

Innate and adaptive immunity to *Candida albicans*: A new view of an old paradigm

Antonella Mencacci, Elio Cenci, Giuseppe Del Sero, Cristiana Fè d'Ostiani, Claudia Montagnoli, Angela Bacci, Francesco Bistoni and Luigina Romani

Microbiology Section, Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Perugia, Italy

Candida albicans is an opportunistic fungal pathogen commonly found in the human gastrointestinal and female lower genital tracts. Underlying acquired immunity to *C. albicans* is usually present in adult immunocompetent individuals, such as the expression of a positive delayed-type hypersensitivity to the fungus, and is presumed to prevent mucosal colonization from progression to symptomatic infection [1,2]. However, if the ability of *C. albicans* to establish a disseminated infection involves neutropenia as a major predisposing factor, its ability to persist in infected tissue may involve primarily downregulation of host cell-mediated adaptive immunity. These data argue for the importance of both innate and adaptive cell-mediated immunity in the control of *C. albicans* infections.

Recent evidence has shed new light on the complex interplay between the innate and adaptive components of the immune system and has revealed complex levels of immunoregulation that were previously unappreciated. In this review, we have summarized recent findings on: i) the protective and nonprotective immune effector mechanisms in mice with *C. albicans* infection; ii) their regulation by cytokines; and iii) the possible role of the innate immune system in mediating differentiation of disparate CD4⁺ T helper (Th) cell subsets.

PROTECTIVE AND NONPROTECTIVE IMMUNE EFFECTOR MECHANISMS

In conditions in which the animals are overwhelmed by a high dose of the yeast given intravenously, mice of different inbred strains die within a few hours, so that any contribution of cell-mediated immunity could barely be assessed. However, upon intravenous challenge with carefully selected fungal doses [3,4], or with low-virulence yeast cells [5], or upon intragastric challenge [6], an infection of a longer course can be obtained, so that it is possible to evaluate the contribution of both nonspecific and specific cellular immune mechanisms in the control

of infection and its pathology. In the case of infection with low-virulence *C. albicans*, susceptibility and resistance to primary and secondary disseminated infections vary among the different inbred strains of mice. C57BL/6 and BALB/c mice are resistant to primary infection and acquire resistance to reinfection, while C3H/HeJ and CBA/J mice show moderate resistance to primary and secondary infections. In contrast, DBA/2, 129/Sv/Ev, and SJL mice are highly susceptible to primary infection. This genetically determined resistance and susceptibility to infection has been found to correlate with the balance occurring between specific CD4⁺ Th1 and Th2 responses [5-10]. The predominance of Th1 cell responses leads to resistance and onset of protective immunity, while Th2 responses are associated with disease exacerbation and pathology. Cytokines produced by Th1 cells activate neutrophils and macrophages to a fungicidal state, whereas those produced by Th2 cells exacerbate the disease because of their deactivating properties for fungicidal effector cells [11]. Thus, Th1 and Th2 cells, by critically providing cytokines with activating and deactivating signals to fungicidal phagocytes, may be instrumental in mobilizing and activating the proper anticandidal effector mechanism at the site of infection. Interestingly, the interaction of circulating yeast cells with phagocytic cells of the host may also be promoted by protective antibodies [12]. It appears that phagocyte-dependent resistance to *C. albicans* may be regulated at the effector level by sets of opposing cytokines, such as IFN- γ and IL-4/IL-10. For example, IFN- γ activates inducible nitric oxide (NO) synthase in macrophages leading to the production of reactive nitrogen radicals that inhibit growth of the fungus [13]. Both IL-4 and IL-10 inhibit the IFN- γ -dependent NO production by macrophages and impair fungal growth restriction by the host [13,14]. Evidence for the occurrence of an imbalanced Th cytokine production and regulation to *Candida* has also been obtained in patients with chronic mucocutaneous [15,16] or hepatosplenic [17] candidiasis, in which the manifestations observed (i.e., impairment of cell-mediated immunity, high levels of *Candida*-specific antibodies, and susceptibility to certain types of bacterial infections) could be easily accommodated within the framework of the Th1/Th2 paradigm to the infection.

