ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2014, 52, 555–564
doi: 10.1093/mmy/myu029
Advance Access Publication Date: 14 July 2014
Review Article

Review Article

New insights into innate immune control of systemic candidiasis

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Received 16 January 2014; Revised 16 March 2014; Accepted 1 April 2014

Abstract

Systemic infection caused by *Candida* species is the fourth leading cause of nosocomial bloodstream infection in modern hospitals and carries high morbidity and mortality despite antifungal therapy. A recent surge of immunological studies in the mouse models of systemic candidiasis and the parallel discovery and phenotypic characterization of inherited genetic disorders in antifungal immune factors that are associated with enhanced susceptibility or resistance to the infection have provided new insights into the cellular and molecular basis of protective innate immune responses against *Candida*. In this review, the new developments in our understanding of how the mammalian immune system responds to systemic *Candida* challenge are synthesized and important future research directions are highlighted.

Key words: systemic candidiasis, innate immunity, neutrophils, monocytes/macrophages.

Introduction

Systemic candidiasis, mainly caused by the commensal yeast *Candida albicans*, is the most common deep-seated human fungal infection in the developed world [1–4]. Advances in modern medicine and the associated increase in immunosuppressed and debilitated patient populations have contributed to the considerable rise in the incidence of systemic candidiasis over the past decades. It is now estimated that more than 50 000 new cases of systemic candidiasis occur in the United States every year (incidence, approximately 20 cases/10 000 population), with an associated annual cost of more than $2 billion [1–4]. There are no available licensed fungal vaccines to prevent disease, and mortality of affected patients exceeds 30%–40% despite the administration of antifungal agents that have potent activity *in vitro* and in *in vivo* preclinical studies [1,2,5]. Therefore, the estimated number of deaths associated with systemic candidiasis exceeds 15 000 per year in the United States, which is comparable to or greater than the number of deaths caused by acquired immunodeficiency syndrome (AIDS) or systemic staphylococcal infections [6,7]. Thus, the substantial burden of systemic candidiasis in humans underscores the importance of gaining a better understanding of the immune pathogenesis of the infection with an aim to devise targeted immune-based strategies in order to augment the current antifungal drug-based treatment regimens in infected patients.

Pathogenesis of systemic candidiasis in humans

In humans, two distinct syndromes of systemic candidiasis develop with different pathogenesis. Mouse models have been established in order to study the molecular and cellular basis of effective host defense against the infection [8–12]. The first syndrome of systemic candidiasis is gastrointestinal tract derived and is exclusively seen in immunosuppressed patients. It is typically seen in the setting of heavy antibiotic preexposure and chemotherapy-induced mucositis and neutropenia in individuals with hematological malignancy and/or hematopoietic stem cell transplantation [1,2,13,14]. Antibiotics are known to increase *Candida* colonization in the gut lumen of mice and humans to levels up to 10^7–10^8 colony forming units/gram of stool [11,15]. In fact, recent elegant work in mice has highlighted the critical role of metabolic products from specific gut microbiota such as lactobacilli in priming interleukin (IL)-22–dependent mucosal immune responses by innate lymphoid cells via the aryl hydrocarbon receptor, which is fundamental for protection against uncontrolled *Candida* local expansion [16]. Yet, additional work is needed in order to define how the various classes of antibiotics that are used in immunosuppressed patients differentially impact the bacterial ecology in the intestine and influence innate antifungal immune responses locally.

