INFECTION AND IMMUNITY, Mar. 1996, p. 945–951 Vol. 64, No. 3
0019-9567/96/$04.00+0

Effects of Interleukin-10 on Human Peripheral Blood Mononuclear Cell Responses to *Cryptococcus neoformans*, *Candida albicans*, and Lipopolysaccharide

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Received 5 October 1995/Returned for modification 28 November 1995/Accepted 20 December 1995

Deactivation of mononuclear phagocytes is critical to limit the inflammatory response but can be detrimental in the face of progressive infection. We compared the effects of the deactivating cytokine interleukin 10 (IL-10) on human peripheral blood mononuclear cell (PBMC) responses to lipopolysaccharide (LPS), *Cryptococcus neoformans*, and *Candida albicans*. IL-10 effected dose-dependent inhibition of tumor necrosis factor alpha (TNF-α) release in PBMC stimulated by LPS and *C. neoformans*, with significant inhibition seen with 0.1 U/ml and greater than 90% inhibition noted with 10 U/ml. In contrast, even at doses as high as 100 U/ml, IL-10 inhibited TNF-α release in response to *C. albicans* by only 50%. IL-10 profoundly inhibited release of IL-1β from PBMC stimulated by all three stimuli. TNF-α mRNA and release was inhibited even if IL-10 was added up to 8 h after cryptococcal stimulation. In contrast, inhibition of IL-1β mRNA was of lesser magnitude and occurred only when IL-10 was added within 2 h of cryptococcal stimulation. IL-10 inhibited translocation of NF-κB in response to LPS but not the fungal stimuli. All three stimuli induced IL-10 production in PBMC, although over 10-fold less IL-10 was released in response to *C. neoformans* compared with LPS and *C. albicans*. Thus, while IL-10 has deactivating effects on PBMC responses to all three stimuli, disparate stimulus- and response-specific patterns of deactivation are seen. Inhibition by IL-10 of proinflammatory cytokine release appears to occur at the level of gene transcription for TNF-α and both transcriptionally and posttranscriptionally for IL-1β.

The opportunistic fungi *Cryptococcus neoformans* and *Candida albicans* have a marked propensity to cause infections in persons with impaired cell-mediated immunity (CMI), especially those with AIDS. In particular, cryptococcosis has emerged as the most frequent life-threatening mycotic infections in persons with AIDS, afflicting at least 5 to 10% of AIDS patients in the United States and an even higher percentage in Africa and other parts of the developing world (5, 7, 24). Mucocutaneous candidiasis affects the majority of persons with AIDS at some point during the course of their illness and, while rarely life threatening, can cause considerable morbidity (39). Despite advances in therapy, cryptococcosis and candidiasis can be difficult infections to successfully treat, especially in the severely immunosuppressed. While clinical and experimental data convincingly demonstrate that CMI is crucial in host defenses against these mycoses, the specific mechanisms by which an intact CMI response results in protection are only incompletely defined.

A central mechanism by which the CMI response is thought to protect the host against mycoses is through the production of proinflammatory cytokines (e.g., tumor necrosis factor alpha [TNF-α] and interleukin-1β [IL-1β]) which recruit and activate leukocytes to inhibit and kill invading fungi. TNF-α and IL-1β promote inflammation, through the induction of cell adhesion molecules, neutrophil and macrophage chemotactic factors, acute-phase proteins, and the generation of other proinflammatory cytokines (12, 45). Recent reports from our laboratory and elsewhere have documented that *C. neoformans* and *C. albicans* are potent stimulators of TNF-α release from mononuclear phagocytes (30, 31, 35). Administration of neutralizing antibody against TNF-α was deleterious in murine models of cryptococcosis and candidiasis (6, 31, 43).

When produced in appropriate quantities, proinflammatory cytokines play a beneficial role as mediators of host resistance to infectious agents. However, overproduction can lead to local and systemic toxicity. Moreover, TNF-α and IL-1 induce human immunodeficiency virus (HIV) replication in latently infected cells, and it has been postulated that release of these cytokines in vivo could accelerate HIV progression (19, 37). Thus, strategies designed to decrease the release of proinflammatory cytokines offer the promise of improving outcome by reducing the morbidity associated with the inflammatory response and, in persons with AIDS, diminishing viral replication. One such strategy being studied is the use of IL-10 (23).

IL-10 is produced by $CD4^+$ T helper type 2 (Th2) cells, $CD8^+$ T cells, monocytes, macrophages, and B cells. IL-10 deactivates macrophages directly by influencing macrophage recruitment, viability, morphology, phagocytosis, expression of cytokine receptors and major histocompatibility complex molecules, antigen presentation, production of monokines, generation of reactive oxygen and nitrogen intermediates, and killing of microbes and tumor cells (2, 20). IL-10 can also act indirectly by suppressing T-cell proliferation and/or gamma interferon production. In this study, we examined the effect of IL-10 on selected human peripheral blood mononuclear cell (PBMC) responses to *C. neoformans* and *C. albicans.*

MATERIALS AND METHODS

Materials. All reagents were obtained from Sigma Chemical Co. (St. Louis, Mo.) unless stated otherwise. All experiments were performed under conditions carefully designed to minimize endotoxin contamination as described previously

