The Role of Endogenous Interleukin (IL)–18, IL-12, IL-1β, and Tumor Necrosis Factor–α in the Production of Interferon-γ Induced by Candida albicans in Human Whole-Blood Cultures

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Despite the importance of interferon (IFN)–γ, tumor necrosis factor (TNF), and interleukin (IL)–18 for host defense against candidiasis, the pathways leading to their stimulation by Candida albicans are unclear. In a whole-blood model, IL-18 neutralization by IL-18 binding protein decreased C. albicans–induced IFN-γ synthesis by 72%. Similarly, neutralization of IL-12 or IL-1β by either neutralizing antibodies or IL-1 receptor antagonist also reduced (by 65%) IFN-γ production. Neutralization of TNF by TNF binding proteins resulted in only a 36% reduction of IFN-γ synthesis. In contrast, production of TNF and IL-8 was largely unaffected by these cytokine inhibitors. Thus, C. albicans stimulates IFN-γ production in an IL-18–, IL-12–, and IL-1β–dependent manner, whereas production of TNF and IL-8 is independent of these cytokines. Blocking the biologic activities of IL-18, IL-12, and IL-1β in patients (e.g., for treatment of autoimmune diseases) may result in increased susceptibility to C. albicans infection.

Infections caused by pathogenic fungi, especially Candida albicans, are being recognized with increasing frequency in patients with a variety of underlying diseases [1]. Although new antifungal drugs have been developed, mortality due to disseminated candidiasis remains high, especially in immunocompromised hosts [2]. Therefore, a rational approach would be to develop therapeutic strategies aimed to augment and restore host defense mechanisms in these patients, in addition to administration of conventional antifungal therapy [3]. To achieve this, a better understanding of the existing intrinsic mechanisms responsible for the defense against invasive Candida infection is urgently needed.

The host response to C. albicans infection is a complex interplay between cellular and humoral immunity and usually provides a sufficient defense against the microorganism in the healthy host. Heat-killed C. albicans or mannoproteins derived from the yeast cell wall induce interferon (IFN)–γ production from human mononuclear cells [4, 5], and IFN-γ is a key cytokine for innate as well as acquired resistance to candidiasis [6]. In vitro studies have demonstrated stimulatory effects of IFN-γ on the phagocytosis and killing of C. albicans by neutrophils and macrophages [7, 8], and administration of IFN-γ to mice infected with C. albicans has had a beneficial effect on the outcome of the infection [6]. The important role of endogenous IFN-γ in the resistance against both gastrointestinal and systemic candidiasis has also been demonstrated by the increased susceptibility of knockout mice, deficient in IFN-γ or IFN-γ receptors, infected with the yeast [9–12]. In addition to IFN-γ, proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin (IL)–1 and chemokines such as IL-8 also play an important role in the host defense against C. albicans infection [7, 13, 14].

Despite its crucial importance for defense against candidiasis, little is known about regulation of IFN-γ after stimulation of leukocytes by C. albicans. An important step in the regulation of IFN-γ production is the synthesis of IL-12 after stimulation of monocytes and macrophages by C. albicans [15]. However, although IL-12 is required for IFN-γ production and the development of a protective Th1 response during Candida infection [11], recent studies show that additional costimuli are needed for an efficient synthesis of IFN-γ. IL-18 is a newly described cytokine that serves as a costimulus for IFN-γ production by itself, has an important costimulatory activity in the context of microbial stimulation [16–19] and that, unlike IL-12, is constitutively expressed in human cells [20]. When endogenous IL-18 is blocked by administration of neutralizing antibodies or IL-18 binding protein, there is little, if any, IFN-γ production, despite the presence of normal IL-12 concentrations [21]. These findings have been confirmed by studies of IL-18– or IL-1β converting enzyme (ICE)–deficient mice [22–24]. In addition, several studies report that IL-1β, although not able to induce IFN-γ production by itself, has an important costimulatory activity on the IL-12–mediated induction of IFN-γ by T lymphocytes [25–27] and NK cells [28]. Similarly, TNF is also a costimulus...
of IFN-γ production in the context of IL-12 or bacterial stimulation [27, 29]. To our knowledge, the role of these cytokines in the regulation of IFN-γ synthesis by C. albicans remains unknown.