In addition to antibiotic exposure that increases the intraluminal *Candida* burden in the gut, the breach in the integrity of the gastrointestinal mucosal barrier and the phagocyte impairment induced by cytotoxic chemotherapy both facilitate *Candida* translocation to the systemic circulation, giving rise to hepatosplenic candidiasis. In agreement, animal studies have demonstrated the requirement for both mucosal disruption and phagocyte depletion in the development of gastrointestinal tract–derived hepatosplenic candidiasis in mice [11]. As implied by its name, human autopsy studies have revealed that the liver and spleen (but also the kidneys) are the major organs affected in more than 90% of the patients with this condition [9]. Strikingly, *Candida* systemic translocation from the gut appears to also occur in nonimmunosuppressed individuals when given an extremely large oral *Candida* load. This was demonstrated by the voluntary ingestion of more than 10^{12} live yeast cells of *C. albicans* by a healthy physician in 1969 who subsequently developed self-limited fungemia and funguria [17]. This challenge experiment shows that *Candida* may penetrate via an intact mucosal barrier when the yeast luminal concentration exceeds a certain threshold, even in the absence of antibiotic preexposure and phagocyte perturbation. The incidence of hepatosplenic candidiasis has dramatically declined in recent years due to the introduction of widespread *Candida*-active azole prophylaxis in high-risk immunocompromised patients [18]. However, more research is required in order to elucidate the microbiota-derived cues and host innate immune mechanisms that control *Candida* local gastrointestinal expansion and systemic translocation. Such knowledge could lead to the development of probiotic- and immune-based strategies in immunosuppressed patients for the prevention and/or treatment of systemic candidiasis of gastrointestinal origin without the widespread use of azole prophylaxis, which is known to result in emergence of antifungal resistance [19].

The second syndrome of systemic candidiasis in humans is skin derived and is seen most often in acutely ill nonneutropenic patients in the intensive care unit [1,2,20]. The universal presence of a central venous catheter in these patients compromises the integrity of the cutaneous barrier and allows for invasion of *Candida* in the systemic circulation. In contrast to gut-derived candidiasis in patients with hematological malignancy, human autopsy studies have shown that the kidney (>90%) but not the liver or spleen (<10%–20%) is the principal affected organ in skin-derived candidemic patients [8], attesting to the protective role of resident phagocytes and neutrophils in hepatic and splenic anti-*Candida* host defense. A mouse model of disseminated candidiasis that introduces *Candida* yeast cells via the lateral tail vein has been extensively used to study fungal virulence, pharmacology, and immunity over the past decades [12]. Despite its inherent limitation that a large fungal load is delivered to induce disseminated infection in mice, which are *Candida*-naive, this model mimics skin-derived human systemic candidiasis, with kidney being the primary target organ [12,20,21]. In this review, the recent advances in our understanding of innate immune factors that mediate protection against systemic candidiasis are outlined with a focus on skin-derived disease, which comprises the majority of clinical cases of systemic candidiasis in modern hospitals.

Innate immune recognition of *Candida*: The complex interplay of multiple receptors

A major advance in the field of fungal immunology over the past decade has been the explosion in the discovery and functional characterization of an array of soluble and membrane-bound pattern recognition receptors (PRRs) that recognize *Candida* (and other pathogenic fungi). Each PRR senses specific extracellular (eg, β-glucans, mannans) or intracellular (eg, DNA, RNA) pathogen-associated molecular patterns (PAMPs) of *Candida* yeast and/or pseudohyphal elements (summarized and illustrated in detail in refs. 22 and 23). With regard to soluble PRRs, deficiency in the complement components C3 and C5 in mice has been shown to result in increased mortality as a consequence of impaired anti-*Candida* resistance and exuberant infection-driven immunopathology, respectively [24,25]. Furthermore,

Toll-like receptor (TLR) signaling through TLR2 (in interaction with TLR1 and TLR6), TLR4, and TLR7 and the downstream adaptor protein MyD88 are indispensable for survival in the mouse model of systemic candidiasis by promoting yeast phagocytosis and/or production of proinflammatory cytokines and chemokines [26–29]. More recently, the discovery of the nonredundant *in vivo* role of the C-type lectins (CLRs) dectin-1, dectin-2, dectin-3, mincle, and mannose receptor and the downstream signaling adaptor molecules syk and CARD9 (caspase-associated recruitment domain) has highlighted the importance of CLR-induced signaling in innate immune control of *Candida* in mice [30–34]. Last, *Candida* infection also activates the inflammasome through both β-glucan and secreted aspartic protease (ie, Sap2 and Sap6) sensing [35,36]. Specifically, Nlrp3 (via both the caspase-1 pathway and the noncanonical caspase-8 pathway) and Nlrp10 but not Nlrc4, Nlrp6, or Nlrp12 are critical for host defense *in vivo* [36–38].