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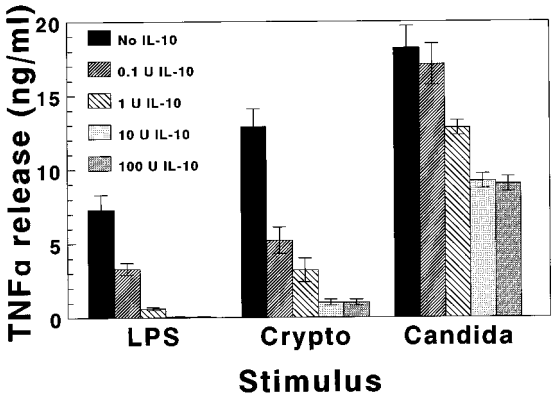

FIG. 1. Effect of IL-10 on TNF-α release from stimulated PBMC. PBMC (2.5×10^5) were stimulated with 100 ng of LPS per ml, *C. neoformans* (Crypto; 2.5×10^6), or *C. albicans* (Candida; 5×10^5) in the absence or presence of the indicated concentrations of IL-10. After 18 h, supernatants were collected and assayed for TNF-α by ELISA. Data are the means ± standard errors of three experiments, each of which was performed in triplicate. Mean TNF-α release from unstimulated PBMC was below the limits of detection of the assay (<0.1 ng/ml). For each stimulus, $P < 0.001$ comparing no IL-10 with any concentration of IL-10 except for *C. albicans* and 0.1 U of IL-10.

(30). RPMI 1640 and phosphate-buffered saline (PBS) were obtained from Biowhittaker, Inc. (Walkersville, Md.) and contained less than 0.005 endotoxin unit per ml. Lipopolysaccharide (LPS) from *Escherichia coli* O111:B4 and the LPS antagonist *Rhodobacter sphaeroides* lipid A (RSLA; a gift of Nilo Qureshi, Middleton VA Hospital, Madison, Wis.) were prepared in pyrogen-free PBS (Biowhittaker) as described previously (16, 30, 40). Complement-sufficient pooled human serum containing less than 100 pg of endotoxin per ml was purchased from Biowhittaker. Unless otherwise noted, all incubations were performed with RPMI 1640 containing 5 to 10% pooled human serum at 37°C in humidified air supplemented with 5% CO_2.

Antibodies, cytokines, and plasmids. Recombinant IL-10 was purchased from Biosource International (Camarillo, Calif.). Bioactivity of IL-10 was 5×10^5 U/mg as determined by proliferation of MC-9 cells costimulated with murine IL-4. Monoclonal antibody against human TNF-α was the gift of Miles Inc. (West Haven, Conn.). Polyclonal anti-TNF-α was purchased from Genzyme (Boston, Mass.). Monoclonal antibody against human IL-1β was a gift of Cistron Biotechnology, Inc. (Pine Brook, N.J.). Polyclonal anti-IL-1β was the gift of Jack Schmidt (Merck Research Laboratories, Rahway, N.J.). The full-length cDNA plasmid for TNF-α was the gift of Leo Lina (Cetus Corp., Emeryville, Calif.) and was prepared as a 0.6-kb *Hin*dIII-*Kpn* fragment for Northern (RNA) blot hybridization of extracted RNA. The full-length cDNA plasmid for IL-1β was the gift of Michael Tocci (Merck Research Laboratories) and prepared as a 1.5-kb *Eco*RI-*Pst*I fragment.

Fungi. *C. neoformans* serotype A strain 145 (27, 30, 36) was grown in RPMI 1640 (without bicarbonate) (pH 6.0) at 37°C. Under such conditions, capsule thickness averaged 1.2 μm, as measured with a light microscope equipped with a calibrated ocular micrometer following negative staining with India ink (30). A well-described isolate of *C. albicans* (28, 44) was grown in the yeast phase on Sabouraud dextrose agar at 25°C. Fungi were harvested after 4 days of growth, washed at least five times in PBS, heat killed at 50°C for 30 min, and stored at 4°C. Overgrowth of cultures during the 18-h incubations precluded use of live fungi.

Isolation of leukocyte populations. Leukocyte populations were purified as in previous studies by standard techniques (26, 30). Peripheral human blood was obtained from healthy volunteers by venipuncture. For each set of experiments, no donor was used more than once. Blood was anticoagulated with 10 U of pyrogen-free heparin (Elkins-Sinn, Inc., Cherry Hill, N.J.) per ml and centrifuged at $500 \times g$ for 15 min, and the leukocyte-rich buffy coat was harvested. PBMC were collected following centrifugation of the buffy coat over a gradient of Ficoll-Hypaque. In some experiments, PBMC were depleted of T cells and some natural killer cells on the basis of their capacity to rosette neuraminidase-treated sheep erythrocytes. T cells and some natural killer cells express CD2 and rosette sheep erythrocytes, whereas other cell types, including monocytes, do not (26).

