Interleukin-15 Activates Proinflammatory and Antimicrobial Functions in Polymorphonuclear Cells

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Interleukin-15 (IL-15) is a recently discovered cytokine produced by a wide range of different cell types including fibroblasts, keratinocytes, endothelial cells, and macrophages in response to lipopolysaccharide or microbial infection. This suggests that IL-15 may play a crucial role in the activation of phagocytic cells against pathogens. We studied polymorphonuclear leukocyte (PMN) activation by IL-15, evaluated as enhancement of PMN anti-Candida activity as well as IL-8 production, following stimulation with the cytokine. The PMN response to IL-15 depends on binding to the IL-15 receptor. Our experiments show that binding of a biotinylated human IL-15–immunoglobulin G2b IgG2b fusion protein was competed by the addition of human recombinant IL-15 (rIL-15) or of human rIL-2, suggesting that IL-15 binding to PMN might involve the IL-2β and IL-2γ chains, which have been shown to be constitutively expressed by PMN. In addition, we show by reverse transcription-PCR and by flow cytometry with a specific anti-IL-15Rα chain monoclonal antibody that PMN express the IL-15Rα chain at the mRNA and protein levels. Incubation with IL-15 activated PMN to secrete the chemotactic factor IL-8, and the amount secreted was increased by costimulation with heat-inactivated Candida albicans. In addition, IL-15 primed the metabolic burst of PMN in response to formyl-methionyl-leucyl-phenylalanine but was not sufficient to trigger the respiratory burst or to increase the production of superoxide in PMN exposed to C. albicans. IL-15 also increased the ability of PMN to phagocytose heat-killed C. albicans organisms in a dose-dependent manner, without opsonization by antibodies or complement-derived products. In the same concentration range, IL-15 was as effective as gamma interferon (IFN-γ) and IL-2 in increasing the C. albicans growth-inhibitory activity of PMN. Taken together, these results suggest that IL-15 is a potent stimulant of both proinflammatory and antifungal activities of PMN, activating several antimicrobial functions of PMN involved in the cellular response against C. albicans.

Interleukin-15 (IL-15) is a recently discovered cytokine that shares many biological activities with IL-2 and requires both β and γ chains of the IL-2 receptor (IL-2R) for binding and signaling (1, 22). However, the IL-15R complex includes a specific α subunit (IL-15Rα), distinct from the IL-2Rα chain (11, 13). IL-15 stimulates the growth of activated T, B, and NK cells and tumor-infiltrating lymphocytes (2, 23, 24), acts as a chemoattractant for T lymphocytes (40), induces lymphokine-activated killer activity in NK cells, and induces the generation of cytolytic effector cells (6, 11). There is increasing evidence that IL-15 can also affect phagocytic cells. We have recently shown that IL-15 acts as a proinflammatory cytokine that induces monocytes to secrete IL-8 and monocyte chemotactic protein 1 (4), while other investigators have shown that it induces morphological changes and delays apoptosis in polymorphonuclear leukocytes (PMN) (20). One major difference between IL-2 and IL-15 is their cellular source. Whereas IL-2 is produced principally by T cells, IL-15 mRNA is present in macrophages and many nonlymphoid tissues including placenta, skeletal muscle, and epithelial and fibroblast cell lines (23). IL-15 expression is induced in macrophages by microbial activators such as lipopolysaccharide, mycobacteria, or Toxoplasma gondii (17, 30). These observations suggest that IL-15 may play a role in the activation of the immune response to infection. PMN form the first line of defense in the inflammatory response against invading pathogens. Neutropenia or PMN dysfunctions result in severe infections, including systemic candidiasis (33). Inflammatory reactions result in the production of cytokines such as tumor necrosis factor alpha, granulocyte colony-stimulating factor, IL-2, gamma interferon (IFN-γ), and IL-8, which further attract and activate incoming PMN (15, 16, 32). The main function of activated PMN is to phagocytose and kill microbial pathogens. However, there is evidence that they can also behave as a secondary source of cytokines (IL-8, IL-12, and TNF-α) which can have important autocrine and paracrine effects (12). In this study, we investigated the expression of the α chain of the IL-15R on PMN and the effect of IL-15 on IL-8 secretion, superoxide anion release, phagocytosis, and candidacidal activity. These studies are important to clarify the role of IL-15 in the early steps of the innate immune response to invading pathogens.

