions with activated endothelial cells and mediates the rolling of neutrophils along vascular endothelium (111). After selectin-mediated rolling, neutrophils prepare for transendothelial migration and express increased β2 integrins like Mac-1 (CD11b/CD18) (112). As the neutrophil reaches its target, activation continued and leads to release of cytokines that recruit other immune cells followed by execution of their microbicidal actions. IL-8 is the most abundant cytokine released by PMN that attracts primarily neutrophils induces activation of PMN, e.g. degranulation (113, 114). Further microbicidal actions include oxidative burst, receptor-mediated phagocytosis, mobilization of anti-microbial peptides and formation of neutrophil extracellular traps (NETs) (109). A pivotal role in microbial killing is taken by the assembly of the NADPH oxidase complex at the membrane of the phagosome, which is necessary for initiation of the reactive oxygen cascade. Mutations in one of the genes encoding for the NADPH oxidase complex lead to deficiency in oxidant formation and results in chronic granulomatous disease (CGD). Patients with CGD are characterized by enhanced susceptibility to bacterial and fungal infections (115). Beside the generation of reactive oxygen species (ROS), neutrophils are equipped with anti-microbial substances stored in so-called granules. These granules can be grouped into three types azurophilic (also known as primary granules), secondary or specific and tertiary granules. The granules differ in their content and their ability to mobilize. Tertiary granules contain gelatinase and mobilize first, while azurophilic granules comprise anti-microbial peptides like myeloperoxidase (MPO) and elastase and show the least degranulation propensity (109). The specific granules are equipped with a wide range of anti-microbial peptides, e.g. lactoferrin, and play an important for the oxidative burst. A controversial and interesting anti-microbial feature of neutrophils is the process of NETosis, an active form of cell death that leads to the release of DNA coated with histones and anti-microbial peptides like elastase to trap and kill microbes. The mechanism is not fully understood and needs to be further elucidated especially since NET formation can also have detrimental effects on the host, e.g. inducing autoimmunity (116, 117).

The role of neutrophils in anti-fungal immunity is indispensable (107). Quantity and qualitative defects in neutrophils, e.g. neutropenia, predispose for fungal infections (118). Neutrophils are the only innate immune cells that inhibit the yeast to hyphae conversion of C. albicans, a process closely linked to pathogenicity of the fungus (119,
Furthermore, *C. albicans* is killed both intra- and extracellularly via oxidative and non-oxidative mechanisms (121). The investigation of invasive candidiasis in a mouse model showed that neutrophils reveal differential effects dependent on the phase of infection. The early presence of neutrophils was protective and led to control of fungal growth but the presence late after infection induced tissue injury and mortality (122, 123). Nevertheless, neutrophils comprise a pivotal role in innate immune defense due to their abundance in circulation and their efficient way to kill and degrade invading pathogens.

### 1.4.2 Monocytes

Peripheral blood monocytes are a heterogeneous population that represents approximately 10% of human leukocytes. They develop from hematopoietic stem cells in the bone marrow and circulate in the blood for 1 to 3 days, permanently surveying the environment and able to react rapidly to alterations and to migrate to inflamed tissue (124). In the absence of an inflammatory signal they constitute a reservoir of myeloid cells and give rise to macrophages or differentiate into dendritic cells (125, 126). In general, their main functions are to retain homeostasis by removing apoptotic or toxic compounds and as inflammatory cells to respond to microbial stimuli by secretion of cytokines (TNF-α, IL-1β, IL-8, IL-6, IL-10) and release of anti-microbial effector molecules (ROS, complement factors, prostaglandins, nitric oxide and proteolytic enzymes) (127-129). Furthermore as accessory cells, they are able to link inflammation and the innate defense to the adaptive immune response by MHC class II antigen-presentation, but to a lesser extent than dendritic cells (130, 131). Monocytes display many scavenger receptors to recognize microorganisms, lipids and dead cells and the chemokine receptor CCR2 that enables monocytes to traffic to sites of infection in response to the chemokine MCP-1/CCL2 (monocyte chemoattractant protein-1) (132). Based on the surface expression of the two receptors CD14 (LPS receptor) and CD16 (FCγ receptor III) monocytes are divided into functional subsets (133). The majority of 80-90% of peripheral blood monocytes are strongly CD14 positive and CD16 negative (CD14$^{\text{high}}$ CD16$^{-}$) so-called classical monocytes. Another subset is the population of CD16 positive monocytes that can be further divided into two groups dependent on the surface expression of CD14 (CD14$^{\text{high}}$ CD16$^{+}$ and CD14$^{\text{dim}}$ CD16$^{+}$). A potent anti-fungal function of the classical CD14$^{\text{high}}$ CD16$^{-}$ monocytes has been demonstrated by Smeekens and colleagues. Only CD14$^{\text{high}}$ CD16$^{-}$ monocytes induced an anti-fungal Th17 response in CD4-positive T cells, mediated by combination of
enhanced mannose receptor (MR) surface expression and increased release of IL-1β and PGE2 (134). So far the contribution of monocytes to innate defense against fungal infections has been underestimated, due to the indispensable role of neutrophils. Certainly, monocytes are less effective than neutrophils in killing C. albicans in an ex vivo whole blood model, but they are able to phagocytose and kill Candida yeast and filaments more efficiently than DC and produce large amounts of pro-inflammatory cytokines (121, 135). Furthermore, recruitment of monocytes to sites of fungal infections has been seen in vivo (132, 136). Recent investigations of the contribution and the temporal requirement of monocytes during disseminated candidiasis revealed that monocytes are important in the first 48 h post infection to control fungal growth in kidney and brain (137).

1.4.3 Dendritic cells

Dendritic cells (DC) are characterized by unique properties that allow them to induce an effective and specific immune response against invading pathogens (138, 139). They are the most effective professional antigen presenting cells (APC) located in almost all peripheral tissues of the body in their immature form (140). DC represent a very heterogeneous population of cells that can be distinguished via surface marker expression. There are two major types: CD11c⁺ myeloid DC (including Langerhans cells, interstitial DC and monocyte-derived DC) and CD123⁺ plasmacytoid DC (141). Plasmacytoid DC express less MHC class molecules and produce great amounts of type I and type III interferons (IFN), thus playing a role in the innate defense against viruses and in tolerance (142). Myeloid DC, especially migratory DC, play a role in recognition of antigens and mainly initiate a pro-inflammatory immune response (143). Within each of these subsets, cells differentiate from precursor cells in the bone marrow to immature DC (iDC) and migrate through the blood to the peripheral tissues, where they form a dense network and control their environment (131). Immature DC take up antigens and foreign bodies via pinocytosis, makropinocytosis, phagocytosis or receptor-mediated endocytosis and generate antigen-loaded MHC molecules (MHC: major histocompatibility complex). Upon activation, e.g. stimulation with an antigen, LPS, TNF-α or CD40L (CD154), DC undergo maturation, express lymphocyte co-stimulatory signals, migrate mainly to the secondary lymph nodes and secrete cytokines to initiate an immune response (131, 144, 145). In comparison to immature DC that are unable to stimulate T cells, mature DC (mDC) show reduced capacity for antigen uptake and processing but increased stimulatory functions.
DC comprise a very small percentage of circulating blood cells, which makes it difficult to isolate sufficient quantities for experimental investigations. Due to the rarity of DC in blood, multiple protocols exist for the \textit{in vitro} expansion of DC. The most commonly used precursor cells for generating myeloid-like DC are CD14$^+$ monocytes (146). In the presence of M-CSF monocytes differentiate into macrophages, but after incubation for 6 days with GM-CSF and IL-4, they develop into immature DC. IL-4 induces the suppression of CD14 on monocytes, while GM-CSF initiates expression of CD1a on the cell surface (147, 148). Under these \textit{in vitro} culture conditions, the cells obtain morphology similar to immature DC and express DC-specific surface markers. Furthermore, it has been shown that they can bind and process antigens, which corresponds to the behavior of immature DC \textit{in vivo} (149). Stimulation of immature DC for about 24 h to 48 h leads to fully mature DC that express no CD14, but highly CD83, CD86 and MHC II molecules (149, 150). Morphologically, DC are characterized as irregularly shaped cells with long cytoplasmic branched projections, so-called dendrites (Figure 3).

![Morphology of monocyte-derived immature and mature DC.](image)

Both images show monocyte-derived DC in different morphological stages. The left image displays immature DC after 6 days of differentiation characterized by small dendrites. After LPS stimulation to mature DC, cells form peripheral extensions and show a stellate morphology. Scale bar indicates 10 µM.
1.4.3.1 DC antigens and antigen presentation

DC are characterized by a variety of surface molecules to suit their different functions, e.g. migration, adhesion, antigen-uptake or antigen presentation. For this thesis, expression profiles of distinct CD (cluster of differentiation) molecules, which are typically expressed on DC were analyzed (Table 1).

DC deliver antigen to processing compartments with the help of a specialized endocytic system and many uptake receptors. Following uptake of antigen DC are able to present antigen on two types of MHC molecules: Class I presents peptides from endogenous proteins to CD8-positive (CD8⁺) cytotoxic T cells and the class II presents cleaved peptides from exogenous proteins to the CD4-positive (CD4⁺) T helper cells (151). The amount of MHC expression correlates with the efficiency of antigen presentation. MHC II proteins are HLA-DR, HLA-DQ, and HLA-DP, which are expressed on immature DC and highly up-regulated on mature DC. Unlike MHC class I and II molecules, CD1 molecules present lipids, glycolipids and lipopeptides to T cells (152-154). They share functional and physical characteristics, but traffic independently from MHC II molecules during DC maturation (155, 156). The number of CD1 genes varies among the species. In humans five CD1 proteins are described for dendritic cells: CD1a, CD1b, CD1c and CD1d, all expressed on the surface and CD1e an intracellular protein (152, 157). In general, the CD1 family can be classified into 3 groups based on amino acid comparisons. Group 1 contains CD1a, CD1b and CD1c that are distinctively expressed on DC and are inducible on monocytes by stimulation with cytokines that induce DC differentiation (156). CD1d belongs to group two and its cellular expression is less established. Group 3 comprises CD1e that is localized within the Golgi compartment of immature DC and in the lysosomes of mature DC. CD1e does not reach the cell surface and may play a physiological role in antigen processing rather than presentation (158, 159). While CD1a, CD1b and CD1c are recognized by conventional T cells, CD1d functions as an antigen-presenting molecule for the T cell subset known as NKT cells (T cell responses to bacterial lipid and glycolipid antigens) (155, 160, 161). Beside the enhanced expression of antigen presenting molecules (CD1, MHC I and II), DC maturation is associated with the up-regulation of surface markers CD80, CD86 and CD40. These co-stimulatory molecules are additionally required in order to effectively stimulate T cells and to influence T cell regulation (160, 162). Both, CD80 and CD86 interact with the ligand CD28, which is expressed on the surface of resting and activated T cells. The interaction of CD40 with its ligand CD40L (CD154) expressed on T cells, leads on the one hand to further activation and proliferation of
T cells and on the other hand to enhanced expression of the co-stimulatory molecules CD80 and CD86 as well as MHC II molecules (163). Moreover, this feedback stimulation induces secretion of T cell polarizing cytokines, e.g. IL-12 (164).

One of the most characteristic antigens for fully matured DC is CD83. The maturation marker is mainly expressed on mature immunocompetent DC, but can also be found on activated B and T cells and neutrophils (165). Human CD83 acts as an enhancer during T cell activation, driving the expansion of newly primed naïve T cells by supporting their long-term survival and function (165, 166). Furthermore, engagement with CD83 enhances the in vitro generation of CTL (cytotoxic T cells) (167). Enhanced CD83 expression on cultured DC has been reported after a number of cellular signals, e.g. cytokines (TNF-α) or LPS (150, 168).

<table>
<thead>
<tr>
<th>surface marker</th>
<th>monocytes</th>
<th>immature DC</th>
<th>mature DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD86</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>CD1a</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD40</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>CD80</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>CD83</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

Table 1 Surface marker expression during generation of monocyte–derived DC.

The differentiation of DC from monocytes is characterized by a change in surface marker expression. For ease, the expression pattern of surface marker important for antigen presentation and maturation are summarized in this table. (− not expressed on the surface; + expressed; ++ enhanced expression)

1.4.3.2 Activation and polarization of T cells

Fully matured DC are decisive for the proliferation and differentiation of naïve T cells (T cell priming) (169). The priming of naïve T cells can result in CD8+ T effector cells, namely the cytotoxic T cells (CTL) or CD4+ T cells. CD8+ T cells, activated by MHC-I-carrying DC, differentiate into cytotoxic T cells, which clear infected host cells by lysis.
MHC class II-expressing DC activate CD4\(^+\) T cells, which then differentiate into several types, e.g. Th1, Th2, Th17 and regulatory T cells. Th1 cells are responsible for secretion of pro-inflammatory cytokines, which kill mainly intracellular pathogens, while Th2 cells are involved in allergies and parasitic infections and fight against extracellular pathogens. Another potent pro-inflammatory mediator is the Th17 response, which plays a role in autoimmune disorders (asthma, arthritis) and is involved in the immune defense of fungi and extracellular bacteria (170, 171). Furthermore, DC induce regulatory T cells that suppress T cell responses and play a crucial role in immune tolerance (172-175).

The polarization of T cell is defined by 3 signals emanated by mature DC. The first signal is mediated by detection of the specific antigen-presenting MHC complex by the T cell receptor (TCR) through formation of an immunological synapse (176). However, this signal alone is not sufficient to induce proper T cell activation and polarization into different T effector cells. A second signal required for T cell activation combined all co-stimulatory signals based on a ligand-receptor binding, like interaction of CD80/86 on DC with CD28, expressed on naïve T cells or other co-stimulatory receptors (CD40). Due to the strong surface expression of co-stimulatory molecules, DC are more effective in stimulating T cells than other APC like B cells or macrophages. A third signal, not essential for T cell activation, but required for differentiation into certain T effector cells has been shown to be the current cytokine milieu (163, 177). DC release a number of cytokines that are associated with T cell proliferation. For the differentiation of naïve T cells into CD4\(^+\) Th1 cells the secretion of IL-12 (p70) is required (178, 179). For the polarization towards CD4\(^+\) Th2 cells IL-4 and IL-10 are essential and the development to regulatory T cells is controlled by IL-10 (178, 180, 181). The induction of Th17 cells is promoted by IL-1 and IL-23 that can also be released by DC (182, 183). Beside DC other close-by innate immune cells, such as natural killer (NK) cells, NKT cells, mast cells, eosinophils and basophils also affect cytokine environment of the DC-T cell interaction, thus influencing T cell polarization.