In this study, we investigated how much of IFN-γ induction by C. albicans results from direct fungal stimulation, and we assessed the relative importance of indirect stimulation through endogenous IL-12, IL-18, IL-1β, and TNF in this process. Because both IL-18 and IL-1β are secreted as inactive precursors, which require cleavage by ICE to generate the mature active forms, we examined the effect of a specific inhibitor of this enzyme (caspase-1) to assess its contribution to their processing and subsequently to the Candida-induced production of IFN-γ. The role of specific cytokine inhibitors for the modulation of IFN-γ synthesis was assessed by the use of monoclonal antibodies (MAbs) and/or natural antagonists such as IL-18 binding protein (IL-18BP), IL-1 receptor antagonist (IL-1Ra) and TNF binding proteins (TNFbps). In addition, because each of these cytokines stimulates production of proinflammatory cytokines and thereby has potent inflammatory properties, we also assessed their effects on TNF and IL-8 production induced by C. albicans.

Methods

Volunteer selection. The study population comprised 9 healthy, nonsmoking men aged 23–45 years. The volunteers were free of infectious or inflammatory disease and had abstained from cyclooxygenase inhibitors for 2 weeks before the study. Blood was collected by venipuncture into heparinized syringes (sodium heparin; final concentration, 10 U/mL; Elkins-Sinn).

Reagents. We purchased the reversible ICE inhibitor (ICEi) Ac-Tyr-Val-Ala-Asp-2,6-dimethylbenzoyloxymethylketone (Alexis). The ICEi was reconstituted in 10 mM dimethyl sulfoxide (DMSO) and subsequently was diluted to the desired concentration in RPMI. Recombinant IL-18BP (expressed in CHO cells with carboxyl his) was produced as described elsewhere [30]. Recombinant human IL-1Ra was a gift of D. Tracey (Upjohn). Recombinant human TNFbp (4 domain p55 soluble TNF receptor) [31, 32] was provided by C. Edwards (Amgen). The mouse anti-human IL-12 MAb was purchased from R&D Systems. Mouse anti-human IL-1β MAb was provided by Cistron Biotechnology. An irrelevant mouse anti-human IgG antibody was used as a control and did not influence cytokine release. RPMI culture medium (Gibco BRL) was supplemented with 10 mM L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin (Gibco).

C. albicans. C. albicans UC820, a clinical isolate that has been well described [33], was maintained on agar slants at 4°C. Earlier experiments showed that strain UC820 can develop hyphae and pseudohyphae in vitro and in vivo to the same extent as a panel of virulent control C. albicans. C. albicans UC820 was inoculated into 100 mL of Sabouraud broth and was cultured for 24 h at 37°C. After 3 washes with pyrogen-free saline by centrifugation at 1500 g, the number of yeast cells was counted in a hemocytometer; occasional strings of 2 yeasts were counted as 1 cfu of C. albicans. The suspension was diluted to the appropriate concentration with pyrogen-free saline. Microscopy confirmed that the suspension consisted of blastoconidia. The blastoconidia were heat killed during 30 min at 100°C.

Whole-blood cytokine culture. We added 0.5 mL of heparinized blood to 75 × 10-mm polypropylene tubes (Falcon). The cytokine inhibitors were added in various concentrations and mixed, and, after 10 min, 0.5 mL of RPMI culture medium or heat-killed C. albicans blastoconidia (final concentration, 10⁷ organisms/mL) was added. This optimal concentration of C. albicans for cytokine induction was chosen after a pilot experiment with dose-dependent C. albicans stimulation. Each sample was incubated in duplicate. After 24 or 48 h of stationary incubation at 37°C in the presence of 5% CO₂, 0.5 mL of blood was mixed with Triton X-100 (1% final concentration; Bio-Rad Laboratories) to disrupt cell membranes and lyse all cells. Samples were kept at –70°C until cytokine measurement. Total cytokine concentrations were measured in the lysed blood samples treated with Triton X-100 [34]. In a pilot experiment, the effects of the various cytokine antagonists on spontaneous release of cytokines were tested. The spontaneous release of IFN-γ was below the detection limit in the majority of samples, and the cytokine inhibitors did not influence this process. Similarly, no effect of the cytokine antagonists on the spontaneous production of TNF or IL-8 was observed (data not shown).