Recent studies on the genetic control of host susceptibility and/or resistance to *C. albicans* infection have shed new and important light on the role of antifungal cell-mediated immunity. Besides the fifth component of complement as a major influence on susceptibility and resistance [18], Ashman has recently provided evidence of at least two Mendelian-type, resistance genes that control the host response to systemic *C. albicans* infection in mice [19]. These genes appear to affect distinct parame-

Dirección para correspondencia:

Dr. Luigina Romani
Microbiology Section, Department of Experimental
Medicine and Biochemical Sciences, University of
Perugia, Via del Giochetto, 06122 Perugia, Italy
Tel.: +39 075 585 3411; Fax: +39 075 585 3400;
E-mail: lromani@unipg.it

ters of infection, such as tissue destruction and kidney colonization. Allocation of presumptive "resistant" and "susceptible" alleles of these genes among various inbred strains provides an excellent correlation with the various measures of infection. One important implication of this finding is that effector mechanisms of antifungal resistance would be both genetically determined and site-specific. This is in line with experimental and clinical evidence that induction of antifungal cell-mediated immunity is highly compartmentalized [1,20,21]. Indeed, the susceptibility of mouse strains to systemic candidiasis does not correlate with that to mucosal, gastrointestinal, or vaginal candidiasis [22]. Likewise women susceptible to oral and esophageal candidiasis are generally not more susceptible to vaginal candidiasis [21,23].

REGULATION OF Th1- AND Th2-DEPENDENT IMMUNITY

The role of cytokines. Th1 and Th2 CD4⁺ T cells develop from a common naive CD4⁺ T cell precursor. Several parameters have been reported to influence their pathway of differentiation of CD4⁺ T cell precursors [2, 20]. Among these, cytokines appear to play a major role, acting not only as modulators of antifungal effector functions but also as key regulators in the development of the different Th subsets from precursor Th cells. Studies in mice have shown that development of protective anticandidal Th1 responses requires the concerted actions of several cytokines such as IFN- γ [9,24], TGF- β [25], IL-6 [26], TNF- α [8], and IL-12 [27], in the relative absence of inhibitory Th2 cytokines, such as IL-4 and IL-10, which inhibit development of Th1 responses [28]. Early in infection, neutralization of Th1 cytokines (IFN- γ and IL-12) leads to the onset of Th2 rather than Th1 responses, while neutralization of Th2 cytokines (IL-4 and IL-10) allows for the development of Th1 rather than Th2 cell responses [2,20,29]. However, in highly susceptible mice, exogenous IL-12 does not exert beneficial effects on the course and outcome of disseminated and mucosal infections [27]. Moreover, administration of IL-4 fails to convert an already established Th1 response into a Th2 response [28], and late IL-4 depletion exacerbates chronic infection [30,31]. These findings indicate the existence of complex immunoregulatory circuits underlying cytokine activity in mice with candidiasis. Studies performed in genetically-modified mice, including cytokine-deficient mice, have furthered our understanding of cytokine-mediated regulation of Th cell development and effector functions in candidiasis and have revealed complex levels of immunoregulation previously unappreciated [10]. TNF/LT- α and IL-6 deficiencies render mice highly susceptible to *C. albicans* infections. In contrast, resistance to primary and secondary infections was not impaired in the absence of IL-1 β or IL-10, as occurs in ICE- or IL-10-deficient mice, respectively. Finally, IL-12, IL-4, or functional IFN- γ deficiencies, while not affecting resistance to primary infections, render mice susceptible to reinfection. Resistance or susceptibility to infections correlates with the levels of *Candida* growth in target organs, as well as with the type of Th cytokine production by specific CD4⁺ T lymphocytes [10]. A reduced production of IL-4 and IL-10 and increased production of IFN- γ and IL-2 was observed in mice resisting both primary and secondary infections, such as ICE- and IL-10-deficient mice [10, and unpublished observations]. On the contrary, high-level production of IL-4 and IL-10 and low-level production of Th1 cytokines were observed in TNF/LT- α - and IL-6-deficient mice succumbing to primary infec-