1.4.3.3 Role of DC in anti-fungal immunity

DC link the innate and adaptive immunity for a range of fungal pathogens e.g. *C. albicans*, *Cryptococcus neoformans* or *A. fumigatus*. (184-186). For *C. albicans*, polymorphism is a contributor to interaction with DC (187). In general, DC are able to phagocytose and kill yeast and hyphae by the use of distinct receptors depending on morphology of the fungus (188). Recognition of fungal cell wall components and fungal
uptake by DC is driven by pattern recognition receptors (PRRs) like dectin-1 and 2, DC-SIGN, mannose receptor (MR) and toll-like receptors (TLRs) (189, 190). The C-type lectin receptors dectin-1 and dectin-2 play a prominent role in morphology-specific recognition of *C. albicans*. While dectin-1 signaling is activated by β-(1-3)-glucan, localized in the outer layer of *C. albicans* yeast cells, dectin-2 recognizes mannose-structures and preferentially binds filamentous forms (191). Both dectin-1 and dectin-2 induces a NF-κB-mediated response via SYK-CARD9 signaling. The uptake of fungal cells induces maturation and promotes differentiation of DC. Rather than for killing and elimination of *C. albicans* cells, DC are important for phagocytosis and processing of *C. albicans* antigens for presentation and in turn, initiation of the adaptive immune system. Netea and colleagues showed that human DC are less potent in possessing anti-*Candida* properties compared to monocytes and macrophages (135). This goes along with a limited release of pro-inflammatory cytokines in response to *C. albicans* cells compared to monocytes and macrophages. However, DC discriminate between the yeast and filamentous form of *C. albicans* and direct the Th response according to morphology of the fungus (188). While *C. albicans* yeast cells elicit a protective Th1 response, the filamentous form supports a non-protective Th2 response by inhibiting Th1 differentiation and reduced release of the immunoregulatory cytokine IL-12 (188, 192, 193). Beside the Th1 cell response, also Th17 cells have a role for protection against fungi like *C. albicans*. Th17 cell activation is induced mainly through SYK-CARD9 and mannose receptor signaling and promotes neutrophil and macrophage recruitment (194-196). Interestingly, CD1d-positive DC were shown to activate NKT cells after stimulation with fungi in a dectin-1 dependent manner but independent from fungal lipid antigen presentation (197). Taken together, DC fulfill the concept of an professional antigen-presenting cell and orchestrate the T cell response in anti-fungal immunity in response to morphology of the fungus.
1.5 Aims of this work

Farnesol - the first quorum sensing molecule discovered in an eukaryote - regulates morphology of the fungal pathogen *C. albicans* and exerts effects on other microorganisms. Its role in inter- and intraspecies microbial communication has been largely described while the interaction with human immune cells remains elusive. In this work the impact of farnesol as a model for fungal signaling on human innate immune cells was investigated.

The aims of this work were:

- To define non-pro-apoptotic concentrations of farnesol for innate immune cells and characterize potential pro-apoptotic effects
- Characterize the effect of farnesol on the activation of human innate immune cells (neutrophils, monocytes and dendritic cells)
- Analyze the influence of farnesol on the maturation process of monocyte-derived DC
- Define the role of farnesol as a virulence trait of *C. albicans* by investigation of immune effector mechanism
2 Experimental procedures

2.1 Reagent preparation

Farnesol was obtained as a 4 M stock solution (Sigma-Aldrich) and then diluted in 100% methanol. The working concentrations of farnesol used ranged from 10 µM to 200 µM. Methanol was used as a solvent control indicated as mock-treated cells. N-3-oxo-dodecanoyl-L-Homoserine lactone (HSL) was purchased from Sigma-Aldrich, dissolved in DMSO and used in concentrations up to 50 µM.

2.2 Candida cultivation

C. albicans strains used for co-incubation with immune cells are indicated in table 2. Both C. albicans strains were maintained on YPD agar. For experiments, colonies were transferred to YPD medium (2% D-glucose, 1% peptone, 0.5% yeast extract in water) and cultured at 30°C to stationary phase. Germ tubes were induced by culturing in RPMI 1640 medium for one hour at 37°C. For confrontation with PMN or monocytes germ tubes were washed in phosphate-buffered saline (PBS) and used at a MOI of 0.5.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Genotype</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC5314</td>
<td>wild type (198)</td>
<td>C. albicans wild type</td>
<td>B. Hube, HKI Jena</td>
</tr>
<tr>
<td>CaGFP</td>
<td>CaGFP in SC5314 (121)</td>
<td>ADH1/adh1::pADH1-GFP-SAT1-ADH1t</td>
<td>A. Haeder, ZIK Septomics Jena</td>
</tr>
</tbody>
</table>

Table 2 C. albicans strains used in co-incubation with PMN and monocytes.

2.3 Cell culture

2.3.1 Isolation and stimulation of neutrophils

Human polymorphonuclear cells (PMN) were isolated and purified from peripheral venous blood of healthy donors by density gradient centrifugation using the Polymorphprept™ system (PROGEN Biotechnik GmbH) as described elsewhere (199). All assays were performed in RPMI 1640 (Biochrom AG) with 10 mM L-glutamine.
containing 5% heat inactivated fetal bovine serum (FBS). For the analysis of surface marker and oxidative burst of PMN, stimulation with FOH (50 µM - 200 µM) and the solvent control was performed in cryotubes on a rolling mixer for 30 min. For determination of anti-microbial peptides cell supernatants were harvested after incubation with FOH for 2 h.

2.3.2 Isolation and stimulation of monocytes

Human monocytes were isolated from buffy coats of healthy volunteers, kindly provided by Dagmar Barz (transfusion medicine, Jena). First, primary human peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using BIOCOLL (Biochrom AG). Monocytes were separated from PBMC by negative magnetic bead selection via magnetic cell sorting system (MACS) using the Isolation Kit II (Miltenyi Biotech). Freshly isolated monocytes were immediately used for stimulation assays. All stimulation assays were performed in RPMI 1640 (Biochrom AG) with 10 mM L-glutamine containing 5% or 10% heat inactivated FBS. Surface phenotype and the release of cytokines were determined after treatment with FOH (50 µM and 100 µM) for approximately 24 h.

2.3.3 Differentiation and stimulation of monocyte-derived DC

Human monocytes were isolated from buffy coats of healthy volunteers. First primary human peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using BIOCOLL (Biochrom AG). Monocytes were separated from PBMC by positive magnetic bead selection via magnetic cell sorting system (MACS) using CD14+ microbeads (Miltenyi Biotech) according to the manufacturer's instructions. Freshly isolated monocytes were differentiated to immature dendritic cells (iDC) over 6 days with culture in GM-CSF (800 U/ml, Leukine® sargramostim) and IL-4 (1000 U/ml, Miltenyi Biotech). After generation iDC were confirmed by CD1a-positive (at least 60%) and a CD14-negative staining. Cells were stimulated with the solvent methanol (mock-treated/ solvent control) and FOH in different concentrations (50 µM - 200 µM) at 37°C for 24 h. Culture in LPS for 24 h (10 ng/ml; Sigma-Aldrich) was used for the final maturation step to mature DC (mDC). All stimulation assays were performed in RPMI 1640 (Biochrom AG) with 10 mM L-glutamine containing 5% or 10% heat inactivated FBS. For experiments concerning the impact of farnesol on the differentiation process of monocytes to iDC fresh isolated monocytes were exposed to cytokines for differentiation and in addition to FOH (10 µM - 100 µM) or the solvent
control (see Figure 4). To ensure that farnesol is present during the entire differentiation process FOH was included in the media and therefore the concentration was constant even after media replenishment.

![Diagram showing the generation of monocyte-derived DC in the presence of farnesol.](image)

**Figure 4** Generation of monocyte-derived DC in the presence of farnesol.

### 2.3.4 Cultivation and stimulation of Jurkat cells

For this study, the CD4+ T helper cell line Jurkat was kindly provided by Ignacio Rubio (CMB, Jena). Jurkat cells were cultured in RPMI 1640 (Biochrom AG) with 10 mM L-glutamine supplemented with 10 heat inactivated FBS at 95% relative humidity and 5% CO2. Every second or third day, cells were passaged. Cell counting was performed by trypan blue staining using a Neubauer cell chamber. Jurkat cells were stimulated with different concentrations of FOH (50 µM - 200 µM) for indicated time points.

### 2.4 Flow cytometry

All flow cytometry analyses were performed on a FACSCanto II (BD Bioscience) and the data was analyzed with FlowJo software 7.6.4.

#### 2.4.1 Characterization of surface marker

Phenotypic characterization of PMN, monocytes and DC was performed by flow cytometry using fluorochrome conjugated antibodies. For this purpose 100 µl volume of cell suspension was stained with the following antibodies (see table 3). In parallel, staining with the appropriate isotype was performed as a binding specificity control.
After 15 min staining at 4°C, cells were washed and harvested in CellWASH (BD Bioscience) and subsequently analyzed on a flow cytometer.

<table>
<thead>
<tr>
<th>Antibody/ origin</th>
<th>Isotype/ origin</th>
<th>Concentration</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE anti-human CD1a BioLegend</td>
<td>mouse IgG1 BioLegend</td>
<td>50 µg/ml</td>
<td>DC</td>
</tr>
<tr>
<td>PE anti-human CD80 BioLegend</td>
<td>mouse IgG1 BioLegend</td>
<td>100 µg/ml</td>
<td>DC</td>
</tr>
<tr>
<td>PE anti-human CD116 BD Bioscience</td>
<td>mouse IgG1 BD Bioscience</td>
<td>100 µg/ml</td>
<td>DC</td>
</tr>
<tr>
<td>FITC anti-human CD14 BioLegend</td>
<td>mouse IgG2a BioLegend</td>
<td>100 µg/ml</td>
<td>DC</td>
</tr>
<tr>
<td>FITC anti-human CD40 BioLegend</td>
<td>mouse IgG1 BioLegend</td>
<td>50 µg/ml</td>
<td>DC/monocytes</td>
</tr>
<tr>
<td>APC anti-human CD1d BioLegend</td>
<td>mouse IgG1 BioLegend</td>
<td>100 µg/ml</td>
<td>DC/monocytes</td>
</tr>
<tr>
<td>APC anti-human CD83 BioLegend</td>
<td>mouse IgG1 BD Bioscience</td>
<td>50 µg/ml</td>
<td>DC</td>
</tr>
<tr>
<td>V450 Mouse anti-human CD86 BD Bioscience</td>
<td>mouse IgG1 BD Bioscience</td>
<td>100 µg/ml</td>
<td>DC/monocytes</td>
</tr>
<tr>
<td>PerCP anti-human HLA-DR BioLegend</td>
<td>mouse IgG2a BioLegend</td>
<td>100 µg/ml</td>
<td>DC/monocytes</td>
</tr>
<tr>
<td>APC anti-human CD16 BioLegend</td>
<td>mouse IgG1 BioLegend</td>
<td>5 µg/ml</td>
<td>PMN</td>
</tr>
<tr>
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<td>mouse IgM1 BD Bioscience</td>
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<tr>
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</tr>
<tr>
<td>PE anti-human CD11b BioLegend</td>
<td>mouse IgG1 BioLegend</td>
<td>50 µg/ml</td>
<td>PMN</td>
</tr>
</tbody>
</table>

Table 3 Antibodies and corresponding isotypes used for FACS analysis.

2.4.2 Determination of apoptosis

For determination of apoptosis, cells were harvested and stained by FACS using the Annexin V-FITC Apoptosis Detection Kit II (BD Biosciences) according to the manufacturer’s instructions. The percentage of viable [Annexin V-FITC-negative and
propidium iodide (PI)-negative] and apoptotic [Annexin V-FITC-positive and PI-positive] cells were measured.

2.4.3 Quantification of oxidative Burst

The oxidative burst of PMN was measured using the Bursttest from Orpegen Pharma. After 30 min of incubation with FOH in different concentrations cell suspensions (100 µl) were treated with a 1 to 50 dilution of the fluorogenic substrate dihydrorhodamine 123 (DHR-123). After incubation for 10 min at 37°C formation of reactive oxidant species was monitored by oxidation of DHR-123 to R-123. After analysis with FlowJo software 7.6.4 results were presented as median fluorescence intensities.

2.4.4 Phagocytosis Assay

Phagocytosis of C. albicans by pretreated PMN or monocytes was measured via flow cytometry. First, isolated immune cells were pretreated with FOH for 30 min in case of PMN and for approximately 24 h in case of monocytes. Then, cells were washed by centrifugation (5 min, 250 g) and subsequently co-incubated with GFP-expressing C. albicans cells for 1 h (MOI of 0.5). To distinguish between intra- and extracellular fungal cells, sample preparation was treated with a specific anti-Candida antibody (Acris Ab) that binds to all extracellular fungal cells. Phagocytosis rate was calculated as percentage from all Candida cells associated to either PMN or monocytes.

2.5 XTT assay

To determine the metabolic activity of C. albicans wildtype cells after co-incubation with either PMN or monocytes, reaction was stopped with 800 µl of ice-cold distilled water and subsequently mixed with 200 µl 0.2% Triton X100. Sample preparation was then washed and centrifuged at 14,000 g for 5 min at 4°C. After removing supernatant, cells were resuspended in 800 µl ice-cold distilled water and again centrifuged at 14,000 g for 5 min at 4°C. Subsequently, the cells were incubated with 200 µl XTT solution (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2H-tetrazolium hydroxide) (Sigma-Aldrich). The reagent was previously prepared by solving 0.5 mg/ml XTT in PBS and adding 50 µg/ml Coenzyme Q (all from Sigma-Aldrich). After 1 h of incubation at 37°C, 100 µl of cell-free supernatant was transferred into an optical flat-bottom well plate (in triplicates) and analyzed in a TECAN Infinite 200 plate reader. The absorbance of the cell-free supernatant was measured at a wavelength of 450 nm.
Experimental procedures

The fungal metabolic activity was determined as percentage of the metabolic activity of *Candida* in media.

### 2.6 Measurement of secreted proteins

Supernatants were obtained by centrifugation and stored at -80°C to be used later for cytokine measurements. Following stimulation with farnesol secretion of cytokines and other proteins (MMP12) by monocytes, DC and anti-microbial peptides (lactoferrin, elastase 2 and myeloperoxidase) released by PMN were assessed in cell-free supernatants using the Milliplex® MAP Kits from Millipore and the ProcartaPlex™ from Affymetrix eBioscience according to the manufacturer’s instructions. Soluble CD14 was measured in cell-free supernatants according to manufacturer’s instruction of the Quantikine ELISA Kit from R&D Systems.

### 2.7 Allogeneic mixed lymphocyte reaction

Dendritic cells were generated from peripheral blood monocytes as described before (see 2.3.3). At day 5 iDC were stimulated with LPS (10 ng/ml, Sigma-Aldrich) and pulsed with tetanus toxoid (10 μg/ml, Sigma-Aldrich). On day 6 DC were harvested and fresh CD3-positive T cells were obtained from buffy coats by density gradient centrifugation and separation via AutoMACS device (Miltenyi Biotech) using CD3-positive microbeads according to the manufacturer’s instructions. Purified CD3-positive T cells were suspended in 0.5 μM carboxyfluorescein succinimidyl ester (CFSE, LifeTechnologies) diluted in RPMI 1640, and incubated at 37°C in the dark for 10 min. An equal volume of RPMI 1640 containing 10% human serum (type AB, PAA) was added to inactivate the extracellular CFSE. The cells were washed three times in PBS and then resuspended in the proliferation media consisting of RPMI 1640 medium enriched with 10% human serum (hS) and IL-2 (10 U/ml, Immunotools) and IL-4 (145 U/ml, Miltenyi Biotech) and GM-CSF (140 U/ml). Mature DC were harvested at day six washed with PBS and resuspended in proliferation media as well. CFSE CD3+ T cells were mixed in a ratio of 10:1 (10⁵ T cells to 10⁴ DC per well) in a volume of 1 ml. CFSE⁺-labeled T cells alone were used as a negative control. After day 4 cells where harvested and CFSE fluorescence was analyzed via flow cytometry.
2.8 Cell spreading assay

Cells were seeded into an 8 well ibidi plate coated with fibronectin (10 µg/ml, Sigma-Aldrich) at a density of 2x10^4 cells per well. Following 40 min of incubation, cell spreading was monitored via microscopy. Non-spread cells where defined as small and round, with little or no membrane protrusions while spread cells were defined as larger cells with visible lamellipodia. Bright field images were obtained with a Zeiss LSM 780 confocal microscope, percentage of spread cells was scored.

