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# Expression of Cytokines and Inducible Nitric Oxide Synthase mRNA in the Lungs of Mice Infected with *Cryptococcus neoformans*: Effects of Interleukin-12

KAZUYOSHI KAWAKAMI,* MASAKI TOHYAMA, XIE QIFENG, AND ATSUSHI SAITO

*First Department of Internal Medicine, Faculty of Medicine, University of the Ryukyus, 903-01 Okinawa, Japan*

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**We have recently established a murine model of pulmonary and disseminated infection with a highly virulent strain of *Cryptococcus neoformans* and demonstrated that administration of interleukin-12 (IL-12) protected the animals against infection. In this study, we extended these studies by investigating the host defense mechanisms. In particular, we examined the expression of mRNA for helper T-cell 1 (Th1) cytokines (IL-2, lymphotoxin, and gamma interferon [IFN-γ]), Th2 cytokines (IL-4, -6, and -10), macrophage-derived cytokines (tumor necrosis factor alpha [TNF-α], IL-1β, transforming growth factor β [TGF-β], IL-12p40, and IFN-γ-inducing factor [IGIF]), and inducible nitric oxide synthase (iNOS) in the lungs on days 1, 3, 7, and 14 after infection and following treatment with IL-12. There was little or no expression of mRNAs for Th1 cytokines, TNF-α, IL-12p40, IGIF, and iNOS in the infected mice, but expression increased markedly after treatment with IL-12. In contrast, the mRNAs for Th2 cytokines, IL-1β, and TGF-β were detected at considerable levels during the early stages of infection, and, interestingly, expression was not suppressed by IL-12 but rather augmented, particularly during the late stage. Similar results were also obtained for IFN-γ, IL-4, IL-10, and TNF-α measured in the lung homogenates by enzyme-linked immunosorbent assay. These results suggest that the predominance of expression of Th2 cytokines and TGF-β over Th1 cytokines, TNF-α, IL-12p40, IGIF, and iNOS is associated with severe lethal infection in mice and that administration of IL-12 protects infected animals by stimulating Th1 cytokines.**

The helper T-cell (Th)-derived cytokines are categorized into two groups. The first comprises Th1 cytokines, including interleukin-2 (IL-2), gamma interferon (IFN-γ), and lymphotoxin (LT). The second group comprises Th2 cytokines, including IL-4, -5, -6, -9, -10, and -13 (31). These groups are known to inhibit the biological activities of each other by producing IFN-γ and IL-4 (31). The Th1 cytokines are strongly involved in the enhancement of cell-mediated immunity (31) and play a central role in the host defense mechanisms against various pathogenic microorganisms (40). IL-12 is produced by macrophages and B cells and stimulates natural killer (NK) cells and T cells to produce IFN-γ (46). More importantly, IL-12 plays a critical role in the development of Th1 cells from naive T cells (17). Recently, another IFN-γ-inducing cytokine, termed the IFN-γ-inducing factor (IGIF), with biological activities similar to IL-12 has been identified and is proposed to be called IL-18 (34, 48). On the other hand, the Th2 cytokines inhibit the production and biological activities of Th1 cytokines (31), thus attenuating host defense mechanisms against pathogenic organisms, as previously shown by increased susceptibility of mice to leishmaniasis by immunological manipulations that enhance the dominance of Th2 cells over Th1 cells (2, 25). Thus, the commitment of specific Th cells to differentiation into Th1 or Th2 cells may determine the host susceptibility to particular pathogenic microorganisms.

*Cryptococcus neoformans*, a ubiquitous fungal microorganism, has attracted attention because it causes a life-threatening infection in patients with impaired cell-mediated immunity, such as AIDS (44). In infections caused by this pathogen, cellular immunity, mediated mostly by $CD4^+$ T cells and macrophages, forms a central role in host defense (16, 18, 29). Recent studies from our group as well as other laboratories have demonstrated that IFN-γ is important in protecting mice against this infection through induction of macrophage anticryptococcal activity (22, 37). In mice, such activity is mediated to a large extent by L-arginine-dependent killing mechanisms (1, 45).