tion, and in IL-12p40-, IFN- γ R-, and IL-4-deficient mice succumbing to secondary infection [10,32]. Altogether, these data demonstrate that susceptibility to primary and secondary *C. albicans* infections in knockout mice correlates with the failure to develop anticandidal protective Th1 responses and with the occurrence of non protective, IL-4- and IL-10-producing Th2 cells. Moreover, these studies revealed the existence of a hierarchical pattern of cytokine-mediated regulation of antifungal Th cell development and effector function. Early in infection, production of some proinflammatory cytokines (TNF- α and IL-6), more than others (IL-1 β), appears to be essential for the successful control of infection and the resulting protective Th1-dependent immunity. Both IL-12 production and responsiveness are required for the development of Th1 cells, whose proper activation depends on the presence of physiological IL-4 [31] and IL-10 [32]. Thus, a finely regulated balance of directive cytokines, such as IL-4, IL-10, and IL-12, rather than the relative absence of opposing cytokines, appears to be required for optimal development and maintenance of Th1 reactivity in mice with candidiasis.

The role of the innate immune system. Once considered merely a vestige of ancient antimicrobial systems, made redundant by the evolution of acquired immunity, innate immunity is now considered to actively participate in determining the type of specific immune response to a pathogen [33]. In candidiasis, the initial handling of fungal pathogen by cells of the innate immune system plays a major role in determining CD4⁺ Th development. Indeed, qualitative or quantitative defects of antifungal effector and immunoregulatory functions of phagocytic cells result in the development of anticandidal Th2, rather than Th1, cell responses [34].

The instructive role of the innate immune system in the adaptive immune response to a pathogen may be operative at different levels [33,35]. In candidiasis, regulation of the early fungal burden [30], cytokine production [34,36-38], and expression of costimulatory molecules [8,32] are possible pathways through which the innate immune system may control CD4⁺ Th development. In particular, the finding that neutrophils, more than macrophages, are endowed with the ability to produce directive cytokines, such as IL-10 and IL-12, emphasize the important role of neutrophils in the overall control of infection and its pathology [34,37,38]. Human neutrophils also produce bioactive IL-12 in response to a mannoprotein fraction that is capable of inducing Th1 cytokine expression in peripheral blood mononuclear cells [39]. Because of the large number of neutrophils present in the blood or inflammatory tissues during infection [34], it is likely that neutrophil production of cytokines may influence the early development of the T cell response to *C. albicans*. Ultimately, this could be a likely expectation shared with other cells of the innate immune system, that although devoid of highly specific receptors but responding to more general patterns of microbial molecules, may nevertheless influence the final outcome of T cell differentiation [33]. Accumulating evidence indicates that the role of neutrophils in candidiasis may go beyond their fungicidal effector function [34]. In humans, risk factors for invasive fungal infections are not the same in all neutropenic patients [40]. Chronic systemic candidiasis initiated by neutropenia may persist in spite of normal neutrophil counts and adequate antifungal therapy [41]. Moreover, it has been recognized that some patients, particularly transplant recipients with adequate or even normal neutrophil counts, may be at high risk for invasive mycoses [42,43]. Paraphrasing the current emerging

dilemma concerning the proper management of patients at risk for invasive fungal infections: "Is recovery from neutropenia all that is needed"? [44], it is no longer adequate to reason only in terms of absolute numbers of effector cells, rather number and function should be considered. The ability of neutrophils to directly or indirectly affect the development and effector functions of CD4+ Th cells may likely be an additional function with important implications for immunity and therapy of invasive fungal diseases.

CONCLUSIONS

Immunomodulation by cytokines in the treatment of opportunistic fungal infections represents a promising strategy [45,46]. Considerable evidence in preclinical and clinical settings supports the model of specific immunity to opportunistic fungal infections as being highly susceptible to cytokine influences and reciprocally regulated by cells of the innate immune system [47]. However, i) the risk factors predisposing to invasive fungal infections,

ii) the role and mechanisms of cytokines, either alone or in combination with antifungal drugs, in treating these infections, and iii) the response to cytokine therapy are major undefined issues. The Th1/ Th2 paradigm of acquired immunity, although perhaps somewhat simplistic, is proving essential for a better understanding of immunoregulation in candidiasis and other fungal infections. With a view to either control fungal infectivity or oppose fungus-associated immunopathology, the promotion of fungus-specific Th1 responses appears to be a realistic objective in the development of vaccines and therapy of human fungal infections.

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