In addition to fungal recognition by individual PRRs, synergistic interactions between different PRRs also occur, resulting in augmentation of downstream immune activation. Examples of such interactions that may serve as a means to broaden the repertoire of *Candida* PAMP sensing and/or tailor antifungal immune responses include that of TLR2 with dectin-1, of dectin-2 with dectin-1 and dectin-3, of TLR2 with galectin-3, and of C5a with TLR2 and TLR4 [32,39–42]. In fact, therapeutic modulation of TLR/CLR costimulation has been successfully used in chromoblastomycosis [43], suggesting that a similar concept may hold promise for devising therapeutic interventions in other mycoses including systemic candidiasis.

However, *Candida* sensing by the innate immune system is subject to several complexities. First, a *Candida* PAMP may be sensed by discrete subsets of PRRs that differ depending on the myeloid cell subset and the *Candida* morphogenic state. For example, whereas yeast mannan is recognized by the mannose receptor on macrophages, it is sensed by dectin-2 and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin on dendritic cells. Instead, pseudohyphal mannan recognition is mediated by dectin-2 on both macrophages and dendritic cells [31,44,45]. Second, different myeloid cells use PRRs differentially; for instance, dectin-1-CARD9 signaling activates natural killer (NK)-κB and proinflammatory cytokine secretion in mouse bone marrow–derived dendritic cells, resident peritoneal macrophages, and alveolar macrophages but not in bone marrow–derived macrophages, Flt3-elicited bone marrow–derived dendritic cells, or thioglycollate-elicited peritoneal macrophages [46]. Third, different *Candida* strains possess apparently distinct PAMP structures, which results in heterogeneous patterns of recognition by various TLRs and CLRs both *in vitro* and *in vivo* [22,47]. Our knowledge of the immunological role of individual PRRs has significantly expanded by our use of reductionist approaches in individual immune cell types *ex vivo*. However, it remains elusive how *Candida* recognition is integrated *in vivo* via the multitude of PRRs, how different PRRs drive different responses despite the use of similar signaling pathways, and how divergent this process is among the diverse repertoire of clinical *Candida* strains that infect patients, including the emerging non–*albicans Candida* species. Another dynamic parameter for consideration is how fungal sensing is influenced by *Candida* recognition–evading strategies *in vivo*. Specifically, it has been shown that masking of β-glucan exposure on the *Candida* surface and altered cell wall chitin content both impede CLR-mediated pathogen recognition during systemic infection [48,49]. Importantly, more work using conditional knockout mice that lack specific PRRs in different myeloid cell populations will be required to define the *in vivo* role of different PRRs in cell-specific and organ-specific *Candida* sensing.

Although mouse data have shown that complement, TLR/MyD88, CLR/CARD9, and inflammasome-mediated IL-1β signaling is indispensable for survival in systemic candidiasis, no universal accordance is seen in the corresponding inborn errors of immunity in humans in whom only CARD9 signaling is nonredundant. Thus, patients with *MYD88* mutations who lack both TLR- and IL-1β–dependent signaling and patients with complement deficiencies develop pyogenic bacterial infections but are not susceptible to fungal disease [50,51]. On the other hand, patients with *CARD9* mutations who have impaired myeloid cell proinflammatory cytokine responses and their neutrophils have defective killing capacity specifically against *Candida* are prone to systemic candidiasis [52,53]. In fact, the infection in these individuals appears to have a propensity for the central nervous system for reasons that are not yet entirely understood [52,53]. Of interest, patients with the p.Y238X *DECTIN-1* mutation, which abolishes dectin-1–dependent CARD9 signaling, do not develop systemic candidiasis and thus are not a phenocopy of patients with CARD9 mutations [54]. Therefore, it remains uncertain which CLR or CLRs upstream of CARD9 (either alone or in synergy) are essential for mounting nonredundant anti-*Candida* innate immune responses in humans.

Granulocytes: The role of oxidative and nonoxidative killing and the impact of organ-specific and time-dependent neutrophil recruitment

The crucial role of neutrophils in antifungal immunity was first appreciated more than half a century ago with the advent of neutrophil-depleting cytotoxic chemotherapy for

the treatment of leukemic patients, who, as a result, developed opportunistic fungal infections including systemic candidiasis [13,14]. Since then, multiple studies have shown that neutropenia is a major risk factor for both development of and worse outcome after gastrointestinal tract–derived and skin-derived systemic candidiasis in humans [1,2,55–57].