Recently, we established a murine model of pulmonary and disseminated cryptococcosis and demonstrated that IL-12 protected mice from lethal infection with *C. neoformans* by inducing production of IFN-γ (23). However, the mechanism for the IL-12 effect is not fully understood. In this study, we examined the kinetics involved in generation of Th1- and Th2-associated cytokines, macrophage-derived cytokines, and inducible nitric oxide synthase (iNOS) in an effort to characterize host defense mechanisms against cryptococcal infection. We also examined the effects of IL-12 treatment on the synthesis of these cytokines and iNOS.

## MATERIALS AND METHODS

**Animals.** Female (BALB/c × DBA/2)$F_1$ mice were purchased from SLC Japan (Hamamatsu, Japan) and used at the age of 7 to 10 weeks. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of our university. The anticryptococcal activity of macrophages derived from this strain of mice is not detected in the absence of stimulation with IFN-γ plus lipopolysaccharide, while it is markedly induced after stimulation (3). All mice were housed in a pathogen-free environment and received sterilized food and water at the Laboratory Animal Center for Biomedical Science in University of the Ryukyus.

***C. neoformans.*** A serotype A encapsulated strain of *C. neoformans*, YC-11, was obtained from a patient with pulmonary cryptococcosis. The strain showed thick capsule (7.6 ± 2.3 μm; $n = 59$) when examined shortly after harvesting from the infected lungs (not shown). The yeast cells were cultured on potato dextrose agar plates for 3 to 4 days before use.

**Intratracheal instillation of microorganisms.** Mice were anesthetized by an intraperitoneal injection of 70 mg of pentobarbital (Abbott Laboratories, North

* Corresponding author. Mailing address: First Department of Internal Medicine, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-01, Japan. Phone: 81(98)895-3331, ext. 2438. Fax: 81(98)895-3086.

Chicago, Ill.) per kg of body weight and restrained on a small board. Live *C. neoformans* cells ($10^5$) were inoculated in a volume of 50 μl per mouse by inserting a blunted 25-gauge needle into and parallel to the trachea.

**IL-12.** Recombinant murine IL-12 (specific activity, $2.5 \times 10^8$ Roche units/mg of protein) was kindly provided by Hoffmann-La Roche Inc. (Nutley, N.J.). IL-12 was intraperitonealy administered at a dose of 0.1 μg per mouse daily for 7 days from the day of infection.

**Extraction of RNA and reverse transcription-PCR (RT-PCR).** Total RNA was extracted from the lungs of mice at various time points after instillation of *C. neoformans* by the acid guanidinium thiocyanate-phenol-chloroform method as described by Chomczynski and Sacchi (6). For this purpose, 30 to 70 μg of RNA was obtained from one set of lungs and resuspended in 50 μl of diethylpyrocarbonate-treated distilled water.

Subsequently, reverse transcription was carried out by mixing 5 μg of sample RNA solution (15 μl) with 2 μl of hexadeoxyribonucleotide mixture (GIBCO BRL, Life Technologies, Tokyo, Japan). This solution was incubated for 2 min at 95°C and quickly cooled on ice. In the next step, 12 μl of a solution containing 6 μl of 5× reverse transcriptase buffer (250 mM Tris-HCl [pH 8.3], 375 mM KCl, 15 mM $MgCl_2$ [GIBCO BRL]), 0.5 μl of 200 RNase inhibitor (200 U/ml; GIBCO BRL), 3 μl of 100 mM dithiothreitol, and 2.5 μl of 10 mM deoxynucleoside triphosphate was added, and the tubes were incubated for 2 min at 37°C. We then added 1.0 μl of Moloney murine leukemia virus reverse transcriptase (200,000 U/ml; GIBCO BRL) and incubated the sample for 60 min at 37°C. After receiving 45 μl of 0.7 M NaOH and 40 mM EDTA, the tubes were incubated for 10 min at 65°C and quickly cooled on ice. The resultant cDNA was precipitated with 75% ethanol overnight at −70°C. The precipitates were washed once with 75% ethanol, dried, and resuspended in 50 μl of diethylpyrocarbonate-treated distilled water. The samples were stored at −20°C until use. This reaction was always performed simultaneously for parallel samples from one experiment.