Cytokine release. Leukocytes were mixed with fungi in 96-well polystyrene plates (Costar Corporation, Cambridge, Mass.). Unstimulated leukocytes and LPS served as negative and positive controls, respectively. At defined intervals, supernatants were removed and stored at −70°C until analysis for cytokine release. TNF-α and IL-1β were measured by sandwich enzyme-linked immunosorbent assay (ELISA), using the antibodies listed above, as described in full elsewhere (30, 32). The ELISAs were sensitive over a range of 100 to 3,000 pg of TNF-α and IL-1β per ml of supernatant. IL-10 was measured by ELISA sensitive to 10 pg of IL-10 per ml, using antibody pairs (PharMingen, San Diego, Calif.) according to the manufacturer's protocol.

Isolation and quantitation of mRNA. Total cellular RNA was extracted from 5×10^6 to 10×10^6 cells per sample by using an RNA isolation kit (Tri-reagent; Medical Research Center, Cincinnati, Ohio) and analyzed by Northern blotting as described previously (30). Hybridized probe was visualized by autoradiography and quantitated by densitometry (Molecular Dynamics Personal Densitometer equipped with ImageQuant software; Molecular Dynamics, Sunnyvale, Calif.).

mRNA stability. PBMC (5×10^6) were stimulated with 100 ng of LPS per ml or *C. neoformans* (5×10^7) in six-well plates. At 3 h poststimulation, half the wells received 10 U of IL-10 per ml. At 4 h poststimulation, 5 μg of actinomycin D per ml was added to shut down new gene expression. Total RNA was harvested 0, 0.5, 1, 2, and 3 h after addition of actinomycin D, and 10 μg of each sample was subjected to Northern blot analysis for TNF-α message. Autoradiographic signals were quantified by densitometry as described above.

Electrophoretic mobility shift assay. Nuclear translocation of the transcription factor NF-κB was assayed by electrophoretic mobility shift assay exactly as described previously (8, 18). Briefly, nuclear extracts from stimulated PBMC were prepared in the presence of the protease inhibitors, and protein concentration was determined by using a commercial kit (Bio-Rad Laboratories, Hercules, Calif.). A synthetic oligonucleotide containing the NF-κB binding sequence was end labeled with [α-^{32}P]dATP and [α-^{32}P]dCTP, using the Klenow fragment. Unincorporated nucleotides were removed with a G-25 spin column (Pharmacia Biotech, Piscataway, N.J.). Labeled probe (0.2 ng) and nuclear extract (4 μg) were incubated at room temperature for 30 min in 1× band shift buffer (10 mM Tris-HCl [pH 7.8], 1 mM EDTA, 40 mM KCl, 1 mM dithiothreitol) containing 50 μg of poly(dI-dC) per ml and 5% glycerol. Reactions were then size fractionated by electrophoresis in 4% native polyacrylamide gels, transferred to 3MM filter paper (Whatman Laboratory Products, Clifton, N.J.), dried, and visualized by autoradiography. Samples were quantitated by densitometry as described above. A 25-molar excess of unlabeled probe was used as a specific competitor.

Statistics and presentation of data. Means and standard errors were compared by the two-tailed, two-sample *t* test on a statistical software program (SigmaStat for Windows; Jandel Scientific Software, San Rafael, Calif.). For experiments in which multiple comparisons were made, adjustments for significance were made with the Bonferroni correction.

RESULTS

Effect of IL-10 on TNF-α release. In preliminary experiments, it was determined that maximal TNF-α release occurred at a 10:1 ratio of *C. neoformans* to PBMC (30) and a 2:1 ratio of *C. albicans* to PBMC (data not shown). Next, the effect of IL-10 on TNF-α release from PBMC stimulated by LPS, *C. neoformans*, and *C. albicans* was studied. IL-10 effected dose-dependent inhibition of TNF-α release in PBMC stimulated by LPS and *C. neoformans*, with significant inhibition seen with 0.1 U of IL-10 per ml and greater than 90% inhibition noted with 10 U of IL-10 per ml (Fig. 1). In contrast, even at doses as high as 100 U/ml, IL-10 inhibited TNF-α release in response to *C. albicans* by only 50%. Consistent with previously published experiments (30), fractionation of PBMC based on their capacity to rosette sheep erythrocytes revealed that TNF-α release was associated almost exclusively with the monocyte-enriched fraction. Moreover, inhibition of TNF-α release by IL-10 was similar to that for monocyte-enriched PBMC (data not shown).

As previously demonstrated for *C. neoformans* (30), TNF-α release in response to *C. albicans* was not secondary to endotoxin contamination, as demonstrated by the inability of 1 μg of the LPS antagonist RSLA per ml to inhibit TNF-α release from PBMC (18.3 ± 1.5 and 17.9 ± 1.8 ng of TNF-α per ml [mean ± standard error of three triplicate experiments] with and without RSLA, respectively). In contrast, in concurrent experiments, RSLA inhibited LPS-stimulated TNF-α release from PBMC by 91%.