Neutrophils are the most potent leukocytes for mediating *Candida* killing and the only immune cells shown thus far to be capable of successfully inhibiting the conversion of *Candida* yeast into pseudohyphae [23], a key fungal virulence trait [58]. In fact, the lower myeloperoxidase content and the lack of α-defensins in mouse neutrophils compared with human neutrophils was shown to correlate with the decreased relative ability of mouse neutrophils to inhibit *Candida* filamentation [59], thus underscoring the importance for future work that is aimed at better characterizing the qualitative differences in anti-*Candida* killing mechanisms between mouse and human neutrophils.

Neutrophil ingestion of *Candida* is followed by the rapid assembly of the five subunits of the nicotinamide adenine dinucleotide phosphate (NADPH)–oxidase complex at the phagosomal membrane [60], a process critical not only for yeast killing and restriction of *Candida* filamentation but also for directing phagocyte recruitment into *Candida*-infected tissues [58,61,62]. NADPH oxidase–dependent generation of superoxide anion is followed by formation of hydrogen peroxide, which is then converted to hypochlorous acid by myeloperoxidase. These reactive oxygen species, together with the NADPH oxidase–induced K-flux–dependent activation of candidacidal neutrophil proteases within the phagosome [63], play an important role in oxidative *Candida* killing. In agreement, neutrophils from NADPH oxidase– and myeloperoxidase-deficient mice and humans are unable to efficiently kill *Candida ex vivo* [64–67]. Nonetheless, systemic candidiasis occurs very infrequently in patients with chronic granulomatous disease and complete myeloperoxidase deficiency, the vast majority of whom never develop the infection [67–69], suggesting that *in vivo* nonoxidative killing mechanisms are essential and, at large, sufficient to compensate for the lack of reactive oxygen species' generation [70]. In contrast, patients with chronic granulomatous disease are at high risk for development of invasive aspergillosis and other mold infections, indicating that different fungi differentially depend on phagocyte oxidative mechanisms for effective clearance. Consonant with the indispensable role of nonoxidative killing mechanisms of human neutrophils against *Candida*, patients with CARD9 deficiency who develop systemic candidiasis have impaired neutrophil *Candida* killing despite normal oxidative burst [52].

Our understanding of the molecular basis of neutrophil anti-*Candida* killing mechanisms has significantly advanced in recent years. For example, dectin-1–dependent, calcineurin-mediated nuclear factor of activated T-cells-independent signaling and dectin-1–dependent Mac-1/Vav activation both appear important for oxidative neutrophil killing [71,72]. In addition, neutrophils are able to ensnare and kill *Candida* yeasts and hyphae extracellularly by forming neutrophil extracellular traps (NETs), which consist of a backbone of neutrophil DNA decorated with several granular proteins with antifungal activity, such as myeloperoxidase, elastase, and calprotectin [73]. NET formation appears particularly important for restricting the growth of pseudohyphal elements, which neutrophils cannot internalize due to their large size. Depending on the experimental condition used, both oxidative and nonoxidative mechanisms have been implicated in driving *Candida*-induced NETosis via Rab27a and dectin-1/complement receptor 3/ERK activation, respectively [74–76]. Although mice deficient in calprotectin, a constituent of neutrophil NETs, were shown to be susceptible to systemic candidiasis following bloodstream or pulmonary yeast inoculation [77], more studies are required to uncover "signature" NET-specific molecules, which will enable the investigation of the contribution of NETs in organ-specific anti-*Candida* resistance *in vivo*. More work will also be needed to further define the signaling pathways that mediate oxidative and nonoxidative neutrophil killing against *Candida*, as such knowledge could lead to the development of immune-based strategies to enhance the candidacidal activity of neutrophils in infected patients, while avoiding neutrophil-mediated immunopathology.