MATERIALS AND METHODS

PMN isolation. Whole blood obtained from healthy donors after informed consent was diluted 1:2 with saline (0.9% NaCl), layered on Lymphoprep (Nycomed; Pharma AS, Oslo, Norway), and centrifuged at 400 g for 30 min at room temperature. The PMN layer, on the surface of the erythrocyte cell pellet, was collected, and contaminating erythrocytes were lysed by hypotonic shock in

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sterile distilled water for 30 s at room temperature. The cells were washed twice in phosphate-buffered saline (PBS) before being adjusted to the desired concentration. All cell suspensions contained less than 1% monocytes as determined by monocyteesterase staining. Cell viability was greater than 95% by trypan blue exclusion in 0.4% trypan blue solution and after the addition of 1% heat-inactivated fetal calf serum to RPMI 1640 medium containing 10% heat-inactivated fetal calf serum with 2 mM l-glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 5 mM HEPES buffer (GIBCO Laboratories, Grand Island, N.Y.); this is referred to as complete medium. Therefore, unless otherwise indicated, all the experiments were performed with few or no opsonins present.

Cytokines and reagents. Reconstituted human IL-15 was kindly provided by Tony Troutt (Immunex Corp., Seattle, Wash.). To obtain human IL-15-mouse immunoglobulin G2b (IgG2b) fusion protein, cDNA encoding for IL-15 was fused to genomic DNA encoding for the Fc portion of mouse IgG2b. Biotinylation of human IL-15-mouse IgG2b fusion protein resulted in higher stability of the cytokine without reduction of its biological activity as determined by the CTTL proliferation assay (10). Highly purified human IL-2 was kindly provided by Cetus Corp. (Emeryville, Calif.). Recombinant human IL-8 and IFN-γ were from Peprotech (Rocky Hill, N.J.). fMLP (formyl-methionyl-leucyl-phenylalanine) was from Sigma Chemical Co. (St. Louis, Mo.). All reagents and media were shown to be free of endotoxin by using a standard Limulus amebocyte lysate LAL assay (BioWhittaker, Walkersville, Md.).

Flow cytometry analysis. PMN were preincubated for 30 min at 4°C in PBS containing 2% goat serum plus 0.2% sodium azide, washed twice with 1% bovine serum albumin in PBS, and incubated (2 x 10² cells in 50 μl of PBS) for 60 min at 4°C with the biotinylated IL-15-IgG2b fusion protein (1.3 μg per sample) or with isotope-matched biotin-conjugated IgG (Pharimingen, San Diego, Calif.) with or without the addition of unlabelled IL-15 or IL-2. The cells were then incubated with 1% BSA and further incubated with fluorescein isothiocyanate (FITC)-conjugated avidin (Sigma) for 20 min at 4°C. IL-15 binding was assessed by flow cytometry. To determine the expression of the α chains of the IL-15R and IL-2R, PMN were indirectly labeled for 20 min at 4°C with anti-IL-15R (clone M160; a kind gift of Tony Troutt, Immunex Corp., Seattle, Wash.) or anti-CD25 (Pharimingen) monoclonal antibodies (MAB) followed by washing and incubation with FITC-conjugated goat anti-mouse Ig. R 1-30 (anti-μ, microglobulin) (26) was used as a positive control. Labelled PMN were analyzed by flow cytometric analysis with a FACScan (Becton Dickinson, Immunocytometry System, San Jose, Calif.).