Effect of IL-10 on fungal binding. One possible explanation for the inhibitory effects of IL-10 on TNF release was an effect on binding of the fungi to PBMC. To test this possibility, PBMC were incubated with *C. neoformans* and *C. albicans* in the presence or absence of 10 U of IL-10 per ml, and binding

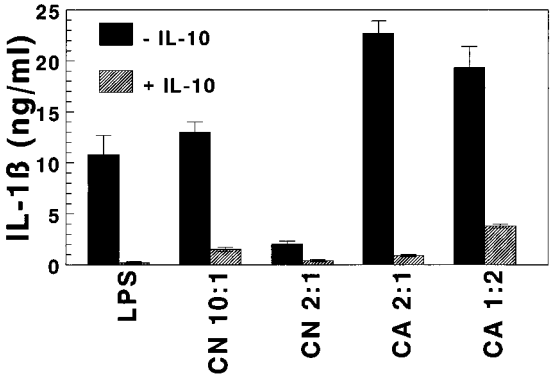

FIG. 2. Effect of IL-10 on IL-1β release from stimulated PBMC. PBMC (2.5×10^5) were stimulated with 100 ng of LPS per ml, or with *C. neoformans* (CN) or *C. albicans* (CA) at the indicated fungus-to-PBMC ratio in the absence (−) or presence (+) of 10 U of IL-10 per ml. After 18 h, supernatants were collected and assayed for IL-1β by ELISA. Data are the means ± standard errors of three experiments, each of which was performed in triplicate. Mean IL-1β release from unstimulated PBMC was below 0.4 ng/ml. $P < 0.001$ comparing samples without and with IL-10 for each stimulus.

was assessed. For both fungi, IL-10 had no significant effect on binding. (Binding indices at a *C. neoformans*-to-PBMC ratio of 2:1 were 59.0 ± 3.2 and 60.7 ± 3.9 with and without IL-10, respectively. Binding indices at a *C. albicans*-to-PBMC ratio of 1:2 were 58.9 ± 8.0 and 66.2 ± 12.8 with and without IL-10, respectively.) Similar results were found at other effector/target cell ratios (data not shown).

Release of IL-1β. We next examined the effect of IL-10 on IL-1β release from PBMC stimulated with *C. neoformans* and *C. albicans* (Fig. 2). Both fungi stimulated IL-1β release, although *C. albicans* was the more potent inducer. Levels of IL-1β were greater than those seen with the LPS positive control even at a 1:2 ratio of *C. albicans* to PBMC. In contrast, IL-1β release in response to a 10:1 ratio of *C. neoformans* to PBMC was similar to that seen with LPS stimulation but decreased by 84% when the ratio was decreased to 2:1. Addition of 10 U of IL-10 per ml profoundly inhibited release of IL-1β from PBMC stimulated by LPS and both fungi.

Time course of effects of IL-10 on TNF-α release. The next set of experiments sought to examine whether IL-10 could still inhibit TNF-α release even if added after the PBMC had already been stimulated. PBMC were stimulated for 18 h with LPS, *C. neoformans*, or *C. albicans*. IL-10 was added to the cell wells at 0, 1, 2, 4, or 8 h into the 18-h incubation, following which TNF-α was measured in the supernatants (Fig. 3A). In parallel wells, supernatants were removed at 0, 1, 2, 4, or 8 h and assayed for TNF-α in order to determine the amount of TNF-α released prior to the addition of the IL-10 (Fig. 3B). Consistent with previous results (30), TNF-α release in response to *C. neoformans* had delayed kinetics compared with that seen with LPS. The kinetics in response to *C. albicans* was similar to that seen with LPS. TNF-α release in response to *C. neoformans* was inhibited by more than 95% even if IL-10 was added up to 2 h after fungal stimulation. Moreover, 50% inhibition was seen when IL-10 was added 8 h into the incubation. Considering that at 8 h TNF-α release in response to *C. neoformans* was 34% of that seen at 18 h, it is apparent that the vast majority of new TNF-α release is inhibited by IL-10 regardless of the time of addition of IL-10. As in Fig. 1, IL-10 also inhibited TNF-α release in response to *C. albicans*, although to a lesser extent than in response to *C. neoformans* and LPS. In agreement with data from Wang et al. (46), potent inhibition was seen even if IL-10 was added up to 2 h after LPS stimulation.

Effect of IL-10 on TNF-α and IL-1β gene expression and stability. Having established that IL-10 inhibits TNF-α and IL-1β release, we next examined whether inhibition occurs at the level of gene expression (Fig. 4). PBMC were stimulated with *C. neoformans* for 1, 2, 4, 8, and 16 h. During the stimulation, IL-10 was omitted or added at 0, 1, 2, 4, or 8 h. RNA was extracted, and Northern analysis was performed. Consistent with previous data (30), in the absence of IL-10, *C. neoformans* stimulated peak TNF-α message at 4 to 8 h. In contrast, IL-1β message appeared slightly earlier, with significant message seen by 2 h and peak message seen at 4 h. Addition of IL-10 at the start of the incubation inhibited peak TNF-α and IL-1β message by 83 and 57%, respectively, as measured by scanning densitometry. The effect of IL-10 on TNF-α message