Importantly, before neutrophils come in contact with and kill *Candida*, they need to successfully traffic to the site of infection via induction of chemotactic factors that guide directional cell movement toward a gradient of increased chemokine concentration present in infected tissues [78]. In fact, Ccl3- and Cxcl1-mediated neutrophil recruitment orchestrated by Th1 and Th17 lymphocytes is the mechanism by which the cell wall protein-based Als3 vaccine [79], which is currently under phase 2 clinical trials [80], appears to mediate protection against the infection. Of interest, only when neutrophils accumulate early within the first 24–48 h after infection are they protective in mice, as subsequent neutrophil recruitment does not confer additional survival benefit [81]. The requirement of prompt neutrophil accumulation for optimal anti-*Candida* host defense correlates with the organ-specific microbiological progression of the infection in mice [21]. Thus, the liver and spleen, which successfully control fungal proliferation and prevent *Candida* filamentation, are able to recruit significant numbers of neutrophils within the first critical 24-h period post-infection

[21]. Instead, the lack of efficient signals for rapid neutrophil recruitment in the kidney is associated with the inability of the organ to control fungal overgrowth and pseudohyphal formation [21]. In fact, the delayed neutrophil recruitment in the kidney at a time when *Candida* has already invaded within renal tubules could render the cells ineffective as neutrophils may have impaired effector function within the tubular urine microenvironment [82,83]. Therefore, defining the chemoattractant signals that are necessary for early protective neutrophil trafficking in *Candida*-infected tissues and elucidating why renal neutrophil recruitment is specifically sluggish are important directions for future research.

While early neutrophil trafficking in infected tissues is protective, neutrophil accumulation late in the course of the infection has been shown to be deleterious in mice [81,84]. Specifically, we recently reported that the chemokine receptor Ccr1 drives late pathogenic neutrophil recruitment from the blood into the kidney, but other yet-unknown chemoattractant receptors are also operational at this phase of the infection [84]. Future work should aim to determine why neutrophils exert detrimental effects late but not early in the course of systemic candidiasis. This time-dependent difference in mediating immunopathology late after infection does not appear to relate to a differential intrinsic capacity for degranulation or release of tissue-damaging reactive oxygen products compared with neutrophils recovered early after infection [84]. Importantly, because pathogenic neutrophil effects have been reported in human renal candidiasis and in a subset of patients with hepatosplenic candidiasis following neutrophil recovery [85–87], discovery of Ccr1 and other molecular factors that mediate neutrophil-induced immunopathology in systemic candidiasis could potentially lead to targeted therapeutic interventions in selected cohorts of patients.

Mononuclear phagocytes: The emerging roles of inflammatory monocytes, resident macrophages, and dendritic cells in anti-*Candida* host defense

Although the indispensable role of neutrophils in host defense against systemic candidiasis has been well accepted for decades, the important contribution of mononuclear phagocytes in innate immune control of the infection has been less recognized. This holds true despite early *in vivo* studies in mice that showed that clodronate-induced depletion of mononuclear phagocytes results in accelerated tissue fungal proliferation and increased mortality [88] and that neutrophil depletion does not adversely affect innate immune control of *Candida* in blood, highlighting the compensatory immune effects of blood monocytes [81].

Despite the remarkable ability of monocytes/macrophages to internalize *Candida* and secrete several proinflammatory cytokines and chemokines, a perception has persisted for years that these cells do not play a key role in immune protection based on the observation that they are not particularly capable of killing *Candida* relative to neutrophils. The majority of studies on the role of mononuclear phagocytes in innate immune control against *Candida* have used thioglycollate-elicited mouse peritoneal macrophages, which indeed do not exert potent candidacidal activity *ex vivo* [57]. Although these cells are easy to obtain for downstream functional studies, they do not come in contact with *Candida* in the course of systemic candidiasis, which raises the question whether they accurately portray the role of mononuclear phagocytes in immune pathogenesis against the infection. To that end, it was recently shown that both bone marrow mouse monocytes, which traffic to sites of *Candida* infection, as well as kidney resident macrophages, which are the first cells to come into contact with *Candida* in the infected kidney, both exert significant candidacidal activity [89,90]. In line with this, human blood classical $CD14^{++}CD16^{-}$ and nonclassical $CD14^{+}CD16^{++}$ monocytes also have candidacidal activity [91]. These data collectively suggest that mononuclear phagocytes from anatomical sites that come in contact with the fungus after infection may be functionally distinct from peritoneal macrophages, attesting to the well-described functional heterogeneity of different mononuclear phagocyte subsets in mice [92]. Therefore, although technically more challenging, future research should incorporate studies of mononuclear phagocytes sorted from target organs of systemic candidiasis.