In a separate experiment, the differential effect of an ICE inhibitor on intracellular and extracellular IL-1β production was investigated. The intracellular IL-1β concentration was investigated by centrifuging the blood after stimulation with C. albicans for 24 h, discarding the supernatant (plasma), and adding an identical volume of RPMI medium on the cell pellet. After the tubes were vortexed, we added Triton X-100 and measured the intracellular cytokines in the cell lysates. The release of extracellular IL-1β was measured in the plasma separated from the cells during the first step of the procedure.

Cytokine measurements. Human IFN-γ, TNF, and IL-8 concentrations were measured by electrochemiluminescence, as described elsewhere [34, 35]. IL-1β was measured by a commercial ELISA kit (Cistron) specific for mature IL-1β. The cross-reactivity of the ELISA with proIL-1β is <20%. We measured IL-12 p40 (both p40 and p40/35 complexes) concentrations by commercial ELISA (R&D Systems).

Statistical analysis. Data are expressed as mean ± SE. Differences between groups were analyzed by Mann-Whitney U test, using the Bonferroni correction for multiple comparisons.

Results

Influence of ICE inhibition on C. albicans–stimulated cytokine production. We investigated the effects of a specific inhibitor of ICE (caspase-1) on the production of Candida-induced IL-1β and IFN-γ. Stimulation of blood with C. albicans induced synthesis of significant amounts of IL-1β (2225 ± 514 pg/mL) and IFN-γ (3762 ± 1038 pg/mL). The ICE inhibition dose-dependently decreased C. albicans–stimulated production of IFN-γ, up to a maximal inhibition of 63% (figure 1, P < .03). The addition of the ICE inhibitor to the whole-blood culture also significantly reduced Candida-induced secreted IL-1β (76% inhibition; P < .02) but not intracellular IL-1β (data not shown). The ICE inhibitor’s
lack of effect on intracellular IL-1β also demonstrates the lack of cell toxicity of the minute amounts of DMSO present in the ICE inhibitor preparation, since toxicity would have released IL-1β from the cells, with subsequent depletion of IL-1β from the intracellular compartment.

**Effect of IL-18BP on C. albicans–induced IFN-γ production.** Previous studies demonstrated that mature IL-18 is required for the lipopolysaccharide (LPS)–induced production of IFN-γ, even in the presence of IL-12 [23, 24]. To investigate the role of endogenous IL-18 for the *Candida*–induced IFN-γ synthesis, increasing concentrations (7.8–500 ng/mL) of IL-18BP were added to the whole-blood cultures. IL-18BP reduced *C. albicans*–induced IFN-γ production in a dose-dependent manner (figure 2). IC_{50} of *C. albicans*–induced IFN-γ production was achieved by 125 ng/mL of IL-18BP, which was therefore used (unless otherwise indicated) in additional experiments in which we studied the effects of IL-18BP and other cytokine inhibitors. Concentrations of IL-18BP >500 ng/mL did not further reduce IFN-γ production (data not shown).

**Role of endogenous IL-1β, IL-12, and TNF in Candida-induced IFN-γ production.** Because IL-18BP was not able to block the induction of IFN-γ by *C. albicans* by >72% (see above), we assumed that other cytokines must be involved in this process. Recent studies suggest that IL-1, although not able to induce IFN-γ by itself, is an important costimulus of IFN-γ production [25–27]. As shown in figure 3A, when IL-1 receptors were blocked by IL-1Ra (10 ng/mL), there was a significant reduction in the stimulation of IFN-γ by *C. albicans*. IL-18BP and IL-1Ra had additive inhibitory effects on IFN-γ production. To differentiate between the effects of endogenous IL-1α and IL-1β, we compared the inhibitory action of IL-1Ra with that of a MAb against human IL-1β on *Candida*-induced IFN-γ. IL-1Ra and the anti–IL-1β MAb were equally potent in inhibiting IFN-γ production induced by *C. albicans* (figure 3B), suggesting that blocking IL-1β and not IL-1α accounts for the ability of IL-1Ra to reduce *Candida*-induced IFN-γ production.

Because the combination of IL-18BP and IL-1Ra was not able to completely block the induction of IFN-γ, we investigated the role of endogenous IL-12 and TNF in this process. Stimulation of blood with *C. albicans* induced 380 ± 103 pg/mL of IL-12. Anti–IL-12 antibodies significantly inhibited (61% reduction of production) *C. albicans*–induced IFN-γ, and the effects of anti–IL-12 and IL-18BP were additive (figure 4A). A similar effect on IFN-γ, although less pronounced (36% inhibition), was found by blocking endogenous TNF by TNFbp (10 mg/mL; figure 4B).