2.9 Transfection of Jurkat cells via electroporation

Transfection of Jurkat cells was performed using the Amaxa Cell Line Nucleofector Kit®. After cell harvest and determination of cell number, 1x10^6 cells per sample were centrifuged (90 x g) for 10min. Cell pellet was carefully resuspended in 100 µl Nucleofector® solution and combined with 2 µg plasmid DNA as indicated (Table 4). Subsequently, cell/DNA suspension was transferred into a cuvette and electroporated in a Nucleofector® II device from LONZA using the optimized program X-001. Post Nucleofection® 500 µl of pre-equilibrated culture medium (RPMI 1640 medium enriched with 10% FBS) were added and the whole suspension was then transferred into a culture well plate and further processed. Transfected Jurkat cells were stimulated with FOH (50 µM) or the solvent control (mock-treated). For transfection with mCherry plasmids (H-, N-, K-Ras) stimulation was performed in 8 well ibidi plates. In case of HA-tagged R-Ras, cells were seeded on poly-L-lysine (Sigma-Aldrich) coated coverslips in a 24 well plate.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Description</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>pmCherry-H-Ras</td>
<td>Ras genes were cloned in fusion with mCherry using pmCherry C1 vector</td>
<td>Ignacio Rubio (200)</td>
</tr>
<tr>
<td>pmCherry-K-Ras</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pmCherry-N-Ras</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pHAl-R-Ras</td>
<td>pCGN vector with R-Ras wt and HA -tag Addgene plasmid 14727</td>
<td>Adrienne Cox (201)</td>
</tr>
</tbody>
</table>

Table 4 Plasmids used for transfection in Jurkat cells.
2.10 Immunofluorescence and microscopic analysis of transfected Jurkat cells

In the case of Ras proteins linked to mCherry, experiments were performed in 8 well ibidi plates and microscopic images were taken directly after incubation without further modifications. Jurkat cells transfected with the HA-tagged R-Ras were performed in a 24 well plate on poly-L-lysine coated coverslips to allow subsequent staining with an anti-HA-antibody before fluorescence microscopy. Therefore, transfected Jurkat cells were fixed with histofix (4% formaldehyde, Roth) for 5 min and washed with PBS (3 times). After permeabilization with 0.2% triton X100 (4 min, at room temperature [RT]) and additional washing with PBS (2 times) cells were stained for 10 min with an anti-HA-tag-PE antibody (monoclonal, dilution 1:11, Miltenyi Biotech) at 4°C. After washing, stained coverslips were mounted in Prolong® Gold antifade reagent and stored at −20°C until imaging. All transfected Jurkat cells were imaged with an LSM 780 confocal laser scanning microscope (Carl Zeiss). Images were acquired using a 20x objective lens. The mCherry was excited with the HeNe 594 nm line and the HA-PE antibody with the Argon 488 nm line. All images were exported as TIF files and subjected to the same processing routine using the ZEN lite 2012 Software (Carl Zeiss).

2.11 Western blot

Cell pellets were lysed in 1x RIPA-lysis buffer supplemented with protease inhibitor (both ROCHE) for 30 min on ice, centrifuged at 4°C and maximum speed for 10 min. Protein concentration of the lysates was determined with a BCA protein assay (Thermo Scientific). Subsequently, equal amounts of protein were loaded on a polyacrylamide gel (Precise™ Gel, 4-20%, Thermo Scientific) and electrophoretically separated. The Spectra multicolor Broad Range Protein Ladder (Thermo Scientific) was used as protein size standard. Western blot was performed according to the sandwich-method. Proteins were transferred onto a polyvinylidene fluoride membrane (PVDF, Immobilon-FL; Merck Millipore) and blocked with I-Block™ solution (Applied Biosystems). Blocked PVDF membranes were incubated with primary antibody overnight at 4°C followed by a 1 h incubation with the fluorochrome-conjugated secondary antibody at RT. Blot images were analyzed with a Fluorchem Q imager (Biozym) and processed with the Alphaview software. Appropriate loading controls were included to confirm same
amounts of protein in each sample. All used antibodies were diluted in I-Block™ solution and are listed in the following tables (Table 5, 6).

<table>
<thead>
<tr>
<th>Primary antibodies</th>
<th>Source</th>
<th>Origin</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>(pan) Ras (RAS10)</td>
<td>mouse</td>
<td>Millipore</td>
<td>1:500</td>
</tr>
<tr>
<td>anti-HA (HA-7)</td>
<td>mouse</td>
<td>Sigma-Aldrich</td>
<td>1:500</td>
</tr>
<tr>
<td>anti-mCherry (1C51)</td>
<td>mouse</td>
<td>Abcam</td>
<td>1:2000</td>
</tr>
<tr>
<td>α-tubulin</td>
<td>rabbit</td>
<td>Cell Signaling Technology</td>
<td>1:1000</td>
</tr>
<tr>
<td>(pan)14-3-3 (H-8)</td>
<td>mouse</td>
<td>Santa Cruz</td>
<td>1:2500</td>
</tr>
</tbody>
</table>

Table 5  Primary antibodies used for western blot.

<table>
<thead>
<tr>
<th>Secondary antibodies</th>
<th>Source</th>
<th>Origin</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-mouse Dylight 649</td>
<td>goat</td>
<td>Jackson ImmunoResearch</td>
<td>1:1000</td>
</tr>
<tr>
<td>anti-mouse Alexa Fluor 568</td>
<td>goat</td>
<td>Life Technologies</td>
<td>1:2000</td>
</tr>
<tr>
<td>anti-rabbit Alexa Fluor 568</td>
<td>goat</td>
<td>Life Technologies</td>
<td>1:2000</td>
</tr>
</tbody>
</table>

Table 6  Secondary antibodies used for western blot.

### 2.12 Microarray based analyses of DC

For array analysis monocytes were differentiated to DC in the presence of 50 µM FOH. At least $1 \times 10^6$ DC were harvested at the following time points: after 3 d, after differentiation for 6 d and after 6 d and additional stimulation with LPS for 6 h. A solvent control was carried out for all three time points. Cells of 4 independent donors were used and isolation and cultivation was performed according to 2.3.2 and 2.3.3. Microarray based analyses of DC were performed in collaboration with Steffi Spielberg (202).
2.12.1 RNA isolation and quantification and Illumina Expression Array

After harvest of cells and removal of culture media cell-pellets were resuspended in RNAprotect (Quiagen) for RNA stabilization and stored at −20°C until further processing. RNA isolation was performed using the RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instructions. Quality and quantification of isolated RNA was measured with a NanoDrop 1000 (Thermo Scientific) and RNA integrity was assessed with a Bioanalyzer 2100 (Agilent). RNA amplification and cRNA transcription were carried out with the Illumina TotalPrep RNA amplification kit (Ambion) according to the manufacturer's instructions. The HumanHT-12 v4 Expression BeadChip kit from Illumina was used for determination of expression levels of RNA samples.

2.12.2 Preprocessing of Illumina HT-12 Chips

The microarray data was further processed in collaboration with Michael Weber. After quality control, the data was analyzed using the statistical software R package lumi, followed by quantile normalization and log2 transformation to identify samples with intensity values in 3 or more probes. With the help of a linear model (Limma package) differential expressed genes were identified. Genes were considered as differentially regulated when they showed an absolute fold change (FC) greater than (−2>FC>2) and simultaneously an adjusted P value <0.05. Annotation of differentially expressed genes was carried out using the Bioconductor annotation package illuminaHumanv4.db. Differentially regulated genes were grouped into functional categories using the Kyoto Encyclopedia of Genes and Genomes (KEGG), a database of a collection of pathways and molecular interactions of different biological processes. Significantly enriched pathways were identified in the set of differentially expressed genes relative to all annotated genes on the microarray. Furthermore, data was visualized in a venn diagram using the venny tool (203) and a heatmap was created using R package ggplot2.

2.13 Statistics

Data was analyzed using GraphPad Prism v5.0. Statistical significance was determined by a two-tailed unpaired Student's t test. The specified error bar represents the standard deviation (SD). A result is considered as significant when the significance level of P is less than 5% (P<0.05). Significance is marked by stars in the graphs (*P<0.05, **P <0.01, ***P <0.001)
3 Results

3.1 Farnesol- limited cell-death promoting effects on innate immune cells

Before farnesol (FOH) was identified as quorum sensing molecule of *C. albicans* it was described as an inhibitor of proliferation and an apoptosis inducing factor in different types of mammalian carcinoma cells (94, 95, 204-206). To test whether this can be also observed for human innate immune cells, the effects of farnesol on the viability of monocytes, DC and PMN were examined using primary human immune cells isolated from blood of healthy volunteers.

Freshly isolated PMN were treated with different concentrations of farnesol for 30 min and afterwards stained with Annexin V-FITC and propidium iodide (PI). The percentage of viable [Annexin V-FITC-negative and PI-negative] cells was quantified and the distribution of cell death was displayed in a dot plot. Annexin V-positive and PI-negative cells were considered as apoptotic, whereas Annexin V- and PI-positive cells represent either late apoptotic cells or necrotic cells. The dot plots in figure 5A show the correlated distribution of the cells in terms of these two parameters. For 50 µM and 100 µM FOH the viability remained at approximately 95%, like the mock-treated control (Figure 5). At 200 µM FOH a slight, but not significant, shift to the Annexin V and PI stained positive population was observed (87.6± 9.9%; *P*=not significant) (Figure 5A).
Similar results could be obtained for primary monocytes. Primary monocytes were treated for 24 h with farnesol in concentrations ranging from 50 µM to 200 µM. Experiments were performed in media with 10% serum. No significant reduction of viability was detectable for concentrations up to 200 µM farnesol (Figure 6A, B). Cell distribution after farnesol treatment looked similar to mock-treated cells as seen in the dot plots from a single representative experiment (Figure 6A).

Figure 5 Farnesol has no impact on viability of PMN.
Fluorescence-activated cell sorting (FACS) analysis of apoptosis in neutrophils incubated with FOH in different concentrations (50 µM, 100 µM, 200 µM), performed in media with 5% serum. (A) Representative scatter plots showing the distribution of Annexin V and PI staining for mock-treated and farnesol-treated PMN. (B) Quantitative analysis of the percentage of viable cells by FACS analysis. Data shown are the means ± standard deviation (SD) from at least 4 independent experiments and normalized to basal level of untreated PMN (set to 100%, white bar).
In contrast to PMN and primary monocytes, immature DC (iDC) were found to be more sensitive to farnesol in high concentrations. Whereas FOH concentrations up to 100 µM showed no influence on the viability of iDC, 200 µM FOH had a significant cell death promoting effect seen in a shift of the population to the PI- and Annexin V-positive region in the dot plot (upper right quadrant) and in an overall reduced viability of 64.8 ± 26.2%; P<0.01 (Figure 7A). To test the effect of farnesol on mature DC (mDC), final maturation was induced by adding 10 ng/ml LPS to iDC for 24 h in addition to farnesol (Figure 7). LPS stimulation resulted in up-regulation of CD83, CD86 and HLA-DR as well as concomitant morphological changes, indicating functional maturation of the cells. These changes were verified via flow cytometry and microscopically along with each apoptosis assay (data not shown). Comparable to iDC, mDC showed no significant alterations of cellular viability in the presence of 50 µM to
100 µM FOH, whereas 200 µM FOH led to a diminished viability of 57.6 ± 28.93% (P < 0.01) (Figure 7A, B).

Figure 7 No impact of farnesol on viability of DC in concentrations up to 100 µM.
FACS analysis of apoptosis in monocyte-derived DC treated with farnesol alone and in addition to LPS. (A) Representative scatter plots showing the distribution of Annexin V and PI staining for untreated and farnesol-treated DCs. (B) Quantitative analysis of the percentage of viable cells by FACS. Filled bars represent samples performed in media with 10% serum, striped bars stand for samples performed in media with 5% serum. Data shown are means ± SD from at least 5 independent experiments and normalized to basal level of untreated cells (set to 100%, white bar) (**P<0.01; *** P<0.001 vs. mock-treated control).
Furthermore, we determined the influence of serum content on farnesol activity. Mosel and colleagues previously demonstrated that in the presence of serum much higher levels of farnesol are needed to be functional on *C. albicans*. The authors assumed this could be due to the non-specific binding capacity of albumin (60). Indeed, using 5% instead of 10% serum we could detect a significant decrease of the viability of monocytes treated with 200 µM FOH (36.0 ± 30.5%; *P*<0.001) (Figure 6B). The viability of iDC after 200 µM FOH treatment in the presence of 5% serum compared to 10% serum (64.8 ± 26.2%) was reduced to 30.0 ± 28.5%; *P*<0.001 (Figure 7B).

To compare the low pro-apoptotic activity of farnesol to another quorum sensing molecule, monocytes and DC were incubated with the QS molecule *N*-3-oxo-dodecanoyl-<i>L</i>-homoserine lactone (HSL) from the Gram-negative bacterium *P. aeruginosa*. In contrast to FOH, the potent pro-apoptotic activator HSL induced significant cell death at a concentration of 25 µM (Figure 8A, B).

**Figure 8** The pro-apoptotic effect of the bacterial QSM HSL on monocytes and DC.

Monocytes (A) and DC (B) were treated for 24 h with HSL ranging from 1 µM to 50 µM. Quantitative analysis of the percentage of viable cells by FACS analysis are displayed. Data shown are the means ± SD from at least 4 independent experiments and normalized to basal level of untreated control (set to 100%, white bars) (**"P"<0.01; ***"P"<0.001 vs. mock-treated control).
Monocytes and DC showed around 30% to 40% reduced viability after treatment with 25 µM HSL for 24 h (monocytes: 62.5 ± 17.6%; iDC: 73.4 ± 24.1%; for both \( P<0.01 \)). Moreover, viability decreased up to 50% after treatment with 50 µM HSL for 24 h. DC treated with HSL in addition to LPS showed significant reduced viability for 50 µM HSL (58.0 ± 11.5%; \( P<0.001 \)) (Figure 8B).

Overall, the viability of PMN, monocytes and DC was not affected by physiological concentrations of farnesol.

### 3.2 Neutrophils become activated in the presence of farnesol

After defining physiological relevant concentrations of farnesol that are not inducing significant levels of cell death in human neutrophils, the activation phenotype of these cells in the presence of the fungal QS molecule was evaluated. PMN were treated with farnesol up to 200 µM for 30 min and analyzed for the surface expression of activation markers like CD66b, CD62L, CD11b and CD16 via flow cytometry (Figure 9). All four surface markers showed significant differences compared to mock-treated PMN indicating activation of the cells. After treatment with 100 µM farnesol, PMN showed an enhanced surface exposure of the degranulation marker CD66b (121.0 ± 16.1%; \( P<0.05 \)) and CD11b (141.5 ± 24.9%; \( P<0.05 \)) compared to mock-treated control. Both markers are known to be enhanced after activation (109). The surface markers CD16 and CD62L showed a concentration dependent decrease seen in the histograms as a shift of the dashed line to the left side compared to the black line indicating the mock-treated cell population (Figure 9A). Both the FCγ receptor III CD16 and the adhesion molecule CD62L are shed from the surface during neutrophil activation (207, 208). In the presence of 100 µM FOH, CD62L showed a decrease of about 75% (25.2 ± 18.5%; \( P<0.01 \)) and for CD16 a down-regulation of approximately 40% (59.3 ± 14.1%; \( P<0.05 \)) on the cell surface compared to the mock-treated control could be observed (Figure 9B).
Beside an activated surface phenotype, activation of neutrophils could also be demonstrated by investigation of certain effector mechanisms. The rapid generation of reactive oxygen species (ROS) is one of the main effector mechanisms of PMN. After incubation with different concentrations of farnesol for 30 min the PMN mount a concentration dependent increase in ROS compared to the mock-treated control. Farnesol at 50 µM was able to induce a significantly higher production of ROS, shown as increase of mean fluorescence intensity (130.0 ± 5.5% compared to mock-treated control; \( P<0.001 \)) (Figure 10A).