Two recent studies shed light on the important role of recruited inflammatory monocytes and resident kidney macrophages in innate immune control of systemic candidiasis [89,90]. These studies focused on the chemokine receptors Ccr2 and Cx3cr1, which are "signature" molecules for mouse inflammatory and resident monocytes/macrophages, respectively, and have been shown to correlate with human blood classical and nonclassical monocytes, respectively [78]. Elegant work from the Hohl lab showed that Ccr2-deficient mice, which are unable to mobilize inflammatory monocytes into the infected kidney, had a modest increase in mortality compared with wild-type animals. When Ccr2-DTR mice were used, which in addition to inflammatory monocytes also lack all Ccr2-expressing NK cells and subsets of resident macrophages and dendritic cells, an overwhelming infection ensued, with universal mouse mortality and inexorable fungal proliferation in tissue [89]. Strikingly, transfer of $Ccr2^{+}$ bone marrow inflammatory monocytes in Ccr2-DTR mice rescued the mice from mortality. Of interest, the protective effects of Ccr2-expressing

mononuclear phagocytes were only seen at the onset of infection but not after day 2 post-infection [89]. This time-dependent early protection conferred by inflammatory monocytes is likely explained by the almost exclusive translocation of *Candida* within the renal collecting system after the first 24 h of infection [90]. This localization of *Candida* within tubules acts as an immune-evading mechanism because, as shown by confocal microscopy in live infected animals [90], mononuclear phagocytes (including monocytes) never enter the tubular lumen to come in contact with the pathogen. Thus, the rapid invasion of *Candida* within renal tubules allows only a short window of opportunity for recruited inflammatory monocytes to exert their effector function in the infected kidney.

In addition, we reported that Cx3cr1-deficient mice universally succumbed to the infection and were more susceptible to systemic candidiasis compared with wild-type and Ccr2-deficient animals, suggesting that resident kidney macrophages may have a predominant role in innate immune control of the infection relative to recruited inflammatory monocytes [90]. Because both recruited monocytes and resident macrophages have comparable anti-*Candida* killing capacity [89,90], the differential dependence on resident macrophages for host defense *in vivo* is likely because these cells are already positioned at the site of infection and come into contact with the fungus very early after infection, as shown by confocal microscopy in live mice. Specifically, more than 90% of *Candida* yeast and pseudohyphal elements are in contact with resident kidney macrophages just 2 h after intravenous inoculation, before inflammatory monocytes have the opportunity to traffic into the kidney [90]. Cx3cr1 deficiency results in an approximate 50% decrease in resident macrophage accumulation in the kidney, which leads to ineffective contact of the cells with *Candida* early post-infection and unabated fungal growth in the kidney as early as 12 h after infection, at a time when inflammatory monocytes and neutrophils have not yet been recruited in significant numbers [90]. At the mechanistic level, Cx3cr1 promotes accumulation of macrophages in tissue by relaying cell survival signals via Akt-mediated inhibition of caspase-3–dependent apoptosis [90]. In agreement with the role of Cx3cr1 in host defense against systemic candidiasis in mice, the dysfunctional human *CX3CR1-M280* allele was found to be a risk factor for development of candidemia and disseminated candidiasis in two independent patient cohorts, implying that genetic variation at *CX3CR1* may be a novel factor for risk assessment and prognostication of the infection in humans [90].

In addition to monocytes/macrophages, the role of dendritic cells in host defense against systemic candidiasis has not been comprehensively studied, in part, due to the inherent difficulties in achieving specific dendritic cell depletion *in vivo*. Importantly, the recent phenotypic and functional characterization of the mononuclear phagocyte network in the mouse kidney is a critical step toward better defining the role of different myeloid populations in innate immune control of *Candida* [90,93]. These studies have underscored the notable plasticity among the kidney resident macrophage and dendritic cell populations, which possess overlapping macrophage- (ie, phagocytic) and dendritic cell–characteristic (ie, antigen presentation) functional properties [93].