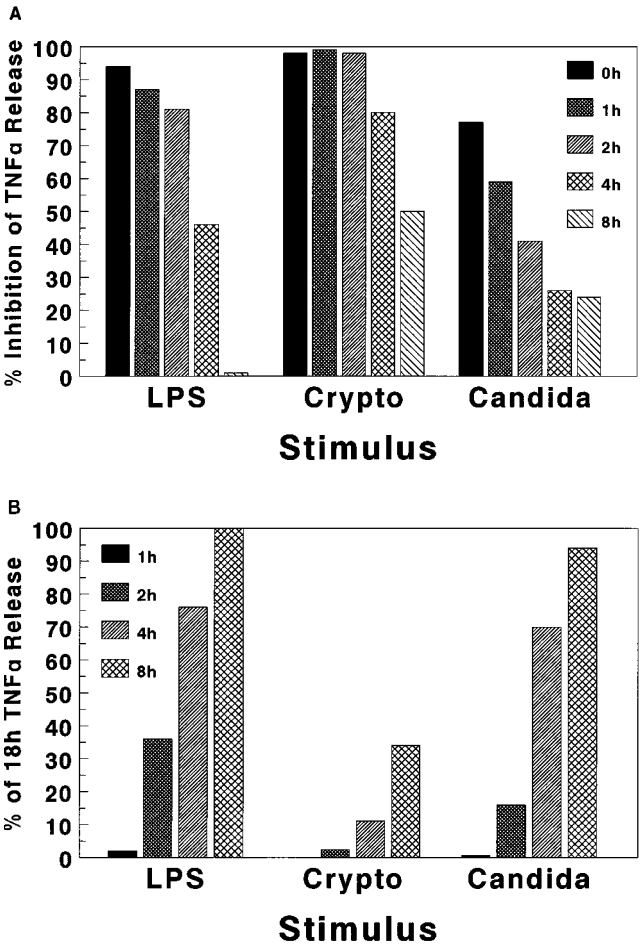

FIG. 3. Effect of time of addition of IL-10 on inhibition of TNF-α release from stimulated PBMC. PBMC (2.5×10^5) were stimulated with 100 ng of LPS per ml, *C. neoformans* (Crypto; 5×10^5), or *C. albicans* (Candida; 5×10^5). (A) At 0, 1, 2, 4, or 8 h into the incubation, 10 U of IL-10 per ml was added. After 18 h, supernatants were collected and assayed for TNF-α by ELISA. Data are expressed as percent inhibition of TNF-α release compared with PBMC stimulated in the absence of IL-10. IL-10 significantly ($P < 0.001$) inhibited TNF-α release in response to all stimuli at all time points for except for LPS at 8 h. (B) IL-10 was omitted. At 0, 1, 2, 4, or 8 h into the incubation, supernatants were collected and assayed for TNF-α by ELISA. Data are expressed as percentage of TNF-α release seen at 18 h. Mean values for TNF-α release when PBMC were incubated for 18 h in the absence of IL-10 with no stimulus, LPS, *C. neoformans*, and *C. albicans* were 0.01, 1, 1.58, 3.75, and 11.38 ng/ml, respectively. Results are from two to five experiments, each performed in triplicate.