As shown above, endogenous IL-18, IL-1β, and IL-12 all appear to contribute to *Candida*-induced IFN-γ. We next assessed whether IFN-γ production can be totally blocked by simultaneous inhibition of these 3 cytokines. The combination of IL-18BP, IL-1Ra, and anti–IL-12 antibodies virtually abolished the production of IFN-γ induced by *C. albicans* (figure 5).

**Effect of cytokine inhibitors on C. albicans–induced production of TNF and IL-8.** *C. albicans* induces production of the proinflammatory cytokine TNF (2532 ± 611 pg/mL) and of the chemokine IL-8 (112,488 pg/mL) [5], which also play important roles in the defense against *Candida* infection [14, 36]. Therefore, we investigated the modulation of *C. albicans*–induced TNF and IL-8 in whole-blood cultures. No effect of IL-18BP, IL-1Ra, or anti–IL-12 antibodies on the *Candida*-induced TNF was observed, with the exception of a marginal inhibitory effect of IL-1Ra on TNF production (figure 6). Similarly, IL-8 production was not influenced by IL-18BP, IL-1Ra, anti–IL-12 antibodies or TNFbp (not shown).
Discussion

In this study, we investigated the mechanisms by which *C. albicans* induces production of IFN-γ in whole-blood cultures. The observation that a specific ICE inhibitor reduced the synthesis of IFN-γ demonstrates the importance of IL-18 and IL-1β in this process. Inhibition of IL-18 by its natural antagonist IL-18BP significantly inhibited up to 72% of the production of IFN-γ stimulated by *C. albicans*. This shows the central role played by IL-18 for *Candida*-induced IFN-γ. IL-1β, IL-12, and TNF are additional endogenous costimuli of IFN-γ production. In contrast, induction of TNF and IL-8 by *C. albicans* is largely independent of secretion of these intermediary mediators.

The pivotal role of endogenous IL-18 for the induction of IFN-γ by *C. albicans* is not surprising, given that absence of mature IL-18 in ICE-deficient mice has been accompanied by severely impaired IFN-γ production after LPS stimulation, despite the presence of expected IL-12 concentrations [23, 24]. The central role of IL-18 for IFN-γ synthesis is reinforced by data showing that IL-12 induction of IFN-γ is dependent on caspase-1 processing of the IL-18 precursor [37]. These findings were confirmed by experiments using IL-18−/− deficient mice [22]. The blockade of IFN-γ production when IL-18BP was added to whole-blood cultures underscores the important potential role of this natural antagonist of IL-18 in the inhibition of the Th1 response stimulated by *C. albicans* [21].

The importance of IL-12 for the stimulation of IFN-γ and the development of a Th1 response is well described. *C. albicans*

![Figure 3. Effect of endogenous interleukin (IL)−18 and IL-1β on interferon (IFN)−γ production. A, Whole blood was stimulated with heat-killed *Candida albicans* in the presence of IL-18 binding protein (IL-18BP; 125 ng/mL) and/or IL-1 receptor antagonist (IL-1Ra; 10 μg/mL). IFN-γ was measured 48 h later. B, Differential role of endogenous IL-1α and IL-1β, investigated by comparing the effects of IL-1Ra (10 μg/mL) and anti−IL-1β monoclonal antibodies (aIL-1β; 1.25 μg/mL) on IFN-γ production induced by *C. albicans*. Data are mean ± SEM (n = 9 [A] or 5 [B] healthy volunteer blood donors), expressed as percentage of baseline *C. albicans*−stimulated IFN-γ production (3543 ± 729 pg/mL). *P < .05 vs. *C. albicans* production; †P < .05 vs. *C. albicans* + IL-18BP production, Mann-Whitney U test.](image)