Moreover, two recently characterized inherited immunodeficiencies have further highlighted the relative significance of resident macrophages over circulating monocytes in protection against systemic candidiasis in humans [94–96]. Hence, patients with MonoMAC syndrome due to *GATA2* mutations have profound monocytopenia but preserved tissue-resident macrophages. Although these patients develop systemic fungal disease caused by dimorphic fungi (eg, *Histoplasma*) and inhaled molds (eg, *Aspergillus*), they have not been reported to develop systemic candidiasis to date [94,95]. In addition, patients with autosomal recessive mutations in the *IRF8* gene who also lack circulating monocytes but have preserved tissue-resident macrophages are susceptible to mycobacterial infections but not to systemic candidiasis [96]. Therefore, more research is needed to define the role of human monocytes and monocyte-derived macrophages and dendritic cells in innate immune control against *Candida*.

The role of other innate cells in host defense against systemic candidiasis

In addition to neutrophils and mononuclear phagocytes, much less is known about the contribution of other cells in innate immune control of systemic candidiasis. Recent elegant work using IL-17RA–deficient mice revealed an unexpected role for IL-17–related cytokines in normal NK cell development and an indispensable role for NK cells in protection against the infection [97]. Specifically, NK cells were shown to prime candidacidal activity in neutrophils via a process that required granulocyte macrophage colony-stimulating factor (GM-CSF) secretion by NK cells but no direct neutrophil-NK cell contact [97]. Importantly, transfer of NK cells into mice that lacked normal NK cell responses was sufficient to restore the fungicidal activity of neutrophils and control *Candida* proliferation *in vivo* [97]. In addition to mouse NK cells, human NK cells also exert anti-*Candida* effector function, both directly via NKp30-mediated perforin production and indirectly via priming neutrophil fungicidal activity [98,99]. Thus, whether nonneutropenic patients with systemic candidiasis would

benefit from GM-CSF administration and/or adoptive transfer of NK cells merits investigation. Of note, patients with inborn errors of NK cell function have enhanced susceptibility to viral disease but not to systemic candidiasis [100]. Because of the rarity of these syndromes, future careful phenotypic characterization of their infection susceptibility will be needed to determine whether quantitative and/or qualitative perturbations in human NK cells are associated with development of systemic candidiasis. In addition to NK cells, the role of other innate lymphoid cells in host defense during skin-derived systemic candidiasis merits future investigation.

On the other hand, Jα18KO mice, which specifically lack invariant natural killer T (iNKT) cells, do not have heightened susceptibility to systemic candidiasis [101], although iNKT cells are known to mediate CD1d^{+} dendritic cell–primed innate immune responses against *Candida* via dectin-1- and MyD88-dependent mechanisms [102]. In fact, glycolipid-mediated activation of iNKT cells in *Candida*-infected mice resulted in interferon-gamma–dependent accelerated tissue fungal growth and higher mortality associated with tissue immunopathology and impaired neutrophil production and accumulation in the bone marrow and blood [103]. Moreover, the role of nonhematopoietic stromal cells in host defense against systemic candidiasis merits further investigation. For example, a recently developed renal epithelial cell *in vitro* assay uncovered the important role of these cells in production of the major neutrophil-targeted chemoattractants Cxcl1 and Cxcl2 [104]. Also, IL-22–mediated protection of renal epithelial cell integrity ameliorated renal tissue injury and conferred protection against systemic candidiasis [105]. Last, because endothelial cells play important roles in modulating *Candida* invasion and immune cell trafficking into infected tissue [106,107], future research is needed to discern the molecular factors on endothelial cells that regulate these processes during systemic candidiasis *in vivo*.

Conclusions

Recent research has uncovered several cellular and molecular factors that are important for the regulation of innate immune responses during systemic candidiasis in mice. These immunological studies have highlighted the distinct role of various immune-sensing receptors and myeloid cells in promoting *Candida* clearance. The parallel discovery of inherited immunodeficiencies with associated susceptibility or resistance to systemic candidiasis has provided the opportunity to interrogate the relevance of the corresponding mouse findings in human disease. Development of a detailed mechanistic understanding of how the mammalian innate immune system responds to systemic *Candida* challenge should aide in the development of novel immune-based risk stratification, prognostication, and therapeutic strategies in patients suffering from systemic candidiasis.

Acknowledgments

This work was supported by the Division of Intramural Research of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Declaration of interest

The author reports no conflicts of interest. The author alone is responsible for the content and the writing of the paper.

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