RT-PCR analysis. RNA extraction and reverse transcription-PCR (RT-PCR) analysis were performed as previously described (9). Briefly, total RNA was purified with TRIzol (GIBCO/BRL) as specified by the manufacturer. cDNA synthesis was performed with 2 μg of RNA in a total volume of 20 μl containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 0.1 M dithiothreitol, 40 U of Superscript II reverse transcriptase (Life Technologies). The reaction mixture was incubated at 90°C for 5 min. A 2-μl aliquot of the cDNA obtained was amplified in a 50-μl reaction mixture containing 500 mM KCl, 100 mM Tris-HCl (pH 8.8), 25 mM KCl, 3 mM MgCl₂, 2 μM each deoxynucleoside triphosphate, 2 mg of BSA (Pharmacia) per ml, 200 nM each primer, and 5 U of Taq DNA polymerase (Life Technologies). The reaction mixture was incubated at 42°C for 50 min, and the reaction was stopped by heating at 90°C for 5 min. A 2-μl aliquot of the cDNA obtained was amplified in a 50-μl reaction mixture containing 500 mM KCl, 100 mM Tris-HCl (pH 8.8), 25 mM KCl, 3 mM MgCl₂, 2 μM each deoxynucleoside triphosphate, 2 mg of BSA (Pharmacia) per ml, 200 nM each primer, and 5 U of Taq DNA polymerase (Life Technologies). The mixture was cycled with 50 μl of sterile mineral oil. To ensure that equivalent amounts of cDNA were used in each reaction, PCR was also performed with 10-fold-diluted cDNA sample and the product was adjusted to the same cycle levels. The following oligonucleotides were used in the PCR: IL-15R sense (5′-GGACGGGCGACCCCTTCCACAGTAA-3′) and IL-15R antisense (5′-GCCA GCGGGCGCTTTTGTGGCG-3′), with cycling conditions of 1 min at 94°C, 1 min at 62°C, and 1 min at 72°C for 35 cycles. α-Actin sense (5′-CAGTAGT TTGCTGATGAC-3′) and β-actin antisense (5′-GAAGATG TTTGCTGATGAC-3′), with cycling conditions of 1 min at 94°C, 90 s at 62°C, and 2 min at 72°C for 27 cycles. A sample (15 μl) of each PCR mixture was electrophoresed through a 2% agarose gel and visualized with ethidium bromide.

Culture of C. albicans. Candida albicans CA2 was kindly supplied by A. Cassone (Istituto Superiore di Sanita, Rome, Italy) and was grown by weekly transfer in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum and 200 μg of streptomycin per ml, 200 U of Superscript II reverse transcriptase (Life Technologies.) The cells were washed twice in sterile distilled water for 30 s at room temperature. The cells were resuspended in complete medium at 2 x 10⁶ cells/ml. To ensure that equivalent amounts of cDNA were used in each reaction, PCR was also performed with 10-fold-diluted cDNA sample and the product was adjusted to the same cycle levels. A cutoff level was settled on the basis of the intrinsic fluorescence of resting PMN. Heat-killed C. albicans was fluorescein labeled as previously described (31). Briefly, yeast cells were resuspended in carbonate buffer (pH 10) containing 1 mg of FITC per ml and incubated at room temperature for 2 h. FITC-labeled Candida cells were separated from free fluorescein by extensive washing with PBS. PMN (10⁶ cells/ml) were incubated (10) with FITC-labeled Candida cells for 30 min at 37°C, washed twice, and kept at 4°C in PBS until examined by flow cytometry (FACSscan). When necessary, C. albicans cells opsonized by incubation in 50% AB human serum at 37°C for 30 min and washed with PBS. Cells kept at 4°C with FITC-labeled C. albicans were used as a negative control because they do not phagocytose the organisms, as confirmed in parallel examinations by fluorescence microscopy. PMN were gated on their light-scattering properties; at least 10,000 events were acquired. To differentiate unengulfed or cell-adherent organisms, ethidium bromide was added at 50 μg/ml. In fact, ingested particles of FITC-C. albicans maintained their green fluorescence whereas particles bound to the cell surface but not internalized were quenched by addition of the red fluorochrome (18). The extent of phagocytosis was assessed as the increase of the mean channel fluorescence of total PMN. An aliquot of each sample was cytocentrifuged and stained with Giemsa for a visual check of phagocytosis under a light microscope.