![Figure 4. Effect of endogenous interleukin (IL)−18, IL-12, and tumor necrosis factor (TNF) on interferon (IFN)−γ production. Whole blood was stimulated with heat-killed *Candida albicans* in the presence of IL-18 binding protein (IL-18BP; 125 ng/mL) and/or anti−IL-12 antibodies (aIL-12; 2.5 mg/mL) (A) or in the presence of TNF-binding proteins (TNFbp; 10 mg/mL) (B). IFN-γ level was measured 48 h later. Data are mean ± SEM (n = 9 healthy volunteer blood donors), expressed as percentage of baseline *C. albicans*−stimulated IFN-γ production (3543 ± 729 pg/mL). *P < .05 vs. *C. albicans* production; †P < .01 vs. *C. albicans* + IL-18BP production, Mann-Whitney U test.](image)
Figure 5. Maximal inhibition of interferon (IFN)-γ production. Whole blood was stimulated with heat-killed Candida albicans in the presence of interleukin-18 binding protein (IL-18BP; 500 ng/mL), IL-1 receptor antagonist (IL-1Ra; 10 mg/mL), anti–IL-12 (aIL-12; 5 mg/mL), or a combination of all 3 cytokine inhibitors (Max inh). IFN-γ was measured 48 h later. Data are mean ± SEM (n = 4 healthy volunteer blood donors), expressed as percentage of baseline C. albicans–stimulated IFN-γ production. *P < .05 vs. C. albicans production; *P < .01 vs. stimulation by C. albicans, together with a single cytokine inhibitor; *P < .05 vs. unstimulated cells, Mann-Whitney U test.

Stimulation of whole blood with C. albicans resulted in the production of IL-12, and blockade of endogenous IL-12 with MAbs led to decreased production of IFN-γ. This demonstrates an important role of endogenous IL-12 during the induction of IFN-γ by Candida. The synergy of IL-12 and IL-18 for IFN-γ production is partly due to up-regulation by IL-12 of the α and β chains of the IL-18 receptor [39]. Others have shown that induction of CD40 expression and CD40/CD40L interactions are central for the stimulation of IL-12 by C. albicans [38].

An important observation is that the blocking action of endogenous IL-1 by either IL-1Ra or anti–IL-1β antibodies also reduced the stimulation of IFN-γ by C. albicans. IL-1 is a proinflammatory cytokine that has costimulatory effects on IFN-γ production [26, 27, 40]. The equal capacity of IL-1Ra and anti–IL-1β antibodies to inhibit IFN-γ suggests that IL-1β and not IL-1α plays a role in the induction of Candida-stimulated IFN-γ. Although IL-1β by itself cannot stimulate production of IFN-γ, recent data indicate that addition of IL-1β to IL-12 potentiates the induction of IFN-γ [26, 27, 40]. The only data suggesting an IFN-γ–stimulating effect of IL-1α were obtained in murine cells [28], whereas IL-1β potentiates IFN-γ production by human cells [26].

Our data are supported by those of Hunter et al. [25], who reported inhibition of IFN-γ by anti–IL-1β antibodies when stimulation was induced by bacterial stimuli such as LPS, heat-killed Toxoplasma gondii, Salmonella typhimurium, Legionella pneumophila, and Yersinia pseudotuberculosis. In contrast, others were unable to find an effect of anti–IL-1β antibodies when IFN-γ production was induced by Listeria monocytogenes [41] or heat-killed Staphylococcus epidermidis (R. J. L. Stuyt, unpublished data). It is, therefore, likely that IL-1β plays an important costimulatory role for IFN-γ induction by some, although not all, microbial stimuli. In this respect, IL-1β acts similarly to IL-18, which may be explained by the similarities in the structure of their receptor complexes, which consist of a binding chain (IL-1R type 1 and IL-18Rα) and a signaling chain (IL-1R accessory protein and IL-18Rβ, respectively) [42]. Both signaling chains contain the TLR/IL-1 receptor domain, which stimulates similar intracellular pathways [43, 44].

Figure 6. Effect of cytokine inhibitors on tumor necrosis factor (TNF) production. Whole blood was stimulated with heat-killed Candida albicans in the presence of interleukin-18 binding protein (IL-18BP; 125 ng/mL), IL-1 receptor antagonist (IL-1Ra; 10 mg/mL), or anti–IL-12 (aIL-12; 5 mg/mL). TNF level was measured 24 h later. Data are mean ± SEM (n = 9 healthy volunteer blood donors), expressed as percentage of baseline C. albicans–stimulated TNF production (2532 ± 611 pg/mL). *P < .05, Mann-Whitney U test.