**Figure 9 Farnesol induces activation of PMN.**

(A) Histogram profiles show changes in the surface expression levels of activation markers CD66b, CD11b, CD16 and CD62L of primary PMN treated with 100 µM FOH for 30 min. Grey filled histograms indicate isotype control, black open histograms show expression of mock-treated samples and the dashed lines show the FOH-treated samples. (B) Levels of activation markers after FOH treatment for 30 min were quantified. All bars show means ± SD of at least 4 independent experiments with PMN from different donors normalized to the basal level of untreated cells (set to 100%, white bar) (*\( P<0.05 \); **\( P<0.01 \); ***\( P<0.001 \) vs. mock-treated control).
The release of anti-microbial peptides and proteins stored within cytoplasmic granules is a non-oxidative effector mechanism of PMN. To determine the ability of farnesol to induce degranulation, PMN were incubated with farnesol in different concentrations for 2 h and levels of myeloperoxidase (MPO), elastase 2 and lactoferrin within the supernatant were quantified. MPO and elastase 2, both stored in azurophilic granules, showed a concentration dependent increased release after farnesol treatment compared to mock-treated cells (Figure 10B). Similar results were obtained for the anti-microbial protein lactoferrin, stored in specific granules. For 200 µM of farnesol we measured a significant increase for all secreted peptides (MPO: $275.7 \pm 77.0\%$, $P<0.001$; elastase 2: $245.6 \pm 75.2\%$, $P<0.01$; lactoferrin: $543.0 \pm 140.9\%$, $P<0.01$).
To assess whether the activation of PMN after FOH treatment affects their fungicidal activity, phagocytosis and killing of *C. albicans* were quantified after co-incubation for 1 h (Figure 11A, B). Therefore, PMN were pretreated with FOH in different concentrations for 30 min. After a washing step cells were subsequently confronted with *C. albicans* cells (MOI 0.5). Phagocytosis was assessed by flow cytometry and quantified as percentage of all associated *C. albicans* cells. As shown in figure 11A a slight concentration dependent decrease in phagocytosis of *C. albicans* cells by PMN was measured (Figure 11A). For pretreated PMN with 200 µM FOH phagocytosis was reduced up to 12% compared to mock-treated cells.

The killing ability of PMN was determined by measuring the change in metabolic activity of the fungus. A reduced metabolic activity of the fungus implied an increased killing by PMN. The ability of FOH pretreated PMN to kill *Candida* was to some extent diminished as seen in a weak increase of metabolic activity of the fungus (Figure 11B). However, this is slightly contradictory to the activation of neutrophils by farnesol.

**Figure 11** Killing and phagocytosis of *C. albicans* by farnesol-treated PMN.

(A) Phagocytosis of *C. albicans* by PMN pretreated for 30 min with FOH in concentrations ranging from 50 µM to 200 µM was measured by flow cytometry. GFP-expressing *C. albicans* cells were used and extracellular fungi were stained with a specific anti-*Candida* antibody after 1 h confrontation. (B) The XTT metabolic activity assay was used to determine fungal killing of *C. albicans* cells after 1 h confrontation with either mock-treated or FOH treated PMN. Values were normalized to 100% metabolic activity of fungal cells in media (white bar) and correspond to means ± SD from 7 independent experiments. (*P*<0.05 vs. mock-treated control)
3.3 Farnesol activates monocytes and induces release of pro-inflammatory cytokines

To analyze the impact of farnesol on primary monocyte activation, monocytes were incubated with farnesol in different concentrations for 24 h and studied with respect to expression of various surface activation markers, co-stimulatory and adhesion molecules. Significant changes in surface expression could only be detected for the co-stimulatory molecule CD86 and the activation marker HLA-DR (Figure 12). Both markers increased after farnesol treatment. For treatment with 100 µM farnesol CD86 showed approximately 58% increased surface expression compared to mock-treated monocytes (157.7 ± 25.5%; *P<0.001). For HLA-DR, the increase was even more pronounced (180.2 ± 75.7%; *P<0.05) (Figure 12).

Figure 12 Farnesol induces an activated surface phenotype on monocytes.

(A) Representative histogram profiles of expression of the activation markers CD86 and HLA-DR on primary monocytes treated with 100 µM FOH are shown. Grey filled histograms indicate isotype control, black open histograms show basal expression of mock-treated samples and the dashed line stands for the samples treated with 100 µM FOH. (B) After 24 h of incubation with FOH levels of activation markers were quantified and shown as means ± SD, normalized to an untreated control (set to 100%, white bar). (*P<0.05; *** P<0.001 vs. mock-treated control)
Aside from an activated surface phenotype alterations in the cytokine profile released by monocytes after farnesol treatment were investigated. A broad set of 19 cytokines and chemokines were measured. The incubation of monocytes with 50 µM FOH for 24 h resulted in 4 times higher levels of IL-6, IL-8, MIP-1β, GRO and a 3-fold increased release of MIP-1α and TNF-α, which are important to initiate an inflammatory response and to recruit other immune cells, e.g. PMN (Figure 13). For MCP-1 this elevated release was most pronounced with a 12-fold higher release for cells treated with FOH. In contrast, FOH had no effect on the release of IL-1α, IL-1β and the anti-inflammatory cytokine IL-10 (data not shown).

**Figure 13 Farnesol induces the release of pro-inflammatory cytokines in monocytes.**
Cell culture supernatants were harvested after 24 h of incubation of monocytes with 50 µM FOH and analyzed for cytokines levels. All bars show means ± SD from 4 independent experiments with monocytes of different donors. Cytokine levels are absolute values in pg/ml. (*P<0.05; **P<0.001 vs. mock-treated control)
Like for PMN the ability of monocytes pretreated with FOH to phagocytose and kill *C. albicans* was determined after 1 h of co-incubation. Therefore, cells were washed after a pretreatment with FOH in different concentrations for 24 h, followed by co-incubation with *C. albicans* cells (MOI 0.5) for 1 h. Similar to PMN, phagocytosis of *C. albicans* by monocytes was only slightly altered in a concentration dependent manner (Figure 14A). No significant differences could be observed for the metabolic activity of *C. albicans* after co-incubation with pretreated PMN (Figure 14B).

These data indicate that farnesol induces activation of monocytes by modulating the pro-inflammatory cytokine profile and enhancing the surface exposure of activation markers CD86 and HLA-DR. However, the low-level activation is not sufficient to enhance fungicidal activity of monocytes.
3.4 Farnesol activates immature DC and impairs IL-12 release

As shown in figure 7 DC are more susceptible towards farnesol compared to monocytes and PMN concerning viability. With respect to activation, iDC displayed an increase in expression of the surface markers CD86 and HLA-DR after incubation with farnesol in different concentrations for 24 h, similar to monocytes (Figure 15). For the co-stimulatory molecule CD86 we observed a significantly enhanced surface exposure after treatment with 100 µM farnesol (190.2 ± 61.57%; \( P<0.05 \)). The MHC II molecule HLA-DR also showed an increase of more than 40% (138.3 ± 21.50%; \( P<0.05 \)) compared to the mock-treated control (95.0 ± 7.0%). However, after stimulating iDC with LPS in addition to farnesol, the activated surface phenotype was not visible anymore. No significant alteration in surface expression of HLA-DR and CD86 was detectable (Figure 15).

Figure 15 Impact of farnesol on surface phenotype of DC.

Maturation and activation markers of immature DC treated with farnesol alone and in the presence of LPS for 24 h were analyzed. Histogram profiles (A) and levels of markers (B) are shown. Graphs show means ± SD normalized to an untreated control (set to 100%, white bar) (*\( P<0.05 \); **\( P<0.01 \) vs. mock-treated control).
Furthermore, the release of cytokines from iDC incubated for 24 h with farnesol in addition to LPS, a trigger for final maturation, was quantified. Farnesol neither had an impact on pro-inflammatory cytokines like IL-1, IL-6, IL-8, MIP-1α, MIP-1β and TNF-α nor on secretion of anti-inflammatory cytokines like IL-10 and IL-4 in the presence of LPS. Interestingly, a significant reduction in the secretion of IL-12 was observed (Figure 16). A 4-fold lower release of IL-12p70 and a 2-fold lower secretion of IL-12p40, a subunit of IL-12p70 (209), were determined compared to mock-treated control. As IL-12 is known for its immunoregulatory function to direct the type of adaptive immune response by inducing Th1 cells its impaired release may suggest a modulation of anti-fungal immunity by farnesol.

![Figure 16](image)

**Figure 16 DC treated with farnesol show an impaired IL-12 release.**
Cytokine release of iDC was measured after stimulation with LPS in addition to farnesol for 24 h, shown as absolute values in pg/ml. (*P<0.05; **P<0.01 vs. mock-treated cells)

### 3.5 Impact of farnesol on DC differentiation

In the previously described experiments, immature DC were treated after differentiation from monocytes in the presence of cytokines (IL-4, GM-CSF) with farnesol for 24 h and in addition with LPS. These analyses focused on the effects of FOH on iDC and mDC, respectively. However, to investigate the impact of farnesol on the differentiation process from monocytes to DC, cells were exposed to farnesol or the solvent control in addition to the treatment with cytokines for differentiation. To ensure that farnesol is present during the entire differentiation process farnesol was included in the media.
3.5.1 Viability and surface phenotype of DC exposed to farnesol during differentiation

First, viability of iDC generated in the presence of FOH in concentrations ranging from 10 µM to 100 µM was determined (Figure 17). As shown in the dot plots no distinct changes in cell distribution could be observed for concentrations up to 50 µM FOH (Figure 17A). However, a clear accumulation of Annexin V- and PI-positive cells was detected for iDC generated in the presence of 100 µM FOH and resulted in a reduced viability of 78.0 ± 15.7%, $P<0.01$. To exclude cell-death promoting farnesol effects, subsequent experiments were performed with FOH in concentrations up to 50 µM.

**Figure 17 Viability of DC differentiated in the presence of farnesol.**

FACS analysis of apoptosis in iDC after differentiation in the presence of FOH (10 µM - 100 µM) is displayed. (A) Representative scatter plots showing the distribution of Annexin V and PI staining. (B) Quantitative data of viable cells are shown as means ± SD from at least 4 independent experiments and normalized to basal level of untreated DC (set to 100%, white bar). (**$P<0.01$ vs. mock-treated control)**
Flow cytometric immunophenotyping enabled the analysis of surface markers that are important for DC maturation and function. The surface phenotype of DC was evaluated after 6 days of differentiation in the presence of FOH (10 µM - 50 µM) and after final maturation induced by LPS stimulation for 24 h (Figure 18, 19).

CD1a, a transmembrane glycoprotein strongly induced on the cell surface after exposure to GM-CSF and IL-4, exhibited the most prominent change (156). After differentiation in the presence of 50 µM FOH, CD1a was almost not exposed to the cell surface compared to mock-treated cells (FOH 50 µM: 1.3 ± 0.7%; \(P<0.001\)). This effect could also be monitored for exposure to lower FOH application rates in a concentration dependent manner (Figure 18). The CD1a level did not change after LPS stimulation (FOH 50 µM: 1.7 ± 0.8%; \(P<0.001\)). Furthermore, alteration of surface molecules like the maturation marker CD83, the co-stimulatory molecules CD40, CD80, CD86 and the MHC II molecule HLA-DR which are important for DC to execute their antigen-presenting role, were determined (Figure 18). Both CD40 and CD80 showed a reduced expression on the cell surface after 6 d of differentiation in the presence of 50 µM FOH (CD40: 73.0 ± 17.2%; \(P<0.05\); CD80: 71.0 ± 21.5%; \(P<0.01\)). This was still visible and more pronounced after stimulation with LPS (CD40: 69.9 ± 12.7%; CD80: 47.7 ± 22.0%; for both \(P<0.01\)). Moreover, farnesol-treated DC also failed to completely up-regulate the maturation marker CD83 on their surface after LPS stimulation (FOH 50 µM: 31.4 ± 19.7%; \(P<0.001\)) (Figure 18). In contrast, after 6 days of differentiation, the myeloid marker CD14 and the activation marker CD86, which are distinctive markers for monocytes and are typically down-regulated during differentiation, retained on the surface of DC generated in the presence 50 µM FOH compared to mock-treated cells (CD14: 113.5 ± 22.5%; \(P<0.01\); CD86: 400.0 ± 184.5%; \(P<0.001\)) (Figure 19). Moreover the MHC-II molecule HLA-DR showed increased expression, too (FOH 50 µM: HLA-DR 159.3 ± 31.2%; \(P<0.001\)). These findings were consistent with the effects determined for iDC treated with farnesol after differentiation (see section 3.4, Figure 15). CD14 is constantly present and significantly enhanced after exposure to LPS (387.5 ± 252.7%; \(P<0.01\)). In contrast, surface expression of CD86 and HLA-DR was markedly impaired after LPS stimulation of DC differentiated in the presence of farnesol (CD86: 47.2 ± 31.0% and 26.9 ± 12.3%; for both \(P<0.01\)) (Figure 19).
Figure 18  Surface expression of CD1a, CD40, CD80, CD83 on DC generated in the presence of farnesol.

Immunophenotype of DC generated in the presence of farnesol was evaluated. For each surface marker (CD1a, CD40, CD80, CD83) quantitative analysis and a representative histogram profile after 6 d of differentiation (left) and in addition after 24 h stimulation with LPS of 6 d old DC (right) are shown. Quantitative data correspond to the means ± SD from at least 5 independent experiments, normalized to basal level of untreated DC (set to 100%, white bar). (*P<0.05; **P<0.01; ***P<0.001 vs. mock-treated control)
Figure 19 Surface expression of CD14, CD86 and HLA-DR on DC generated in the presence of farnesol

Immunophenotype of DC generated in the presence of farnesol was evaluated. For each surface marker (CD14, CD86, HLA-DR) quantitative analysis and a representative histogram profile after 6 d of maturation (left) and in addition after 24 h stimulation with LPS (right) are shown. Quantitative data correspond to the means ± SD from at least 5 independent experiments and normalized to basal level of untreated DC (white bar). (*P<0.05; **P<0.01; ***P<0.001 vs. mock-treated control)
3.5.2 Transcriptional profiling of DC generated in the presence of farnesol

Transcriptional profiling offers a powerful approach to elucidate the cause of the considerable changes in surface marker expression in response to farnesol treatment of DC on a transcriptome level and allows an overview of altered cellular functions. The transcriptome of DC generated in the presence of farnesol was investigated in collaboration with Steffi Spielberg (202). To differentiate between the impact of farnesol on the transcription profile during monocyte to DC differentiation and during maturation from immature DC to mature DC, cells were collected after 3 d and 6 d of generation and after exposure to LPS for 6 h (6 d + 6 h LPS). Four biological replicates were generated for each defined time point by using four different donors. After RNA isolation and cRNA synthesis a whole-genome expression direct hybridization assay based on a bead chip array (Illumina® HumanHT-12v4 Expression BeadChip) was performed to detect differentially expressed genes (DEG) in DC generated in the presence of farnesol. To be considered for further analysis as DEG, a $P$ value of less than 0.05 and a fold change of 2 ($-2 > FC > 2$) were employed. A total of 704 differentially regulated genes were identified. Distinguishing between the initial differentiation process from monocytes to iDC (0 d - 6 d) and the maturation to mDC (after LPS stimulation) we found 381 differentially regulated genes during differentiation of which 25 genes were differently expressed after 3 days and additional 356 genes after 6 days of generation. An extra 323 genes were differentially expressed after 6 h LPS exposure (Figure 20).

![Venn diagram](image)

**Figure 20** Venn diagram showing the number of differentially expressed genes of DC generated in the presence of farnesol.

The overlap between genes of defined time points (3 d, 6 d and 6 d + 6 h LPS) with differential expression of more than 2-fold are shown.
The heat map in figure 21 was generated to show distinct expression patterns of DC generated in the presence of FOH 50 µM. Therefore, a representative set of DEG was selected, which closely mirrored the whole microarray analysis. Comparing all imposed time points the activation pattern revealed the greatest response after LPS-stimulation (time point 6 d + 6 h LPS) (Figure 21).