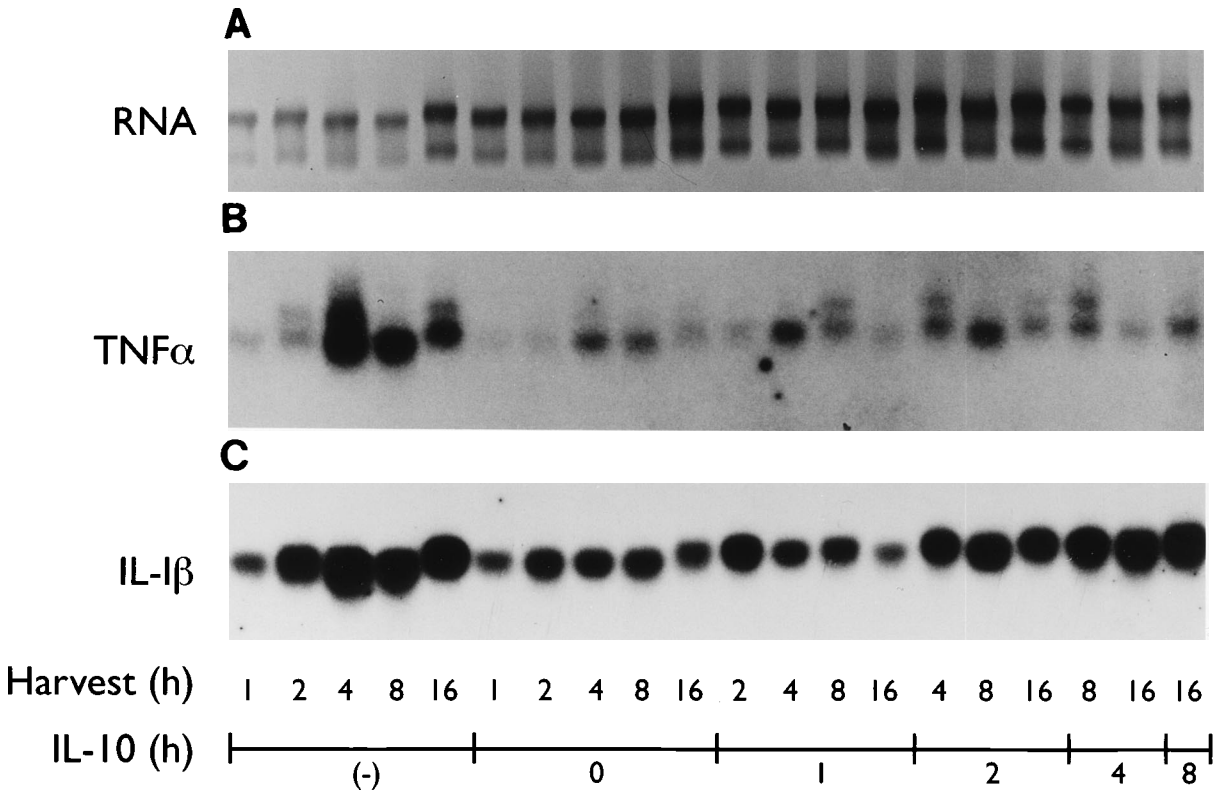

FIG. 4. Effect of time of administration of IL-10 on TNF-α and IL-1β gene expression. PBMC (5×10^6) were stimulated with *C. neoformans* (10^7) at time zero, and cells were harvested at 1, 2, 4, 8, or 16 h. IL-10 at 10 U/ml was omitted (−) or added at 0, 1, 2, 4, or 8 h. (A) Ethidium bromide-stained RNA gel; (B) autoradiogram of Northern blot probed for TNF-α mRNA; (C) autoradiogram of the same Northern blot probed for IL-1β mRNA. Not shown on the gels, TNF-α and IL-1β messages in unstimulated PBMC were 57 and 65%, respectively, of the messages seen with PBMC stimulated for 1 h with *C. neoformans* in the absence of IL-10, as measured by scanning densitometry. Results are representative of two experiments.

continued to be seen even when IL-10 was added up to 8 h after cryptococcal stimulation. In contrast, inhibition of IL-1β message occurred only when IL-10 was added within 2 h of cryptococcal stimulation, and the magnitude of the inhibition was not as great as that seen with TNF-α message. For *C. albicans*-stimulated PBMC, 10 U of IL-10 per ml inhibited peak mRNA for TNF-α and IL-1β by 37 and 17%, respectively.

The experiments described above demonstrate that IL-10 profoundly reduces the amount of TNF-α message in PBMC stimulated by *C. neoformans*. We next sought to determine if the reduced message was due to decreased gene transcription or decreased message stability. PBMC were incubated with *C. neoformans* or LPS in the presence and absence of IL-10. RNA synthesis was stopped by the addition of actinomycin D, and the rate of loss of TNF-α mRNA was evaluated by Northern analysis. In three independent experiments, the half-life of TNF-α mRNA induced by *C. neoformans* and LPS was between 30 min and 1 h regardless of whether IL-10 was present. Thus, the reduced level of TNF-α mRNA seen following IL-10 treatment is not due to decreased TNF-α mRNA stability.

NF-κB. Nuclear translocation of the transcription factor NF-κB is thought to help initiate TNF-α and IL-1β gene transcription (22). The next set of experiments sought to determine if the mechanism of IL-10 inhibition of TNF-α and IL-1β gene expression and release was via inhibition of NF-κB translocation (Fig. 5). PBMC were incubated with or without IL-10 in the presence of no stimulus, LPS, *C. neoformans*, or *C. albicans*. After 1 and 4 h, the reaction was terminated and nuclear extracts were analyzed for the presence of translocated NF-κB. Consistent with previously reported results (11, 13, 47), NF-κB was found in unstimulated PBMC. Stimulation of PBMC with LPS, *C. neoformans*, and *C. albicans* resulted in increases in the amount of translocated NF-κB at both 1 and 4 h. Addition of 10 U of IL-10 per ml had no significant effect on NF-κB translocation in response to the two fungi at either time point. However, IL-10 did inhibit LPS-stimulated NF-κB activation at 1 h by 53% (mean of four experiments). Depletion of monocytes from PBMC by sequential adherence to plastic and nylon wool totally abolished NF-κB translocation in response to the three stimuli (data not shown).

IL-10 release. The experiments described above demonstrated the profound effects of IL-10 on PBMC responses to LPS and fungi. The final set of experiments examined IL-10 production during stimulation of PBMC with LPS, *C. neoformans*, and *C. albicans*. While all three stimuli induced IL-10 release from PBMC, the amount of cytokine released was more than 10-fold less when *C. neoformans* was the stimulus than when LPS and *C. albicans* were the stimuli (Fig. 6). Moreover, IL-10 release in response to *C. neoformans* required a high ratio of *C. neoformans* to PBMC (10:1); at ratios of 2:1 or lower, detectable IL-10 release was not observed (data not shown). In contrast, at a *C. albicans*-to-PBMC ratio of 1:2, release of IL-10 approached that seen when 100 ng of LPS per ml was used as the stimulus.