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Despite the important roles played by endogenous IL-18, IL-12, and IL-1β for the induction of IFN-γ after C. albicans stimulation, some stimulation of IFN-γ synthesis by C. albicans (independent of intermediary endogenous cytokines) is still present after simultaneous blockade with IL-18BP, IL-1Ra, and anti–IL-12 antibodies. Several mechanisms may explain this phenomenon.

First, TNF can act as a stimulus of IFN-γ production [45], and TNFbp partially inhibited Candida induction of IFN-γ. However, this inhibition was less effective than that provided by blockade of IL-18, IL-12, and IL-1β. Because TNF induces IL-1β, and IL-1β, but not TNF, can reconstitute IL-12–induced IFN-γ production in the presence of IL-10 [27], it has been suggested that, in addition to its direct effect, TNF may stimulate IFN-γ synthesis through intermediary induction of endogenous IL-1β [27]. Second, other cytokines (e.g., IL-15 or perhaps the IL-12–like cytokine IL-23) could play a mediator role, since both have costimulatory effects on IFN-γ synthesis [46, 47]. The synergy of IL-15 with IL-12 for the induction of IFN-γ has been proposed to be due to increased expression of either IL-12Rβ1 or CD40

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by IL-15 [48]. Therefore, it is possible that IL-15 plays a role in the induction of IFN-γ by C. albicans. Third, an additional mechanism circumventing the need for macrophage-derived costimulatory cytokines for IFN-γ stimulation is the possibility that C. albicans may directly stimulate lymphocytes and NK cells to synthesize IFN-γ via a non-major histocompatibility complex (MHC)–dependent pathway [4]. A candidate C. albicans receptor on lymphocytes is CD11β/CD18 [49].

In addition to the role of endogenous cytokines in IFN-γ stimulation, we investigated the regulation of TNF and IL-8 during stimulation of whole blood with C. albicans, since these cytokines are important in the recruitment of neutrophils and monocytes, as well as in phagocytosis and killing of Candida during infection [7, 14]. In contrast to IFN-γ, induction of IL-8 was found to be independent of intermediary production of endogenous IL-18, IL-12, IL-1, and TNF. A marginal inhibition of Candida-induced TNF was exerted by IL-1Ra, which is in accordance with the stimulatory effects of IL-1 on TNF production [50]. TNF synthesis induced by Candida was independent of endogenous IL-18 and IL-12.

The use of whole-blood cultures to study C. albicans–induced cytokine production has important advantages, because the culture conditions most closely recreate the in vivo situation. In this system, cell populations that are important for the defense against Candida organisms (e.g., neutrophils, monocytes, and lymphocytes, along with complement, antibodies, and other serum factors) can interact with each other and with the fungus. It has been proposed that initial phagocytosis of C. albicans by blood monocytes and neutrophils induces synthesis of proinflammatory cytokines such as IL-18, IL-12, and IL-1β, which, in turn, are able to stimulate IFN-γ synthesis from T lymphocytes. Additional signals may be given by TNF, which can stimulate IL-1β synthesis, and by IL-15 and IL-23, which act as an additional IFN-γ costimuli [46–48].

The theoretical and practical consequences of our findings may be important, since IFN-γ is a cytokine important for defense against invasive candidiasis. IFN-γ induces reactive oxygen intermediates [51], increases the candidicidal properties of granulocytes [7], and potentiates the extracellular killing of candidal hyphae [52]. In addition, IFN-γ has a role in macrophage activation by induction of reactive nitrogen intermediates [53], enhancement of expression of MHC class II molecules [54] and Fc receptors [55], and enhancement of cytotoxicity [56]. Mice lacking IFN-γ or IFN-γR are more susceptible to both gastrointestinal and systemic candidiasis [9–12].

Recent studies demonstrate the central role played by proinflammatory cytokines such as TNF, IL-1β, and IL-18 for the pathogenesis of autoimmune diseases such as rheumatoid arthritis [57, 58] or Crohn’s disease [59, 60]. Large clinical trials show that blocking TNF and/or IL-1 is an effective therapeutic strategy in these diseases [61], and experimental data suggest beneficial effects of IL-18 blockade [62, 63]. However, experimental data suggest that TNF, IL-18, and IL-1β are important for the defense against both bacterial [64, 65] and fungal [66] infections, including invasive candidiasis [67], and disseminated candidiasis has emerged as a rare, yet severe, complication of anti-TNF therapy [68]. Therefore, the possibility of the occurrence of fungal infections should be considered during anticytokine therapy.

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