**Figure 21** Heat map depicting a representative expression profile in DC generated in the presence of farnesol.

Heat map generated from microarray data reflecting gene expression values at all 3 time points (3 d, 6 d, 6 d+6 h LPS) of DC generated in the presence of FOH in comparison to mock-treated cells. The expression pattern (blue to yellow) represents the spectrum of down-regulated to up-regulated expression of a representative set of DEG. All displayed DEG have a P value <0.05.
KEGG (Kyoto Encyclopedia of Genes and Genomes)-based analysis of the transcriptome could verify gene groups of mainly immunological processes. A high number of differentially regulated genes are involved in processes like hematopoietic cell lineage, cytokine-cytokine receptor interaction, antigen processing and presentation and cell adhesion molecules, indicating broad functional implications of farnesol treatment (Table 7-8).

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Gene count</th>
<th>P value</th>
<th>E score</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematopoietic cell lineage</td>
<td>13</td>
<td>$1.0 \times 10^{-6}$</td>
<td>6.26</td>
<td>CD14, CD1A, CD1D, CD1E, CD36, CR1, CSF2RA, HLA-DRB3, HLA-DRB4, IL1R1, IL1R2, IL9R, ITGA3</td>
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<tr>
<td>Phagosome</td>
<td>14</td>
<td>$4.9 \times 10^{-5}$</td>
<td>4.31</td>
<td>CD14, CD36, CTSL1, CYBB, FCGR2B, HLA-DQA1, HLA-DRB3, HLA-DRB4, LAMP1, OLR1, RAB7B, SCARB1, THBS3, TLR4</td>
</tr>
<tr>
<td>Antigen processing and presentation</td>
<td>8</td>
<td>$2.0 \times 10^{-3}$</td>
<td>2.71</td>
<td>CD74, CTSL1, HLA-DQA1, HLA-DRB3, HLA-DRB4, HSPA5, IFI30, RFXAP</td>
</tr>
<tr>
<td>NF-κB signaling pathway</td>
<td>8</td>
<td>$3.5 \times 10^{-3}$</td>
<td>2.46</td>
<td>BIRC3, BLNK, CD14, IL1R1, IRAK1, LTB, PRKCB, TLR4</td>
</tr>
<tr>
<td>Cytokine-cytokine receptor interaction</td>
<td>14</td>
<td>$4.1 \times 10^{-3}$</td>
<td>2.39</td>
<td>CCL2, CCL24, CCL26, CCL8, CSF2RA, IFNAR2, IL13RA1, IL17RB, IL1R1, IL1R2, IL1RAP, IL9R, LTB, TNFRSF4</td>
</tr>
</tbody>
</table>

Table 7  KEGG pathway enrichment analysis of DEG at 6 d time point.
Shown are the top 5 enriched groups identified by functional annotation via KEGG. The differential regulated genes for each category are shown as well as the fold enrichment (E score) and P values ($P \leq 0.05$).
<table>
<thead>
<tr>
<th>Pathway</th>
<th>Gene count</th>
<th>P value</th>
<th>E score</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
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<td>Cytokine-cytokine receptor interaction</td>
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<td>1.0 × 10^{-6}</td>
<td>9.59</td>
<td>CCL1, CCL19, CCL2, CCL24, CCL26, CCL5, CCL7, CCR1, CCR6, CD40, CD70, CSF2RA, CXCL1, CXCL9, IFNB1, IL13RA1, IL15, IL17RB, IL1R2, IL1RAP, IL28A, IL28B, IL29, IL9R, LTA, LTB, TNFRSF12A, TNFRSF21, TNFRSF4, TNFRSF14</td>
</tr>
<tr>
<td>Hematopoietic cell lineage</td>
<td>13</td>
<td>1.0 × 10^{-5}</td>
<td>5.00</td>
<td>CD14, CD1A, CD1D, CD1E, CD55, CR1, CSF2RA, HLA-DRB3, HLA-DRB4, IL1R2, IL9R, ITGA3, ITGA6</td>
</tr>
<tr>
<td>NF-kappa B signaling pathway</td>
<td>11</td>
<td>3.5 × 10^{-4}</td>
<td>3.45</td>
<td>BIRC3, CCL19, CD14, CD40, DDX58, IRAK1, LTA, LTB, MAP3K14, PRKCB, TNFRSF14</td>
</tr>
<tr>
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<td>2.62</td>
<td>ATP6V0A1, CD14, CLEC7A, CTSL1, CYBB, HLA-DOB, HLA-DQA1, HLA-DRB3, HLA-DRB4, LAMP1, OLR1, RAB7B, THBS3</td>
</tr>
<tr>
<td>TNF signaling pathway</td>
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<td>3.6 × 10^{-3}</td>
<td>2.44</td>
<td>BIRC3, CCL2, CCL5, CREB5, CXCL1, IL15, LTA, MAP3K14, MLKL, MMP9</td>
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<tr>
<td>Cell adhesion molecules (CAMs)</td>
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<td>8.8 × 10^{-3}</td>
<td>2.06</td>
<td>CD40, CLDN1, CLDN23, HLA-DOB, HLA-DQA1, HLA-DRB3, HLA-DRB4, ITGA6, ITGAL, PDCD1LG2, VCAN</td>
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<tr>
<td>Antigen processing and presentation</td>
<td>7</td>
<td>2.6 × 10^{-2}</td>
<td>1.59</td>
<td>CD74, CTSL1, HLA-DOB, HLA-DQA1, HLA-DRB3, HLA-DRB4, HSPA1B</td>
</tr>
</tbody>
</table>

Table 8 KEGG pathway enrichment analysis of DEG at 6 d + 6 h LPS time point.
Shown are the top 7 enriched groups identified by functional annotation via KEGG. The differential regulated genes for each category are shown as well as the fold enrichment (E score) and P values (P≤0.05).
The analysis of the early transcriptome (3 d) showed up-regulated genes coding for the chemokine CCL2/MCP-1 (fold-change [FC]: +4.99) and the surface molecule CD1d (FC: +4.47) and down-regulated genes involved in antigen processing and presentation (CD74 FC: −2.6; CD1E FC: −2.86) as well as the C-type lectin receptor (CLEC1A, FC: −2.85).

With progressive differentiation into iDC (6 d) increased up-regulation of the human gene CD1D (FC: +17.6) and CCL2/MCP-1 (FC: +17.85) was detected. Furthermore, data showed enrichment of genes involved in antigen processing and presentation and cytokine-cytokine receptor interaction seen in up-regulation of CD14 (FC: +13.41) and down-regulation of CD1A, CD1E and CD80 (FC: −20.08, FC: −11.46 and FC: −2.02, respectively) and in the induction of genes coding for chemokines like CCL26/MIP-4α (FC: +9.77) and CCL24/eotaxin-2 (FC: +6.0). Moreover, several genes with functions in cell adhesion and migration were down-regulated like MMP12 (FC: −44.67), MMP25 (FC: −7.6), ADAM19 (FC: −11.11) and CLDN1 (FC: −10.45).

Stimulation with LPS for 6 h (6 d + 6 h LPS) revealed DEG mainly associated with cytokine-cytokine interactions. Genes coding for proteins with chemotactic activity for neutrophils (CXCL1 FC: −4.01) monocytes (CCL1 FC: −25.46) or activated T cells (CXCL9 FC: −19.84) and gene encoding the chemokine receptor CCR6 (FC: −2.42) were significantly reduced. Concerning genes involved in antigen processing and presentation the FC of CD1A and CD1E showed further decrease (FC: −24.14; FC: −14.68) and the gene encoding the co-stimulatory molecule CD40 revealed down-regulation (FC: −2.97) in the transcriptome data. In addition, the number of genes coding for C-type lectin receptors including CLEC7A/dectin-1 (FC: −2.32), which is of decisive importance for recognition of fungal pathogens, decreased after LPS stimulation of DC generated in the presence of farnesol (210). Furthermore an up-regulation of the gene coding for the matrix metallopeptidase (MMP) inhibitor TIMP1 (FC: +4.12) and a down-regulation of the gene encoding the adhesion molecule AMICA1 (FC: −3.05) were found after LPS stimulation of DC differentiated in the presence of farnesol.

In summary, during the differentiation process from monocytes to iDC (3 d and 6 d) regulated genes are associated with antigen processing and presentation, cell adhesion and migration followed by fungal recognition and chemokine and cytokine signaling genes at later time points (6 d + 6 h LPS).
3.5.3 Transcriptional profiling confirmed shift in antigen presenting strategy of DC generated in the presence of farnesol

The results of the transcriptome data are in line with the altered surface phenotype of DC generated in the presence of farnesol. The strong down-regulation of CD1A in the array confirmed the almost complete absence of the glycoprotein CD1a on the surface of iDC generated in the presence of FOH (see Figure 18). Furthermore, the decreased expression of the co-stimulatory molecules CD80 and CD40 on the cell surface reflected the down-regulation of both genes in the array (Figure 18).

To further validate the microarray findings, we first investigated the surface expression of CD1d, that is strongly up-regulated at all 3 time points (3 d, 6 d and 6 d+6 h LPS) compared to the transcriptome profile of the mock-treated control. In our experimental setting freshly isolated human monocytes showed low but measurable surface levels of CD1d (Figure 22A, B). During normal differentiation CD1d levels are not affected compared to CD1a that is strongly induced on the cell surface. However, after farnesol treatment iDC showed an increased expression of CD1d (at 6 d time point: 350.3 ± 303.7%; P<0.01) compared to mock-treated control. Even at the early differentiation stage (3 d) a 78.9% higher expression was detectable (176.0 ± 105.4%; P<0.01). This increase was also maintained during the maturation process from iDC to mDC (Figure 22A, B). In addition, a strong up-regulation of the gene encoding CD14 in the transcriptome data was observed after 6 days and after stimulation with LPS for 6 h (FC: +14.41 and +8.39, respectively). This was confirmed by measuring a significantly increased expression of CD14 on the cell surface after LPS stimulation of DC generated in the presence of FOH (387.5 ± 252.72%; P<0.01; see Figure 19). Moreover, cell culture supernatants were tested for soluble CD14 (sCD14), which either appears after protease-mediated shedding of CD14 or after direct secretion (211). An 18-fold increase of sCD14 could be observed for DC generated in the presence of FOH compared to mock-treated samples (Figure 22C).

Overall, the increased surface expression of CD1d implies a shift in immune response that is further confirmed by enhanced expression of CD14 and an increased release of soluble CD14 that has potential immunoregulatory function.
3.5.4 Farnesol modulates cytokine release indicating a shift in immune response

As mentioned before, the results of the transcriptomic profile showed a difference in the expression profile of various genes encoding cytokines. The release of cytokines is very important for DC to interact with their environment especially other immune cells, e.g., T cells. A broad set of 19 cytokines was tested to determine if the presence of farnesol has an impact on the cytokine profile during maturation of dendritic cells and to validate the transcriptomic data. The data of this study revealed significantly elevated secretion of pro-inflammatory cytokines as well as chemokines by DC in response to
farnesol (Fig. 23). The most pronounced difference in cytokine secretion was detected for the chemokine MCP-1 (CCL2). The 15-fold higher release goes along with the transcriptomic data where it belongs to the most up-regulated genes. In addition, further DEG encoding chemokines were found to be differentially secreted by FOH-treated DC. MCP-3 and eotaxin-2, both chemoattractants for eosinophils (212), were up-regulated in the array and showed increased levels in cell culture supernatants. In contrast, the chemokines I-309 (CCL1) and MIG (CXCL9) were down-regulated on the transcriptome level and were significantly reduced in supernatants of DC generated in the presence of FOH. For the chemokine RANTES a 4-fold decreased secretion compared to the mock-treated control, also reflected the down-regulation in the transcriptome. Moreover, the IL-1 receptor antagonist (IL-1RA), which is an inhibitor of the pro-inflammatory effect of IL-1β showed more than a 4-fold up-regulation on transcriptomic level and protein level (Figure 23A, B). Taken together, the changes in secretion of chemokines validated the observed changes on the transcriptome level (Figure 23B).

For the following cytokines an impact of farnesol on the protein level, but not on the transcriptional level was found. For the pro-inflammatory cytokines IL-6, IL-8, MIP-1α, MIP-1β a 2-fold up-regulation compared to the mock-treated control was determined. More than 4-fold higher levels of GM-CSF, G-CSF and TNF-α were released by DC generated in the presence of 50 µM farnesol (Figure 23A). Interestingly, the key immunoregulatory cytokine IL-12 (IL-12p70) revealed reduced levels after farnesol treatment. We observed a more than 12-fold lower release of IL-12p70 that goes along with a 2-fold decrease of the subunit IL-12p40 (209). Similar results were obtained for IL-12 levels of DC treated with farnesol after differentiation (see section 3.4; Figure 16). In contrast, the anti-inflammatory cytokine IL-10 showed a significantly increased release of approximately 3-fold (Figure 23A). The altered cytokine profile indicates that farnesol influences DC to release cytokines that promote inflammation besides dampening a protective Th1 response, which is important for fungal clearance.
Figure 23 Cytokine levels of DC generated in the presence of farnesol.

(A) Secretion of cytokines was measured in cell culture supernatants after 24 h LPS stimulation of mock-treated iDC and iDC generated in the presence of 50 µM FOH. Bars show means ± SD of at least 4 independent experiments with cells from different donors. (B) Fold change (FC) of chemokine transcript expression levels (time point 6 d+6 h LPS) of FOH-treated (50 µM) relative to mock-treated cells compared to cytokine release into the supernatant (after 24 h LPS stimulation) are plotted. (*P<0.05; **P<0.01; ***P<0.001 vs. mock-treated control)
3.5.5 Farnesol-treated DC are restricted in their migrational behavior

Along with a matured phenotype and an adequate cytokine release DC need to be mobile to accomplish their immune function. Migration is one indispensable attribute, which enables DC to reach T cell-rich areas. The array data revealed a strong down-regulation of genes encoding for metalloproteinases (MMP12, MMP25, ADAM9) and adhesion molecules like CLDN1 and AMICA1, which are important for migration of DC. First, levels of MMP12, a matrix metalloproteinase were evaluated. DC generated in the presence of FOH released more than 80% less MMP12 after LPS stimulation compared to mock-treated cells (12.7 ± 10.9%; \( P<0.001 \)) (Figure 24).

![MMP12](image)

**Figure 24 Secretion of MMP12 is reduced in the presence of farnesol.**

MMP12 level were measured in cell culture supernatants after 24 h LPS stimulation of mock-treated iDC and iDC generated in the presence of 50 µM FOH. Bars show means ± SD of at least 7 independent experiments with cells from different donors. (**P<0.001 vs. mock-treated control)**

For establishing cell contacts followed by induction of T cell responses DC need a functional actin cytoskeleton (213). To observe changes in actin cytoskeleton an *in vitro* cell-spreading assay was performed. Cells were seeded onto a surface coated with the extracellular matrix protein e.g. fibronectin. They first attach to the ECM and then spread rapidly by forming filopodia-like protrusions. To elucidate if cell-spreading is disrupted in DC generated in the presence of FOH 50 µM, cells were allowed to attach and spread on fibronectin coated slides for 40 min. Microscopic analyses revealed that DC generated in the presence of farnesol were not able to form protrusions compared to mock-treated cells (Figure 25). Predominantly, FOH-treated cells developed a rounded phenotype, while mock-treated cells formed filopodial protrusions...
Less than 20% of cells (18.2 ± 5.3%, \( P<0.001 \)) showed a spread phenotype compared to 88.4 ± 9.9% for mock-treated cells (Figure 25B).