DISCUSSION

The studies presented herein demonstrate the potent ability of IL-10 to inhibit human PBMC responses to the opportunis-

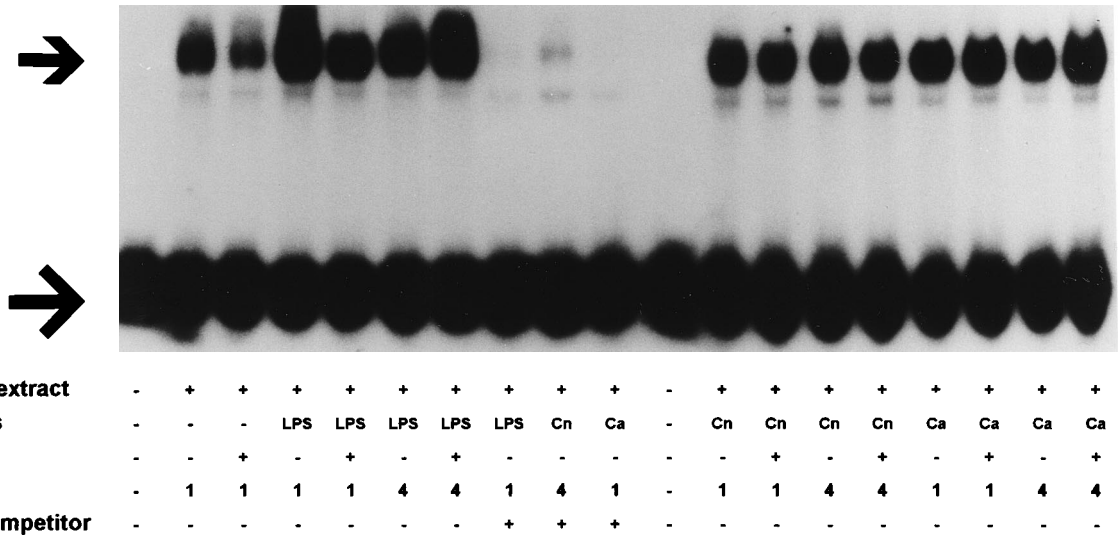

FIG. 5. Effect of IL-10 on NF-κB nuclear translocation in stimulated PBMC. PBMC (5×10^6) were left unstimulated or stimulated with 100 ng of LPS per ml, *C. neoformans* (Cn; 5×10^7), or *C. albicans* (Ca, 10^7). After 1 and 4 h of incubation, nuclear extracts were prepared and analyzed by electrophoretic mobility shift assay as described in Materials and Methods. A 25-molar excess of unlabeled probe served as a specific (Spec.) competitor. The top arrow points to NF-κB, while the bottom arrow points to free probe. The gel shown is representative of three experiments, all of which yielded similar results.

tic fungal pathogens *C. neoformans* and *C. albicans*. IL-10 inhibited release of the proinflammatory cytokines TNF-α and IL-1β from PBMC stimulated by the two fungi, although for TNF-α release, the inhibitory effect of IL-10 was less pronounced when *C. albicans* was the stimulus. Our data begin to elucidate the intracellular mechanisms by which IL-10 inhibit TNF-α and IL-1β release in PBMC stimulated by *C. neoformans* and *C. albicans*. By Northern blot analysis, inhibition of TNF-α occurred at or before the transcriptional level. Similar findings have been reported for LPS-stimulated monocytes (10, 21, 46). In contrast, while IL-10 inhibited IL-1β release in *C. neoformans*-stimulated PBMC by nearly 90%, it inhibited IL-1β mRNA by only 57%. Thus, IL-10 appears to cause inhibition of IL-1β at both the translational and posttranslational levels. Moreover, IL-10 profoundly inhibited TNF-α and (to a lesser extent) IL-1β gene expression in *C. neoformans*-stimulated PBMC even if added after binding had already occurred. IL-10 had no significant effects on TNF-α mRNA stability.

Uncoupling of the transcription factor NF-κB from its cytoplasmic inhibitor IκB is thought to help initiate TNF-α and IL-1β gene transcription, allowing translocation of NF-κB to the nucleus, where it binds to promoter regions on the genes (22). A variety of stimuli, including LPS and, in an autocrine fashion, TNF-α, have been reported to activate NF-κB. In this study, we found that nuclear translocation of NF-κB occurred in response to *C. neoformans* and *C. albicans* but that IL-10 had insignificant effects on NF-κB translocation stimulated by the two fungi. In contrast, in agreement with recent data from Wang et al. (47), IL-10 inhibited NF-κB translocation in response to LPS. While approximately 50% inhibition was seen 1 h after stimulation, an effect of IL-10 on LPS-stimulated NF-κB activation was no longer observed at 4 h. Taken together, our data strongly suggest that IL-10 inhibits release of fungus-stimulated TNF-α at the transcriptional level but largely independently of NF-κB. However, inhibition of LPS-stimulated TNF-α release by IL-10 may be due, at least in part, to inhibition of NF-κB translocation.