Results

IL-15 binding to PMN. To assess IL-15 binding, PMN were incubated with biotinylated human IL-15-IgG2b fusion protein, alone or in the presence of increasing concentrations of human recombinant IL-15 (rIL-15) or human rIL-2 (Fig. 1A). PMN incubated with IL-15-IgG2b fusion protein showed a significant increase in green fluorescence compared to cells incubated with control IgG (P < 0.05). Both IL-15 and IL-2 can inhibit the binding of IL-15-IgG2b fusion protein to PMN. Addition of a two-fold excess (1 μg) of unlabeled recombinant IL-15 was approximately twice as effective as addition of an equal amount of IL-2 in blocking the fusion protein binding (24 and 12% differences, respectively, in median channel value shift to the left). These results suggest that unstimulated PMN express IL-15-binding sites and that both IL-2Rβ and IL-2Rγ may form part of the IL-15R complex. Besides β and γ chains, IL-15R contains a distinct and specific IL-15Rα chain which has been described recently (22). We studied PMN expression of the IL-15Rα subunit at both RNA and protein levels (Fig. 1B and C). By RT-PCR, IL-15Rα mRNA was detected in unstimulated PMN. By FACS analysis with a specific anti-IL- 15Rα chain MAB, the IL-15Rα chain was expressed at a detectable level by ~20% of unstimulated PMN.

Effects of IL-15 on IL-8 production by PMN. PMN activation in response to IL-15 was evaluated by measuring IL-8 production by PMN. As shown in Fig. 2, a basal level of IL-8 was detectable in supernatants of PMN cultured in complete
The addition of IL-15 at concentrations as low as 100 ng/ml led to increased IL-8 release after 6 h of stimulation, and greater amounts were detectable after 24 h. We then studied the effects of \textit{C. albicans} on IL-8 release following PMN activation by IL-15. Heat-inactivated \textit{C. albicans} alone was as potent as 1,000 ng of IL-15 per ml. PMN treated with IL-15 and heat-killed \textit{C. albicans} released significantly greater amounts of IL-8. These results indicate that IL-15 synergizes with inactivated \textit{C. albicans} in inducing IL-8 release by PMN.

**Effects of IL-15 on superoxide anion generation by PMN.**
Cytokines stimulate PMN by inducing the secretion of proinflammatory cytokines and by activating their antimicrobial oxidative pathway (3, 33). To determine whether IL-15 stimulates superoxide anion production by PMN, we used a fluorescent probe (DHR-123) that increases its green fluorescence when exposed to reactive oxygen (35). PMN were preincubated with DHR-123 and then stimulated with IL-15 (1,000 ng/ml), IL-2 (1,000 U/ml), fMLP (10 nM), or medium alone. After 30 min, the fluorescence intensity of resting and stimulated PMN was assessed by FACS. As shown in Fig. 3, the percentages (mean ± standard error [SE]) of positive PMN from four independent donors increased from 3% ± 2% to 89% ± 4% upon fMLP stimulation \((P < 0.05)\), while neither IL-15 nor IL-2 induced superoxide anion production. These results were confirmed by the cytochrome \(c\) reduction method (data not shown). In addition, to examine if IL-15 synergized with either fMLP or \textit{C. albicans} in superoxide anion release, PMN were incubated for 3 h with IL-15 (1,000 ng/ml), IFN-\(\gamma\) (500 U/ml), or medium alone. The cells were then loaded with DHR-123 and stimulated with heat-killed \textit{C. albicans} or 10 nM fMLP for 30 min. As shown in Fig. 4, fMLP induced superoxide anion production in PMN preincubated with medium. The percentages (mean ± SE) of positive cells from three separate donors increased from 2% ± 1% to 25% ± 7% upon fMLP stimulation \((P < 0.05)\). \textit{C. albicans} alone could also induce detectable levels of superoxide, but to a lower extent (2% ± 1% and 8% ± 4%, respectively). Priming of PMN with IL-15 significantly augmented the PMN oxidative burst in response to fMLP (50% ± 5% versus 25% ± 7%; \(P < 0.05\)) but not to \textit{C. albicans} (11% ± 3% versus 8% ± 4%). IFN-\(\gamma\), used as control, was able to prime PMN for additional induction of the oxidative metabolite with either fMLP (53% ± 11% versus 25% ± 7%; \(P < 0.05\)) or \textit{C. albicans}, although to a lower extent (19% ± 3% versus 8% ± 4%; \(P < 0.05\)).