3.5.6 Farnesol impairs the ability of DC to act as a professional antigen-presenting cell

All data reported so far, suggested a defect of DC generated in the presence of farnesol to induce proper T cell responses. To investigate if farnesol has an impact on the function of DC to act as an antigen-presenting cell, the ability to induce T cell proliferation was determined. Therefore, a mixed allogeneic lymphocyte reaction using CFSE labeling was performed. CFSE is a stable cytoplasmic dye that passively diffuses into cells and is distributed equally into daughter cells upon cell division. The fluorescence intensity of CFSE-labeled T cells halves with each generation so that dividing cells can be characterized by a low CFSE signal (CFSE\(^{low}\)). On day 6, mock-treated and farnesol-treated mDC were co-cultured with allogeneic naïve CFSE-labeled CD3\(^+\) T cells for 4 days. The percentage of CFSE\(^{low}\) T cells was measured by flow cytometry as shown in figure 26. While mock-treated mature DC induced T cells to undergo multiple cell division farnesol-treated mDC were significantly less potent in inducing T cell proliferation (Figure 26A). For all tested donors (n=3) farnesol-treated
mDC showed more than 50% reduced percentage of CFSE$_{\text{low}}$ T cells (49.8 ± 24.9%, $P<0.01$) compared to mock-treated mDC (Figure 26B).

Figure 26 Impact of farnesol on antigen presentation of DC.

(A) Representative histograms and dot plots of CFSE fluorescence intensity, gated on CD3+ T cells, are shown for day 4 culture of in vitro stimulation with untreated mDC and mDC generated in the presence of 50 µM FOH. As a negative control CFSE labeled unstimulated T cells are shown. In the histogram each peak represents a round of cell division; percentages of cells that have undergone numbers of divisions are shown above the peaks. (B) Representative dot plots showing percentage of CFSE$_{\text{low}}$ gated cells. Similar results were obtained in 3 independent experiments. Data is normalized to an untreated control (set to 100%, white bar). (**$P<0.01$ vs. mock-treated control)
3.6 Analysis of potential mechanisms for impact of farnesol on the differentiation of monocytes to DC

As farnesol strongly influences the differentiation from primary monocytes to dendritic cells it is of remarkable interest to elucidate the mechanism behind the farnesol effect.

3.6.1 The Ras protein as a potential target for farnesol

Ras proteins are monomeric GTPases that regulate a wide range of cellular processes including cell survival, growth and differentiation by transmitting signals from cell surface receptors to a diversity of effectors (214). In 2012, Singh et al. demonstrated that DC isolated from R-Ras deficient mice were impaired in their ability to prime CD4+ T cell (215). In addition, like FOH-treated DC, R-Ras−/− DC showed attenuated cell spreading and an altered surface phenotype, like lower levels of MHC II (HLA-DR) and CD86 in response to LPS. Taking into account that in C. albicans Ras1 is a potential target for direct inhibition by farnesol we hypothesized that Ras proteins are also a possible point of action in dendritic cells. First, the total endogenous protein level of Ras in DC generated in presence of farnesol compared to mock-treated DC was evaluated to determine if farnesol is inducing degradation of Ras proteins or interferes with their turnover. Therefore, western blot analyses with an anti-pan-Ras antibody, detecting the three isoforms H-, N- and K-Ras were performed. No differences in the expression of Ras could be observed in DC generated in the presence of farnesol compared to mock-treated cells, indicating that farnesol does not induce degradation or interferes with the turnover of Ras proteins (Figure 27).

![Western blot analysis of pan-Ras](image)

**Figure 27 Western blot analysis of pan-Ras**

Western blot analysis of pan-Ras expression in DC generated in the presence of 50 µM FOH (lane 3) compared to mock-treated cells (lane 2). Blot probed with pan-Ras (clone RAS10, 21 kDa). α-tubulin (52 kDa) was used as loading control.
To obtain a more detailed view on the isoforms of Ras and to add R-Ras as a potential target for farnesol, specific monoclonal antibodies for all Ras proteins were employed in western blot analyses. However, not all antibodies were useable in the experimental setting of this study (data not shown). In an alternative attempt, transient transfection was considered to be a useful tool to determine the impact of farnesol on Ras proteins. According to the enhanced susceptibility of primary immune cells to transfection, Jurkat cells, an immortalized human T lymphocyte cell line, was used. To exclude a pro-apoptotic effect of FOH on Jurkat cells, we performed viability analyses with FOH concentrations ranging from 50 µM to 200 µM prior to transfection experiments (Figure 28). Jurkat cells displayed no change in viability for concentrations up to 100 µM. For comparability with the impact of FOH on primary immune cells further experiments were performed with 50 µM FOH.

Figure 28 Viability of Jurkat cells after farnesol treatment.
FACS analysis of apoptosis in Jurkat cells, stimulated with FOH in different concentrations (50 µM - 200 µM) for 24 h. (A) Representative scatter plots showing the distribution of Annexin V and PI staining for untreated and FOH-treated Jurkat cells. (B) Quantitative analysis of the percentage of viable cells, shown as means ± SD from at least 3 independent experiments, normalized to basal level of untreated cells (set to 100%, white bar) (**P<0.01 vs. mock-treated control).
According to an optimized nucleofection protocol from LONZA $1 \times 10^6$ Jurkat cells were transfected via nucleofection with plasmids expressing different Ras isoforms (H-, K-, and N-Ras) linked to the fluorescent protein mCherry or in case of R-Ras linked to the HA-tag. Subsequently, transfected cells were treated either with 50 µM farnesol or the solvent control (mock-treated) for 48 h. Protein preparation and quantification followed by western blot analysis using antibodies either against mCherry or the HA-tag were performed to investigate if farnesol has an impact on the protein levels of H-, K-, N- or R-Ras. (Figure 29 A-D).

**Figure 29 Western blot analysis of Ras fusion protein expression in transfected Jurkat cells treated with farnesol.**

Jurkat cells were transfected with the indicated constructs and treated with FOH 50 µM. Protein expression was assessed 48 h later by either anti-mCherry or anti-HA Western blotting. Blot A-C probed with anti-mCherry (1C51). 14-3-3 (30 kDa) served as loading control. Blot D probed with anti-HA (~31 kDa). α-tubulin (52 kDa) used as loading control.
Comparison of the band intensities of all defined Ras proteins linked to mCherry or a HA-tag showed no expression differences in Jurkat cells treated with FOH and mock-treated cells.

In *C. albicans* it was suggested that farnesol interferes with the farnesylation of Ras proteins, a post-translational modification. Ras proteins undergo post-translational processing to be able to associate with membranes (216). To elucidate if farnesol interferes with e.g. farnesylation of Ras proteins and consequently with plasma membrane association rather than degradation or turnover, Jurkat cells were transfected with plasmids expressing tagged Ras proteins as described above. To track their subcellular localization transfected cells were analyzed 48 h after farnesol treatment via confocal fluorescence microscopy (Figure 30).

![Image](image.png)

**Figure 30** Localization of Ras fusion proteins in transfected Jurkat cells treated with farnesol.

Jurkat cells were transfected with the indicated mCherry Ras construct (A-C) or R-Ras-HA-tag plasmid (D). After 48 h, protein distribution in transfected cells was imaged via confocal microscopy. Scale bars indicate 10 µm.
The treatment of Jurkat cells with farnesol revealed no distinct differences in localization of H-, K- and R-Ras fusion proteins (Figure 30A, C, D). Concerning N-Ras weak differences could be observed displaying less membrane distribution in FOH-treated cells compared to mock-treated cells (Figure 30B). However, in order to give a statement based on quantitative analysis further experiments are required.

3.6.2 Farnesol effects may be mediated in part by desensitizing monocytes to GM-CSF

A detailed examination of transcriptional data obtained from DC generated in the presence of DC revealed another potential target addressed by farnesol. Myeloid dendritic cells are conventionally generated from human peripheral blood monocytes using GM-CSF and IL-4. Both cytokines are necessary to develop a CD14 negative phenotype with an antigen-presenting capacity to stimulate naive T cells (149). Pertaining to the changes in surface phenotype and functionality, DC generated with farnesol seem to be trapped in a monocyte-like state. As differentiation is regulated by these cytokines a subpar response to GM-CSF and IL-4 could be one possible point of action addressed by farnesol. A closer look at the transcriptome data revealed a more than 3-fold down-regulation of the gene encoding the GM-CSF receptor (CSF2R) after six days of differentiation in the presence of FOH and also after stimulation with LPS for 6 h (at 6 d: FC: -3.08; at 6 d+6 h LPS: FC: -3.56). No changes in transcriptome profile of the gene coding for the IL-4 receptor (IL-4R) could be observed. Subsequently, surface expression of both receptors was analyzed via flow cytometry. The GM-CSFR showed a more than 50% decreased expression (44.5 ± 22.4%; P<0.001) on the cell surface of FOH-treated DC after LPS stimulation (Figure 31). In line with the microarray data iDC generated in the presence of farnesol showed no alteration in IL-4R surface exposure (data not shown).
Figure 31 Impact of farnesol on the surface expression of GM-CSFR.
Surface receptor expression of GM-CSFR is shown after 24 h LPS stimulation of mock-treated DC and DC generated in the presence of FOH (50 µM). (A) Data shown are the means ± SD normalized to level of untreated cells (set to 100%, white bar). (B) Representative histogram showing the decrease in surface expression of GM-CSFR. (***P<0.001 vs. to mock-treated control)
4 Discussion

The use of quorum sensing systems to coordinate the expression of virulence factors during infection of a host may confer a survival advantage for pathogenic bacteria (9, 217). The most studied fungal quorum sensing molecule is farnesol, discovered by Hornby et al. (21). Its role in controlling C. albicans morphology and interactions with other microbes has been well analyzed, but the impact on human host cells remains elusive. In 2001, Hornby and colleagues defined two contradicting hypotheses concerning the role of farnesol in Candida pathogenesis, stating that farnesol may be a possible therapeutic compound due to its ability to block the transition from yeast to filamentous form or may be acting indirectly as a virulence factor (21).

The aim of this study was to systematically analyze the impact of farnesol on human innate immune cells to elucidate the role of farnesol as a fungal QSM during interaction with the host.

4.1 Low pro-apoptotic action of farnesol in human innate immune cells

Farnesol, as a member of non-sterol isoprenoids, has been shown to impair proliferation of a number of cell lines and to induce apoptosis in several tumor-derived cell lines (94-96, 204-206, 218). The results of this study revealed that farnesol has low pro-apoptotic activity for human innate immune cells in the presence of serum. No significant changes in the viability of PMN and monocytes could be observed for concentrations up to 200 µM FOH. DC were responsive to farnesol, as they show an induction of apoptosis at 200 µM FOH. The low pro-apoptotic effects on primary immune cells could be attributed to the presence of serum (60). Mosel and colleagues defined concentrations which block germ tube formation in C. albicans in the presence and the absence of serum and demonstrated that much higher levels of farnesol are needed to be effective on C. albicans in the presence of serum. For RPMI containing 10% serum, a concentration of about 150 µM FOH was necessary to block germ tube formation of C. albicans to 50%, which normally is achieved by 1 µM in RPMI without serum (60). In the experimental setting of this study reduced serum levels (RPMI+5%) resulted in a decrease of viability when FOH was used at the highest concentration of 200 µM. Mosel and colleagues hypothesized that the presence of albumins in serum interferes with farnesol due to its nonspecific lipid binding capacity and that FOH accumulates in membranes, potentially leading to physiological artifacts at higher concentrations (250 µM - 500 µM). Concentrations of FOH that might be present in vivo remain elusive but it has been hypothesized that biofilms of C. albicans grown in vitro
can produce up to 1 mM FOH, a concentration significantly higher than 55 µM that has previously been measured in planktonic cultures grown in media containing serum (64). Considering the fact that C. albicans biofilms on implanted devices e.g. catheters are causes of Candida infections, the concentration of FOH, at least in some areas of the active site of infection, may be equivalent to or higher than 50 µM. Furthermore, given that FOH will be exposed to serum in the human body and might interact with lipids and membranes, concentrations up to 100 µM FOH that exhibit no pro-apoptotic effects on innate immune cells are reliable and physiologically relevant.

The fact that farnesol is not pro-apoptotic in non-malignant primary cells but causes significant cell death in a broad range of malignant cells has been described before (58, 97). Adany and colleagues reported a selective toxic effect on neoplastic cells for concentrations ranging from 10 µM to 33 µM that might be mediated through inhibition of phosphatidylcholine biosynthesis (97, 219). Similar anti-cancer properties have been reported by several studies promoting the concept that FOH can function as chemopreventive or therapeutic agent against several types of cancer (55, 220-222). In addition, anti-tumor effects of FOH have been demonstrated in animal models but no clinical trials have been reported to my knowledge (59, 223). The underlying mechanisms have not been fully unveiled and further studies are required to elucidate the selective apoptotic effect and to clarify the therapeutic or chemopreventive use of FOH in human cancer (224).

In contrast to farnesol but in line with previous studies the bacterial QSM HSL was found to be a strong pro-apoptotic stimulus in primary immune cells. The results presented here showed significant cell death at low concentrations of 25 µM. Despite structural similarities of HSL to FOH and its ability to mimic the farnesol effect on C. albicans (87), the Gram-negative QSM has been shown to induce apoptosis at concentrations of 50 µM in phagocytes, i.e. in mouse macrophages and neutrophils and might therefore serve as a supporting tool in host defense evasion by P. aeruginosa (225). However, other studies analyzing the impact of HSL on immune cells showed discrepant results observing no apoptosis in neutrophils in concentrations up to 100 µM HSL (226, 227).

In summary, the use of appropriate physiologically relevant concentrations of QSM like FOH or HSL in interaction with the host should be defined in order to avoid nonspecific and cytotoxic effects.
4.2 Low grade activation of innate immune cells by farnesol

Upon activation neutrophils are able to mobilize an arsenal of antimicrobial effector mechanisms. For this, the hierarchical release of granule subsets is initiated, accompanied by up-regulation of receptors on the cell surface and the generation of an oxidative burst; all necessary to overcome invading microorganisms. To exhibit neutrophil activation and degranulation after FOH stimulation, expression of surface marker (CD66b, CD11b, CD62L, CD16), release of ROS and antimicrobial peptides (MPO, elastase 2, lactoferrin) were investigated and revealed a general activation pattern. Analyzing phagocytosis and killing of fungi after the confrontation of FOH-pretreated neutrophils with *C. albicans* revealed whether this general activation induced by FOH has consequences for the fungicidal ability of PMN. Despite this, the fungicidal activity of neutrophils towards *C. albicans* seemed to be only marginally modified by farnesol. Phagocytosis and killing were slightly reduced but no significant changes could be observed in concentrations up to 100 µM, indicating a low-grade activation by FOH. A qualitatively comparable activation pattern has been shown after confrontation of primary neutrophils with *C. albicans* germ tubes by Duggan and colleagues (228). They reported a strong induction of reactive oxygen intermediates and a massive degranulation represented by up-regulated surface exposure of CD66b and increased concentrations of myeloperoxidase, lactoferrin and elastase. An explanation might be differences in the extent of neutrophil activation. The farnesol-induced activation pattern seemed to be much lower compared to *C. albicans* and not sufficient to modulate fungicidal activity towards *C. albicans*. In contrast, tyrosol another QSM of *C. albicans* has been shown to impair neutrophilic killing of the fungus *in vitro* by inhibiting the oxidative burst (47, 229). Furthermore, neutrophil activation has been investigated for the gram-negative QSM HSL. Wagner and colleagues observed that HSL from *P. aeruginosa* in concentrations of 10 µM enhances the host defense by activating PMN seen in up-regulation of CD11b, induction of chemotaxis and enhanced phagocytosis (227). The low concentrations of HSL needed for neutrophil activation indicate a potent role in modulation of immunity in immune cell activation and induction of cell death.