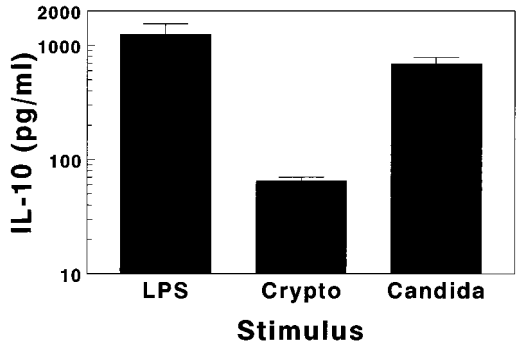

FIG. 6. IL-10 release. PBMC (2.5×10^5) were left unstimulated or stimulated with 100 ng of LPS per ml, *C. neoformans* (Crypto; 2.5×10^6), or *C. albicans* (Candida; 5×10^5). After 18 h, supernatants were collected and assayed for IL-10 by ELISA. IL-10 release from unstimulated PBMC was below the limits of detection of the assay (<10 pg/ml).

The finding that *C. neoformans* and *C. albicans* activate NF-κB in PBMC has potential relevance for persons with AIDS. In addition to its importance as a sentinel event heralding the activation of proinflammatory cytokines, NF-κB activates HIV replication in latently infected cells by binding to NF-κB consensus sequences from the HIV long terminal repeat (38). Thus, the occurrence of cryptococcosis or candidiasis during the course of HIV infection theoretically could accelerate the progression of AIDS by activating viral replication via NF-κB. We have recently found that induction of NF-κB also occurs in human alveolar macrophages stimulated by *C. neoformans* (21a).

Disparate results were noted when PBMC responses to LPS, *C. neoformans*, and *C. albicans* were compared. First, TNF-α and IL-1β release occurred at a lower fungus-to-PBMC ratio with *C. albicans* than with *C. neoformans*. Second, IL-10 was a considerably more potent inhibitor of PBMC TNF-α release when *C. neoformans* and LPS were the stimuli than when *C. albicans* was the stimulus. Third, TNF-α gene expression and release occurred later in response to *C. neoformans* than in response to LPS and *C. albicans*. Finally, LPS and *C. albicans*

were considerably more potent stimulators of IL-10 release than was *C. neoformans.*

The reasons for these disparities are speculative but could relate to different pathways of activation seen in PBMC following exposure to the different stimuli. For example, in the presence of pooled human serum, monocytes are thought to respond to LPS via CD14, to *C. neoformans* via complement receptors, and to *C. albicans* via complement, immunoglobulin, mannose, and β-glucan receptors (3, 8, 25, 29, 33, 48). Regardless of the mechanism, the disparate responses of PBMC to the three stimuli may help explain why the in vivo inflammatory response to each stimulus follows a unique pattern. Moreover, these results underscore the need for careful study of individual stimuli, as extrapolations cannot necessarily be made from one stimulus to another.

Few studies examining the role of IL-10 in cryptococcosis and candidiasis have been published. In a murine model of cryptococcosis, Huffnagle et al. found that CD4 deficiency resulted in loss of lung T cells producing Th2 cytokines (IL-4, IL-5, and IL-10), although residual $CD8^+$ cells were still able to produce Th1 cytokines (IL-2 and gamma interferon) (17). Ausiello et al. were unable to detect IL-10 gene expression in human PBMC stimulated with a mannoprotein fraction of *C. albicans* (1). However, Mencacci et al. found that $CD4^+$ splenocytes from mice immunized with whole *C. albicans* produced significantly more gamma interferon in response to the mannoprotein fraction when neutralizing anti-IL-10 antibodies were added to the cultures (35). Moreover, IL-10 was found in the sera of mice 3 days following an intravenous challenge with *C. albicans* but was undetectable in sera from uninfected controls (41). Suppression of the Th2 response to *C. albicans* by administration of staphylococcal enterotoxin B was associated with reduced amounts of IL-4 and IL-10 and was protective in a murine model of systemic candidiasis (41).

IL-10 has been reported to inhibit murine macrophage microbicidal activity against a variety of pathogens, including *C. albicans* and *Schistosoma mansoni* (4, 14). The mechanism is via inhibition of the effector molecule nitric oxide. However, these results are of questionable relevance for human mononuclear phagocytes, which do not appear to generate sufficient nitric oxide for antimicrobial activity (9, 42). Consistent with these observations, we have been unable to demonstrate inhibitory or stimulatory effects of IL-10 on the antimicrobial activity of human mononuclear phagocytes against *C. neoformans* (25a).

The results reported herein support the experimental use of IL-10 to inhibit release of TNF-α and IL-1β in patients with cryptococcosis and candidiasis. Our findings demonstrating the ability of IL-10 to shut down cytokine gene expression and release even after fungal challenge has already taken place is an added benefit, especially considering that TNF-α release is delayed following stimulation with fungi compared with endotoxin (30, 34). Enthusiasm for the use of IL-10 in systemic fungal diseases must be tempered by the findings that in animal models of cryptococcosis and candidiasis, TNF-α appears to be beneficial whereas IL-10 is deleterious (4, 6, 31, 41, 43). However, in the animal models, antifungal agents were not used. Thus, one potential approach would be to administer IL-10 to control the inflammatory response and antifungal agents to control the fungus. A similar approach has been proposed for septic shock (15).

ACKNOWLEDGMENTS

This work was supported by grants AI25780, AI28408, and GM47127 from the National Institutes of Health. D.T.G. is the recipient of an American Cancer Society Junior Faculty Award.

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Editor: D. H. Howard