**Enhancement of \textit{C. albicans} phagocytosis by IL-15.** We next investigated whether IL-15 stimulation of PMN increased the rate of phagocytosis of \textit{C. albicans}. PMN were preincubated with increasing concentrations of IL-15 (10 to 1,000 ng/ml), IL-2 (50 or 1,000 U/ml), or medium alone; after 30 min, heat-inactivated FITC-conjugated \textit{C. albicans} was added. As shown in Fig. 5, IL-15 induced a dose-dependent increase in phagocytosis by PMN, as indicated by the increase in the green
fluorescence mean channel from three independent experiments. As previously reported, IL-2 did not induce a significant increase in PMN phagocytosis at 50 U/ml (19) but was active at 1,000 U/ml. The percentage (mean ± SE) of PMN that phagocytosed C. albicans increased from 45% ± 3% to 66% ± 4% upon IL-15 stimulation or to 56% ± 3% upon IL-2 stimulation. These results indicate that IL-15 significantly increased the number of phagocytosing cells (P < 0.05) as well as the rate of phagocytosis. In the presence of opsonins, basal phagocytosis was high in resting cells (82% ± 4%), but it increased in IL-15-stimulated PMN (94% ± 1%) (P < 0.05); the mean channel fluorescence increased from 60 ± 6 to 180 ± 10 (P < 0.05).

Enhancement of PMN candidacidal activity by IL-15. To assess the effect of IL-15 on the antifungal activity of PMN, cells were incubated for 3 h with increasing concentrations of IL-15 (10 to 1,000 ng/ml) before the addition of C. albicans. Untreated PMN showed low levels of antifungal activity, which were increased by IL-15 treatment (Fig. 6A). PMN activation by IL-15 was dose dependent; concentrations as low as 100 ng/ml were sufficient to increase the killing of yeasts by PMN.

The effect of IL-15 on the antifungal activity of PMN was compared with the effect of other PMN stimulants such as IFN-γ and IL-2. PMN were preincubated with IFN-γ (500 U/ml), IL-2 (1,000 U/ml), or IL-15 (1,000 ng/ml), of concentrations known to induce maximal PMN activation. The PMN were then incubated with C. albicans for assessment of function. Figure 6B shows that IL-15 had similar potency to IFN-γ and IL-2.

DISCUSSION

In this paper, we demonstrate that IL-15 enhances the response of PMN to the yeast C. albicans. Our binding experiments have shown that both IL-15 and IL-2 partially inhibited the binding of IL-15–IgG2b fusion protein to PMN, suggesting...
that the receptor complexes of both of these cytokines involve the IL-2Rβ and IL-2Rγ chains, as previously reported (21). However, IL-2 and IL-15 utilize different α-chain receptor subunits (11, 22). By using specific MAbs, we showed that, unlike the IL-2Rα chain, the IL-15Rα chain is detectable on unstimulated PMN, although at a low level (15, 25). Taken together, these results can account for the fact that IL-2 competed less than IL-15 in our binding experiments. They also corroborate the hypothesis of Girard et al., which proposes that PMN express a specific IL-15Rα chain, as suggested by the different effects of the two cytokines in the induction of morphological alterations and in the delay of apoptosis in PMN (20).