Monocytes can contribute to immunity without differentiation into macrophages or DC (230). They express molecules like the human leukocyte antigen-DR molecule (HLA-DR) or the co-stimulatory molecule CD86 that can be induced upon activation by different stimuli and are known to be indicators of immune competence (231). Similar to PMN, monocytes displayed an activated surface phenotype after FOH treatment confirmed by a significant increase of CD86 and HLA-DR on the cell surface.
Furthermore, FOH-treated monocytes released enhanced levels of mainly pro-inflammatory chemokines e.g. TNF-α, MIP-1α, MIP-1β or MCP-1. This increased release of distinct cytokines suggests a pro-inflammatory property of FOH to influence the cytokine balance and the induction of diverse responses by different cells. However, even though activation was observed, FOH did not significantly affect the ability of monocytes to phagocytose and kill *C. albicans* cells. Taken together, these results indicate that farnesol is able to induce activation of PMN and monocytes without impairing the functionality of these immune cells to eliminate *C. albicans*.

In addition to monocytes, monocyte-derived iDC were analyzed in this study. In general, iDC express little or no CD86, which increases on the surface upon stimulation (232). After incubation with farnesol iDC exhibited an increase in the surface expression of CD86 and HLA-DR. However, this enhanced surface expression seemed to be compensated by LPS stimulation, which induces final maturation of iDC to mDC. Differences in the expression of CD86 or HLA-DR were not visible anymore. Nevertheless, alterations in cytokine release were analyzed after stimulation of iDC with LPS in addition to FOH. Interestingly, IL-12, an important immunoregulatory cytokine showed reduced levels, while the release of other pro- and anti-inflammatory cytokines stayed unaffected after FOH treatment. The expression of IL-12 during infection is known to influence innate immunity and to determine the outcome of adaptive immune response (209). Especially in fungal infections it is critical in inducing a Th1 response, implying that FOH might play an immunomodulatory role (233).

### 4.3 Farnesol impairs differentiation of monocytes to DC

The results of this study showed a strong impact of FOH on the differentiation process from monocytes to DC. In contrast to the low-grade activation of neutrophils and monocytes, DC generated in the presence of farnesol displayed a monocyte-like phenotype. They retained CD14 on the cell surface, showed almost no expression of CD1a, diminished surface expression of co-stimulatory molecules (CD80, CD86, CD40) and reduced levels of the peptide-presenting molecule HLA-DR and the maturation marker CD83. Mature DC generated in the presence of farnesol are not able to reach a fully mature level which is in line with an impaired immune response. The co-stimulatory molecules CD40, CD80, CD86 and the maturation marker CD83 are critical factors for amplification of T cell responses (160, 163, 167, 234). Specifically, the reduced expression of CD83 could be decisive for the functionality of DC as an APC as it has previously been described to be required for optimum T cell activation (235). Prechtel and Steinkasserer reported that the loss of CD83 leads to a strong
reduction of DC-T cell stimulatory capacity (165). Comparing this immunomodulatory impact of FOH to other microbial QSMs it became apparent that the QSMs of \textit{P. aeruginosa} modulates the phenotype in a similar manner. Consistent with our data HSL induced a decrease in expression of T cell stimulatory molecules CD80 and CD86 and CD40 (236).

Furthermore the maintained CD14 expression and the elevated levels of soluble CD14 in the supernatant of mDC generated in the presence of FOH support a not fully matured phenotype accompanied by functional defects. CD14 is known to be highly expressed on monocytes but absent on DC and sCD14 is able to suppress T cell activation and function by interacting directly with T cells (237, 238). However, these results suggested that FOH triggers differentiation of DC with unique characteristics that are arrested in a monocyte-like state and exhibit weak immunostimulatory activity.

4.4 Farnesol-modulated displacement of CD1 surface expression implies shift in immune response

The most prominent and solid change after differentiation of monocyte-derived DC in the presence of FOH was the modulation of surface marker belonging to the CD1 family. CD1 glycoproteins are specialized in the presentation of lipids, glycolipids and lipopeptides (153, 154). While peptide presentation by MHC II is dependent on maturation, the lipid antigen presentation to CD1 restricted T cells is enabled by both iDC and mDC (156). The results of this study revealed almost no expression of CD1a and enhanced expression of CD1d on DC generated in the presence of FOH. Interestingly, a CD1a negative DC subset, characterized by distinct functions, has been described before. Chang and colleagues showed that monocytes-derived CD1a positive and CD1a negative DC subsets differ in their cytokine production profile and further in their capacity to direct Th cell differentiation (239, 240). CD1a surface expression defines an IL-12 producing population of human DC and Th1 cell polarizing capacity (240). In contrast, CD1a negative DC lack IL-12 production but produce increased levels of IL-10 compared to the CD1a positive subset, which coincides with the FOH-induced changes in our study. Both the reduced IL-12 level and the enhanced IL-10 release by DC generated in the presence of FOH are known to limit the fungal-protective Th1 response (180). This shift of cytokine release away from a Th1 stimulating pattern is likely of immediate functional relevance. Numerous studies have clearly shown that Th1 responses are required for protective immunity and elimination of the fungal pathogen and that DC are known to play a major role in triggering and directing these protective responses (191, 241, 242).
Moreover, the enhanced expression of CD1d seen in the transcriptome and in surface marker expression on DC generated in the presence FOH further promotes a shift in immune response. In general, CD1d is expressed in low levels on DC but sufficient to present lipid antigens to specialized human T cells like NKT cells (243). Moreover, CD1d is differently regulated from group 1 CD1 molecules (CD1a, b, c) (243). While group 1 CD1 molecules like CD1a are up-regulated in GM-CSF and IL-4 treated monocytes, CD1d exhibits no up-regulation under equal conditions (244, 245).

The shift to an enhanced CD1d positive phenotype accompanied by reduced surface expression of CD1a on DC after FOH treatment suggests an alternative effort to achieve an adequate immune response thus favoring activation of NKT cells. The importance of CD1d-restricted NKT cells in anti-fungal immunity has been evaluated by several investigators. In Cryptococcus neoformans infections, NKT cell deficient mice showed a delay in clearance of the fungal pathogen from the lungs (246). Cohen and colleagues suggested that NKT cells are important for early fungal elimination dependent on a IL-12 secretion that is mediated by fungal β 1,3-glucan recognition (197). Controversially, knock out mice, which lack NKT cells showed no differences in fungal burden after *C. albicans* infection, indicating only a minor role of NKT cells in controlling *Candida* infections in mice (247). However, concerning the CD1d restricted T cell activation many studies pointed out that the surrounding cytokines, such as IL-12, released by DC, are critically important for the outcome of the immune response (248-250). The central role of IL-12 is to enhance innate immunity by influencing other immune cells like NK cells and T cells, thus bridging the innate and adaptive immune system. In fungal infections IL-12 acts as a pro-inflammatory cytokine directing a protective Th1 response from naïve CD4+ T cells and inducing high levels of IFN-γ (191, 242). Taking into account that FOH impaired the release of this immunoregulatory cytokine, CD1d restricted NKT cells may rather prevent than promote effective anti-fungal responses.

Beside FOH other microbial QSMs have been shown to affect IL-12 secretion. Both the autoinducer HSL and the PQS molecule produced by *P. aeruginosa*, which also causes biofilm related infections and septicemia, were able to decrease IL-12 levels produced by LPS stimulated bone marrow-derived murine DC but without altering the IL-10 release (236, 251). Interestingly, also *C. albicans* has previously been reported to induce differences in the IL-12, dependent on morphology of fungus (252). Liu and colleagues demonstrated that *C. albicans* germ tubes fail to induce IL-12 in monocytes and that this effect might be mediated by a soluble product of the germinating cells. The so-called *C. albicans* secretory IL-12 inhibitory factor (CA-SIIF) was further characterized and partially purificated by Wang and co-workers (253). CA-SIIF was
identified as a glycoprotein which exhibits inhibition of IL-12 production and also modulated differentiation of monocytes into DC by reducing CD1a expression on human monocyte-derived DC, similar to farnesol.

In addition, previous studies have reported that beside the recognition of microbial pathogens, the lipid environment can interfere with antigen presentation by CD1 molecules (161). Serum lipids can modify the CD1 expression on DC interfering with the peroxisome proliferator-activated receptor-γ (PPARγ) (254). PPARγ belongs to a subset of nuclear hormone receptor family and is known as a lipid-activated transcription factor (255). The induction of DC differentiation leads to an up-regulation of PPARγ that can further be enhanced through exogenous ligands (256). Interestingly, the activation of PPARγ induced CD1d expression and diminished CD1a expression, thus resulting in phenotypic and functional changes e.g. favoring NKT cell activation (254, 257). Furthermore, PPARγ agonists modulate DC specific surface markers by increasing CD86 and HLA-DR and reducing CD80 surface expression (256). These modifications are consistent with the alterations in surface phenotype of iDC generated in the presence of FOH. In addition, other investigators reported about functional inhibition of DC induced by PPAR activation (258). The fact that an opposite regulation of CD1a and CD1d can be induced by a microbial stimulus has been investigated by Roura-Mir and colleagues (259). Mycobacterial lipids induce group 1 CD1 (CD1a, 1b, 1c) expression but not expression of group 2 CD1d. Consequently, the impaired DC phenotype and functionality within our study might be mediated through a FOH-induced activation of PPARγ. That FOH is an activator of PPARs has been shown before in previous studies (57, 260). For example, the farnesol-induced apoptosis in mainly malignant cell types is mediated through nuclear receptors, e.g. the farnesoid X receptor (FXR) and peroxisome proliferator-activated receptors (PPARs) (224, 261). These observations and the fact that PPARγ is up-regulated in our transcriptome data (time point 6d + 6h LPS FC: +5.9) clearly favors the PPARγ activation as a mechanism targeted by FOH.

4.5 Farnesol shifts cytokine profile of DC towards Th2 response

The results presented in this study clearly show that farnesol modulates the cytokine profile of monocyte-derived DC. Beside the induction of pro-inflammatory cytokines (e.g. TNF-α, IL-6, IL-8), the secretion of the immunomodulatory cytokine IL-1 receptor antagonist (IL-1RA) is affected by FOH. DC generated in the presence of FOH and stimulated with LPS showed markedly enhanced IL-1RA level on transcriptomic and protein levels. This member of the IL-1 family is the main natural antagonist of IL-1β,
competing for its binding to the IL-1 receptor. IL-1β is a strong pro-inflammatory cytokine that activates monocytes, macrophages and neutrophils and induces a Th1 and Th17 immune response, both important in disseminated candidiasis (262). The enhanced release of IL-1RA goes along with the previous finding that Candida β-glucan induces IL-1RA production, mainly by CD14+ cells (263). IL-1RA deficiency (DIRA) can lead to severe systemic inflammation, pointing out the important role of IL-1RA in counterbalancing IL-1β. As FOH caused enhanced IL-1RA expression it consequentially influences Th1 and Th17 response both crucial for a protective anti-Candida host defense. Together with the reduced levels of IL-12 and the enhanced release of IL-10 and IL-1RA, farnesol clearly favors an immune response skewed away from Th1 and shifted towards a Th2 phenotype. Further shifts in the expression pattern of cytokines indicate that the farnesol-mediated effects on DC might be beneficial for the fungus. Among the induced proinflammatory cytokines we could observe a dominant release of chemokines which are chemoattractants for eosinophils like MCP-3 and eotaxin 2 (264, 265). Eotaxin 2 can further induce respiratory burst in eosinophils and directly contributes to tissue damage (266). Eosinophils represent potential effector cells in the pathogenesis of allergic asthma associated with fungal pathogens like C. albicans (267). Furthermore previous findings by Navarathna et al. showed that farnesol-treated mice exhibit a surge in eosinophil production (268). Interestingly, Jacobsen and colleagues implied a role for eosinophils in modulating the balance between Th2 and Th17 response induced by DC following allergen exposure (269). This likely contributes to directing an immune response not suited for rapid fungal clearance. Further analyses of this study also revealed an impaired release of chemokines that execute roles in monocytes, T cells and neutrophils attraction and activation. CCL1, a principle monocyte chemoattractant, and MIG that selectively attracts Th1 lymphocytes showed reduced levels (270-272). In addition, the chemokine RANTES that plays a role in trafficking and activating leukocytes like neutrophils and T cells to sites of infection is significantly decreased in the supernatant of DC generated in the presence of FOH. Ellis and colleagues suggested that RANTES might be associated with invasive fungal infections (IFI), as they observed very low concentrations in patients who died from IFI compared to survivors (273).

In summary, these discussed modulations of the cytokine response by farnesol trigger an immune response that steers away from Th1 response and further away from recruiting PMN and monocytes to site of infection, all being crucially important for recovery from fungal infections.
4.6 Farnesol-treated DC are restricted in mobility and T cell-stimulating capacity

Beside the release of cytokines the most important feature of DC is to present antigens and to induce proper T cell proliferation that leads to an adequate immune response against invading pathogens. The alteration in surface phenotype and cytokine release already implied a dysfunctional behavior of DC generated in the presence of FOH and militate for impaired antigen-presentation towards T cells. This is further supported by the fact that the duration of DC-T cell interaction is critical for T cell activation and depends on the degree of DC maturation (274). The basis of a successful DC-T cell interaction is the formation of a stable immunological synapse (176). Therefore, DC need to possess a functional actin cytoskeleton and the mobility to migrate to T cell-rich areas (213). Surveying the ability of FOH-treated DC to spread on fibronectin coated slides revealed a reduced mobility. FOH-treated DC were delayed in spreading and formation of protrusions compared to mock-treated cells, a finding which might indicate a disruption of the actin cytoskeleton. That FOH interferes with actin cytoskeleton has been previously shown by Miquel and co-workers (55). Treatment of malignant A549 cells with 60 µM FOH lead to disorganization of the actin cytoskeleton ending in inhibition of cell proliferation. During this study no interactions with actin fibers were analyzed but this is noteworthy and could be a future approach. Interestingly, the analysis of the transcriptome of FOH-treated DC revealed that the disability to form protrusions might be due to the reduced production of MMP12. MMP12 belongs to the metalloproteases, which support cell migration by remodeling and degradation of ECM, e.g. fibronectin (275, 276). Reduced MMP12 production was previously reported to be associated with a CD1a negative DC phenotype (277). Furthermore, Kis-Toth and co-workers suggested that this reduced production is related to an elevated production of TIMPs, the inhibitors of MMPs that regulate MMP activity (278). This goes in line with our transcriptome data that revealed in addition to a down-regulation of MMP12 an up-regulation of the gene encoding TIMP1 in DC generated in the presence of FOH compared to mock-treated cells. Altogether, the not fully matured phenotype, the change in cytokine release and the restriction in mobility postulated a reduced ability of FOH-treated DC to stimulate T cells. In an allogenic mixed lymphocyte reaction a significantly reduced T cell stimulatory capacity compared to mock-treated DC could be ascertained. The presentation of antigen and to activate T cells is the principle function of DC and enables them to contribute to a protective immune response by bridging the gap between innate and adaptive immunity (279).
Other microbial QSM, like HSL and PQS of *P. aeruginosa* have previously shown to interfere with the T cell stimulating capacity of DC (280). Human LPS-stimulated DC were cultivated after incubation with HSL with specifically allogeneic T cells and showed diminished activation and proliferation of T cells (280). Also Skindersoe and colleagues showed that antigen stimulation of murine LPS-matured DC from mice *in vitro* exhibited reduced T cell proliferation under the influence of HSL (236). More diverse immunological bioactivity on multiple different host cells has been reported for HSL (225, 281, 282). Moreover, Boontham and colleagues pointed out that these direct immunomodulatory effects might have a possible link to severe sepsis (280). They reported that immune changes *in vivo* in patients were comparable with *in vitro* observations induced by HSL and demonstrated the presence of HSL in the sera of patients with severe sepsis implying that QSMs play a role in systemic infections. Regarding FOH no information about the distribution in *Candida* infected tissues is known, but the comparison of our results with previous studies revealed an association of impaired DC maturation and function with the polymorphism of *C. albicans*. Torosantucci and co-workers induced differentiation of monocytes into DC that had either phagocytosed yeast cells or germ tubes of *C. albicans*. Interestingly, after phagocytosis of yeast cells monocytes did not differentiate into DC and exhibit a CD14+/CD1a−/CD83− phenotype, were unable to produce IL-12 and to induce proliferation of naïve T cells but secreted IL-10. Moreover, these cells expressed high levels of TNF-α, IL-6, and IL-8 mRNA transcripts (283). A similar pattern of DC hyperactivation accompanied with impaired maturation has been observed by Ryan and colleagues. They investigated the co-incubation of *C. albicans* or LPS with monocyte-derived DC from APECED and non-APECED patients suffering from chronic mucocutaneous candidiasis (CMC) (284). The polyendocrinopathy candidiasis ectodermal dystrophy (APECED) syndrome is a genetic autoimmune disease, characterized by mutation of the autoimmune regulator (AIRE) gene and CMC as a common symptom (285). The results by Ryan and Torosantucci were consistent with our data. Farnesol-treated DC also released elevated levels of TNFα, IL-8, IL-6 after LPS stimulation and showed less expression of CD83 on the surface resulting in a reduced ability to induce T cell proliferation. Moreover, Ryan and colleagues implied that DC hyperactivation in APECED patients may underlie altered T cell responsiveness, autoimmunity and impaired response to *Candida*. This corroborates the assumption that farnesol acts as a virulence factor of *C. albicans* by interfering with DC maturation resulting in a non-protective immune response. Impeding DC exerting their T cell stimulatory property points to FOH as harmful for the immune system and should be considered as a modulator of the immunity. Along with
its ability as QSM to regulate morphology of the fungus, it might facilitate establishment of *Candida* infection.