PMN microbicidal action involves both oxidative and non-oxidative pathways. Reactive oxygen radicals are released when PMN interact with microbial pathogens or are stimulated via G-protein-coupled receptors (e.g., fMLP receptor) (3, 33). Cytokines such as IFN-γ or G-CSF alone do not induce the respiratory burst, but preincubation of PMN with IFN-γ or G-CSF primes cells for the induction of oxygen radicals by fMLP or by C. albicans. IL-15 was also effective in enhancing the oxidative respiratory burst in response to fMLP, although it failed to induce superoxide anions by itself and to prime PMN for Candida induction of the oxygen radicals. This suggests that either the IL-15 enhancement of PMN anti-Candida activity is mediated through a nonoxidative pathway or the priming effect is not detectable under our experimental conditions. IL-2 is equally effective in the activation of antifungal

**FIG. 4.** Priming of the respiratory burst by IL-15. PMN were preincubated at 37°C with IL-15 (1,000 ng/ml), IFN-γ (500 U/ml), or medium alone for 3 h. They were then loaded with DHR-123 and stimulated with heat-killed Candida (PMN-to-Candida ratio, 10:1), fMLP (10 nM), or medium alone. After 30 min, superoxide anion production was evaluated by flow cytometry as an increase in green fluorescence intensity. The x axis represents the intensity of green fluorescence expressed in a log scale as mean channel, and the y axis represents the number of cells per channel.
functions of PMN (15). As reported by Djeu et al. (15), IL-2-mediated anti-
Candida activity is not related to activation of the metabolic burst because IL-2 does not display any priming activity on the respiratory burst elicited by fMLP. These results indicate that IL-2 and IL-15, although sharing activating properties on PMN, might utilize distinct stimulatory pathways, probably because these two cytokines may activate common receptor components such as the β and γ chains of the IL-2R or distinct receptor subunits like the IL-15Rα chain.

Phagocytosis is considered to be a required step for the intracellular killing of the vegetative form of Candida. We have shown that IL-15 induces a dose-dependent increase of phagocytosis of C. albicans at the same concentration range at which this cytokine induces PMN candidacidal activity. This result suggests that IL-15 may stimulate the phagocytosis and the consequent intracellular killing of yeasts involving nonoxidative mechanisms such as lysosomal antimicrobial peptides and enzymes. We have indirect evidence that the activation of PMN by IL-15 may be associated with the release of antimicrobial peptides (e.g., defensins, CAP37/azurocidin, and lactoferrin) (8). In fact, we observed that PMN activated by IL-15 display an increased expression of CD11b, a marker of specific granules, suggesting that IL-15 probably induces neutrophil degranulation (29a). Another possible mechanism that might be involved in PMN activation by IL-15 is dependent on the expression of the inducible enzyme nitric oxide synthase (iNOS). Although resting PMN do not express iNOS, IL-15 may be capable of inducing its expression. However, in other cell types, the kinetics of iNOS expression are very different from those observed for the anti-Candida activity of IL-15 (28, 38, 39, 41). Besides its antimicrobial activities, IL-15 has proinflammatory properties that may enhance the extent of its anti-
Candida activity in vivo. We showed that IL-15 induces the secretion of the neutrophil chemotactic factor IL-8 within 6 h of stimulation. IL-8 is secreted mostly by monocytes and endothelial cells, but it is also expressed, in smaller amounts, by PMN (7, 37). We recently reported that IL-15 stimulates monocytes to express IL-8 at both the mRNA and protein levels in the same concentration range that is effective in neutrophil activation (4). This suggests that the production of IL-15 in the early steps of the inflammatory response to pathogens might increase the amount of PMN infiltrating the tissues. Besides being a chemotactic factor for PMN, IL-8 itself stimulates antimicrobial functions by enhancing degranulation and anti-Candida activity of PMN (5, 16). Since IL-15 induced IL-8 secretion, we tested the possibility that IL-8 mediates the enhancement of anti-Candida activity induced by IL-15; however, we could not detect any change of IL-15 activity on PMN when IL-8 neutralizing antibodies were added to the culture (data not shown). These results suggest that under our experimental conditions the antifungal activity of IL-15 did not depend on IL-8 secretion. This does not contradict the observed antifungal activity of IL-8, because the concentrations of IL-8 that we detected after 6 h of stimulation with IL-15 are not optimal for activation of PMN anti-Candida activity (16). Upon longer stimulation in vitro or in vivo, IL-8 secretion might be important for the anti-Candida effect of IL-15, but it is likely that in our experimental setting, IL-15 directly activated PMN against C. albicans. Unlike IL-2, IL-15 expression is induced in macrophages by microbial activators such as LPS, mycobacteria, or

![FIG. 5. Phagocytosis of heat-inactivated Candida by PMN stimulated with IL-15. PMN were preincubated with different concentrations of IL-15 (from 10 to 1,000 ng/ml), IL-2 (50 or 1,000 U/ml), or medium alone for 3 h. Heat-inactivated FITC-labeled Candida cells were then added to the cultures at a PMN-to-Candida ratio of 10:1, and the cultures were further incubated for 30 min at 37°C before the extent of phagocytosis was assessed by flow cytometry in the presence of ethidium bromide. The extent of phagocytosis is represented on the y axis as the mean ± SE of green fluorescence mean channel from three independent experiments. Asterisks indicate a significant increase in phagocytosis (*P < 0.05).](http://iai.asm.org/)

![FIG. 6. Enhancement of PMN anti-Candida activity by IL-15. (A) PMN were incubated with increasing concentrations of IL-15 (from 10 to 1,000 ng/ml) for 3 h, exposed to C. albicans, and assayed for anti-Candida activity as described in the text. (B) PMN were stimulated for 3 h with IFN-γ (500 U/ml), IL-2 (1,000 U/ml), IL-15 (1,000 ng/ml), or medium alone before their anti-Candida activity was assessed. Results represent mean percent growth inhibition ± SE for three independent experiments. Asterisks indicate a significant increase in the anti-Candida activity (*P < 0.05).](http://iai.asm.org/)
Toxoplasma gondii (17). We are currently investigating the mechanisms that regulate IL-15 expression by monocytes upon infection with fungi or other microbial pathogens. We found that *C. albicans* up-regulates IL-15 mRNA expression in human monocytes (29b). Taken as a whole, these results suggest that IL-15 may be expressed in the early steps of the aspecific immune response to bacteria and yeasts. IL-15 produced by activated tissue-resident macrophages or by fibroblasts may activate PMN recruited to the site of infection to kill pathogens and determine an additional infiltration of PMN through IL-8 release. In addition, IL-15, which is chemotactic for T cells, may induce T-cell infiltration and maintain their proliferative release. In addition, IL-15, which is chemotactic for T cells, that IL-15 may be expressed in the early steps of the aspecific role in human innate immunity against fungal monocytes (29b). Taken as a whole, these results suggest to IL-15; indeed, Vazquez et al. have recently reported that mechanisms that regulate IL-15 expression by monocytes upon (17). We are currently investigating the disease characterized by increased susceptibility to fungal infections in immunocompromised patients with chronic granulomatous disease (CGD), a genetically inherited disease characterized by increased susceptibility to fungal infections (14). This is dependent on the lack of superoxide anion production by phagocytic cells because of mutations of genes encoding the subunits of NADPH oxidase. In patients with CGD, IFN-γ is currently used for prophylaxis of infections. On the basis of our results, IL-15 might potentiate the antimicrobial functions of PMN by a nonoxidative pathway in CGD patients. It will be possible to test this hypothesis since animal models of CGD have recently become available (29).

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