### 4.7 Potential targets and underlying mechanisms of farnesol

Considering the impact on innate immune cells, unraveling the potential targets and underlying mechanisms addressed by farnesol is of great interest. Starting from what is known about the mechanisms involved in farnesol signaling in *C. albicans*; further experiments of this study focused on the small monomeric GTPase Ras. In *C. albicans*, FOH is postulated to interfere with the Ras1/MAP kinase pathway and Ras itself (73). Endorsed by the investigations of Singh and colleagues that showed a role of small G-protein signaling of the Ras family in DC maturation and functionality, we investigated the turn over, degradation and localization of Ras proteins under the influence of FOH (215). No clear differences in FOH-treated cells compared to mock-treated cells could be observed. Consequently, we could not prove an interaction of FOH with Ras in DC. Nevertheless, as Ras signaling plays a role in FOH mediated regulation of *C. albicans* morphology it should be considered and investigated in more detail and extant.

In a second attempt, the search for another point of action addressed by FOH focused on the initiation of the differentiation process from monocytes to DC. A possible explanation for the FOH effect might be a failed response to maturational stimuli like GM-CSF. The use of IL-4 and GM-CSF as cytokines for differentiation of monocytes to DC lead together to immature CD14 negative DC that are able to develop into mature DC and further induce proliferation of T cells (286). GM-CSF fulfills multiple biological functions in DC development, e.g. proliferation, survival and differentiation, all regulated through a complex molecular network via the GM-CSF receptor (GM-CSFR) (287). The transcriptome analysis of DC generated in the presence of FOH revealed a down-regulation of the GM-CSF receptor but no differences in the expression of the IL-4 receptor, which pointed out that farnesol is not inducing a general down-regulation of cytokine receptors. The reduced expression of GM-CSFR could further be confirmed by flow cytometry on the surface on mDC generated in the presence of farnesol compared to mock-treated mDC. Conti *et al.* reported that there is a positive correlation between the GM-CSFR and the capacity to undergo differentiation (288); supporting the hypothesis that farnesol induces desensitization to GM-CSF that leads to not fully matured and dysfunctional DC. The exact underlying signaling mechanisms and how this contributes to the dysfunctional DC maturation remains elusive. However, all
potential targets discussed above are promising for elucidation of how farnesol interacts with innate immune cells, particularly in case of DC.
5 Conclusions

The multiple roles of farnesol e.g. in morphology of C. albicans or biofilm formation, have raised the potential role of quorum sensing in the ability of the fungus to be virulent and survive in the host. Our findings identify a previously unrecognized immunomodulatory function of farnesol in interaction with the host. Farnesol is not exclusively a fungal QSM but also able to induce activation of neutrophils and monocytes and to influence DC functionality. Farnesol induced a distinct DC subset lacking Th1-promoting cytokines and might therefore be instrumental for C. albicans in directing T cell responses. Interestingly, although farnesol-treated DC fail to induce a proper T cell response the enhanced surface expression of CD1d suggests an alternative way to induce an adequate immune response (Figure 32).

Figure 32 Farnesol modulates DC function and prevents a protective Th1 response.
Farnesol induced a distinct DC subset with an altered surface phenotype, characterized by reduced levels of co-stimulatory molecules, almost no expression of CD1a and diminished levels for CD83 and the MHC II molecule HLA-DR. Furthermore, the lack of Th1 promoting cytokines implies that FOH-treated DC might not be able to induce an immune response suited for anti-fungal clearance. Interestingly, an enhanced CD1d expression indicates an interaction with NKT cells. Whether this interaction contributes to an anti-fungal immune response needs to be further investigated.
Further studies should concentrate on the DC-T cell interaction, specifically the ability of farnesol-treated DC to interact with NKT cells. Furthermore, it would be valuable to analyze the molecular mechanisms underlying the farnesol effect on monocyte-derived DC and characterize in more detail the interaction with *Candida* cells, regarding phagocytosis.

Evading host defenses is a major goal of pathogens and the results of this study strongly support that farnesol can contribute as virulence factor of *C. albicans*. To play a role as a QSM and as virulence determinant links farnesol to the pathogenesis of *Candida* infections and makes it to a potential target in terms of new therapeutic strategies. One promising strategy that is already studied for bacterial QS systems is the so-called quorum quenching, by QS inhibitors (289). To find compounds that are able to inhibit QS signals would offer new antimicrobial agents that inhibit virulence without effecting microbial viability to mitigate selective resistance. The prerequisite to find fungal QS inhibitors is a better understanding of fungal QSMs, like farnesol, and how they interfere with the human host. In the future, it will be intriguing to examine the mechanism addressed by farnesol and whether it can be exploited for biological control of *Candida* infections.
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### 7 List of abbreviations

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<td>Acyl-homoserin-lactones</td>
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<tr>
<td>AIP</td>
<td>Auto-inducing peptides</td>
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<tr>
<td>Als</td>
<td>Agglutinin-like sequence</td>
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<tr>
<td>AMICA</td>
<td>Adhesion molecule interacting with CXADR antigen</td>
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<tr>
<td>APC</td>
<td>Antigen-presenting cell</td>
</tr>
<tr>
<td>APC</td>
<td>Allophycocyanin</td>
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<tr>
<td>APECED</td>
<td>polyendocrinopathy candidiasis ectodermal dystrophy syndrome</td>
</tr>
<tr>
<td>CCL</td>
<td>C-C motif ligand</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
</tr>
<tr>
<td>CLEC</td>
<td>C-type lectin</td>
</tr>
<tr>
<td>CMC</td>
<td>Chronic mucocutaneous candidiasis</td>
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<tr>
<td>cRNA</td>
<td>coding RNA</td>
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<tr>
<td>CXCL</td>
<td>C-X-C motif ligand</td>
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<td>d</td>
<td>Days</td>
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<tr>
<td>DC</td>
<td>Dendritic cells</td>
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<tr>
<td>DMSO</td>
<td>Dimethylsulfoxid</td>
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<tr>
<td>DPP3</td>
<td>Diacylglycerol pyrophosphate phosphatase</td>
</tr>
<tr>
<td>DZ</td>
<td>Dendritische Zellen</td>
</tr>
<tr>
<td>e.g.</td>
<td>exempli gratia</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>et al.</td>
<td>et alii</td>
</tr>
<tr>
<td>E score</td>
<td>Enrichment score</td>
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<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td>FC</td>
<td>Fold change</td>
</tr>
<tr>
<td>FCS</td>
<td>Forward scatter</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>fMLP</td>
<td>N-Formylmethionyl-leucyl-phenylalanine</td>
</tr>
<tr>
<td>FOH</td>
<td>Farnesol</td>
</tr>
<tr>
<td>g</td>
<td>acceleration due to gravity</td>
</tr>
<tr>
<td>GFP</td>
<td>green fluorescent protein</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HA</td>
<td>Hämagglutinin</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Human leukocyte antigen-D related</td>
</tr>
<tr>
<td>HSL</td>
<td>N-(3-oxododecanoyl) homoserine lactone</td>
</tr>
<tr>
<td>iDC</td>
<td>Immature dendritic cells</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodalton</td>
</tr>
<tr>
<td>KEGG</td>
<td>Kyoto Encyclopedia of Genes and Genomes</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>LSM</td>
<td>Confocal laser scanning microscopy</td>
</tr>
<tr>
<td>MCP</td>
<td>Monocyte chemoattractant protein</td>
</tr>
<tr>
<td>mDC</td>
<td>Mature dendritic cells</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>min</td>
<td>Minute(s)</td>
</tr>
<tr>
<td>MIG</td>
<td>Monokine induced by gamma-interferon</td>
</tr>
<tr>
<td>MIP</td>
<td>Macrophage inflammatory protein</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinases</td>
</tr>
<tr>
<td>MOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MOI</td>
<td>Multiplicity of infection</td>
</tr>
<tr>
<td>MPO</td>
<td>Myeloperoxidase</td>
</tr>
<tr>
<td>NETs</td>
<td>Neutrophil extracellular traps</td>
</tr>
<tr>
<td>NKT cell</td>
<td>Natural Killer T cell</td>
</tr>
<tr>
<td>PAMP</td>
<td>Pathogen-associated molecular patterns</td>
</tr>
<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PE</td>
<td>Phycoerythrin</td>
</tr>
<tr>
<td>PerCP</td>
<td>Peridinin chlorophyll</td>
</tr>
<tr>
<td>PI</td>
<td>Propidium iodide</td>
</tr>
<tr>
<td>PMA</td>
<td>Phorbol 12-myristate 13-acetate</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear neutrophil</td>
</tr>
<tr>
<td>PPMC</td>
<td>Person product measure correlation</td>
</tr>
<tr>
<td>PRR</td>
<td>Pathogen recognition receptors</td>
</tr>
<tr>
<td>QS</td>
<td>Quorum sensing</td>
</tr>
<tr>
<td>QSM</td>
<td>Quorum sensing molecule / Quorum-Sensing Molekül</td>
</tr>
<tr>
<td>RANTES</td>
<td>Regulated upon activation, normally T cell expressed and secreted</td>
</tr>
<tr>
<td>RIN</td>
<td>RNA integrity number</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RPMI</td>
<td>Roswell Park Memorial Institute medium</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal RNA</td>
</tr>
<tr>
<td>SAP</td>
<td>Secreted aspartic proteinase</td>
</tr>
<tr>
<td>sCD14</td>
<td>Soluble CD14</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>TCR</td>
<td>T cell receptor</td>
</tr>
<tr>
<td>Th1</td>
<td>Type 1 helper T cell</td>
</tr>
<tr>
<td>Th2</td>
<td>Type 2 helper T cell</td>
</tr>
<tr>
<td>Th17</td>
<td>Type 17 helper T cell</td>
</tr>
<tr>
<td>TIMP</td>
<td>Tissue inhibitor of metalloproteinases</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>Treg</td>
<td>Regulatory T cell</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
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<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>U</td>
<td>Units</td>
</tr>
<tr>
<td>V450</td>
<td>Violett 450 nm</td>
</tr>
<tr>
<td>vs</td>
<td>Versus</td>
</tr>
<tr>
<td>XTT</td>
<td>2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide</td>
</tr>
<tr>
<td>YPD</td>
<td>Yeast peptone dextrose</td>
</tr>
<tr>
<td>α</td>
<td>Alpha</td>
</tr>
<tr>
<td>β</td>
<td>Beta</td>
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<tr>
<td>γ</td>
<td>Gamma</td>
</tr>
<tr>
<td>μM</td>
<td>Micro molar</td>
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9 Appendix

9.1 Curriculum Vitae

PERSONAL INFORMATIONS:

Name: Ines Leonhardt
Marital status: single
Date of birth: 17.10.1984
Birthplace: Jena
Nationality: german

EDUCATION:

1995 to 2003 Staatliches Gymnasium Otto Schott, Jena
Graduation: Abitur
2003 to 2009 Study of nutritional science (diploma) at Friedrich Schiller University Jena, Germany
2008 to 2009 Diploma thesis
TOPIC: Characterization of Candida albicans – neutrophil – interaction using GFP reporter strains and mutants
since 11/2009 Dissertation at ZIK Septomics Jena, (Prof. Dr. Oliver Kurzai)
TOPIC: Impact of the quorum sensing molecule farnesol on human innate immune cells

RESEARCH COURSES:

2006 Work experience at the company „Invigate“ (production of bacterial recombinant proteins)
2007 practical research placement at MIT of University Medical Center Jena, at the Institute for Biochemistry
TOPIC: Cloning of the TSLP receptor single chain into an expression vector / Analysis of the TSLP expression in Salmonella typhimurium stimulated CaCo2 cells
02/2010 Basic Course in Flow Cytometry: Einführung in die Durchflusszytometrie BD FACSCanto™ Durchflusscytometer BD FACSDiva™ Software, BD Bioscience Heidelberg
05/2012 Introduction to the GxPs- with a special focus on GMP and GLP by Dr. Michael Hildebrand of Hildebrand Pharma Consulting
04/2013 Understanding Statistics by Dr. Friedrich Funke, Friedrich Schiller University, Jena

Jena, 06/2015

Ines Leonhardt
9.2 List of scientific presentations and publications

**ORAL PRESENTATIONS:**

**SCIENTIFIC TALKS:**

Leonhardt I, Albrecht A, Hube B (2009), Monitoring nitrogen starvation of *Candida albicans* during interaction with neutrophils, Statusworkshop der Fachgruppe Eukaryontische Krankheitserreger der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM), Tübingen, Germany

Leonhardt I, Kurzai O (2012), Impact of farnesol on human immune cells, 3rd International Conference on Microbial Communication (MiCom), Jena, Germany


**SCIENTIFIC POSTERS:**


Leonhardt I, Kurzai O (2011) Immunomodulation durch mikrobielle Quorum sensing Moleküle, 45. Wissenschaftliche Tagung der Deutschsprachigen Mykologischen Gesellschaft e. V., Kiel, Germany

Leonhardt I, Hünniger K, Kurzai O (2011) Impact of farnesol on human immune cells, 63. Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) e.V., Essen, Germany

Leonhardt I, Hünniger K, Kurzai O (2012) Immunomodulation via microbial quorum sensing molecules, 18th Congress of the International Society for Human and Animal Mycology 2012 (ISHAM ), Berlin, Germany

Leonhardt I, Hünniger K, Rubio I, Kurzai O (2013) The *Candida albicans* quorum sensing molecule is a modulator of innate immune cell function, 6th Trends in Medical Mycology (TIMM), Copenhagen, Denmark
PUBLICATIONS:


9.3 Danksagung

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9.4 Selbständigkeitserklärung

Die geltende Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena ist mir bekannt.


Personen, die mich bei der Auswahl und Auswertung des Materials unterstützt haben, habe ich benannt und in der Danksagung erwähnt.
Alle Personen die bei der Anfertigung der Manuskripte beteiligt waren sind in der Publikationsliste aufgeführt.

Dritte Personen haben weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

Die Hilfe eines Promotionsberaters habe ich nicht in Anspruch genommen.

Diese Arbeit wurde bisher weder an der Friedrich-Schiller-Universität Jena noch an einer anderen Hochschule als Dissertation oder in Form einer Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht.

Jena, 25.06.2015

___________________
Ines Leonhardt