PCR was carried out in an automatic DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.). For amplification of the desired cDNA, gene-specific primers (Table 1) were used. We added 1.0 μl of the sample cDNA solution to 49 μl of the reaction mixture, which contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 10 μg of gelatin per ml, deoxynucleoside triphosphates (each at a concentration of 200 μM), 1.0 μM sense and antisense primer, and 1.25 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus). The preparations in the microtubes were amplified by using a three-temperature PCR system usually consisting of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and extension at 72°C for 1.5 min. PCR conditions of denaturation at 94°C for 1 min, primer annealing at 60°C for 2 min, and extension at 72°C for 3 min were used for transforming growth factor β (TGF-β). The number of cycles was determined for samples not reaching the amplification plateau (32 cycles for hypoxanthine phosphoribosyltransferase [HPRT] and TGF-β; and 35 cycles for the others), as shown in Table 1. The PCR products were electrophoresed on 2% agarose gels, stained with 0.5 μg of ethidium bromide per ml, and observed with a UV transilluminator.

**Measurement of cytokines in lung homogenates.** Mice were sacrificed at days 7 and 14 of infection, and the lungs were excised and then homogenized in 2.0 ml of normal saline with Polytron (Kinematica AG, Littau, Switzerland) at 12,000 rpm for 60 s on ice. The homogenates were centrifuged at $1,600 \times g$ for 10 min, and the supernatants were passed through 0.22-μm-pore-size Millipore filters and assayed for concentrations of IFN-γ, tumor necrosis factor alpha (TNF-α), IL-4, and IL-10, using enzyme-linked immunosorbent assay (ELISA) kits purchased from Genzyme Co. (Cambridge, Mass.) for IFN-γ and from Endogen, Inc. (Cambridge, Mass.), for the others. The detection limits of the assay were 125 pg/ml for IFN-γ, 50 pg/ml for TNF-α, 5 pg/ml for IL-4, and 1.5 U/ml for IL-10.

## RESULTS

**Expression of cytokine and iNOS mRNA in the lungs of mice infected with *C. neoformans*.** In our recent study (23), we established a murine model of pulmonary and disseminated infection with a highly virulent strain of *C. neoformans*. In this model, the fungal cells were directly instilled into the lungs, representing the initial infection site. The microorganisms multiplied rapidly in the lungs, disseminated to the brains within 3 weeks, and induced 100% mortality within 3 to 6 weeks of infection. To examine the kinetics for production of cytokines and iNOS in the lungs after infection with *C. neoformans*, we examined the mRNA expression for Th1 cytokines (including IL-2, LT, and IFN-γ), Th2 cytokines (including IL-4, IL-6, and IL-10), macrophage-derived cytokines (including TNF-α, IL-1β, TGF-β, IL-12p40, and IGIF), and iNOS, which catalyzes the generation of nitric oxide (NO) from L-arginine (15) and mediates the cryptococcocidal activity of IFN-γ-activated macrophages (1, 45). Examination of the lungs was performed on days 1, 3, 7, and 14 after intratracheal instillation of the fungus. As shown in Fig. 1A, there was a marginal expression of IFN-γ mRNA only on day 7 or no expression of IL-2 and LT mRNAs at any time interval, while IL-6 and IL-10 mRNAs were detected on days 1 to 14 and IL-4 mRNA was detected on days 1, 7, and 14 after infection. Among the macrophage-derived cytokines, there was little or no expression of TNF-α, IL-12p40, and IGIF mRNAs, similar to Th1 cytokines, while the TGF-β mRNA was detected on day 1 and then diminished with time (Fig. 1B). The IL-1β mRNA was expressed in the lungs of infected phosphate-buffered saline (PBS)-treated mice on days 1, 3, and 7 (Fig. 1B). On the other hand, there was little or no iNOS mRNA expression in the lungs (Fig. 1C). In mice instilled intratracheally with normal saline (uninfected mice), mRNAs of all cytokines noted above and iNOS were not generated at any time interval, while the HPRT mRNA was detected at a constant level (data not shown).

**Modification of cytokine and iNOS mRNA expression by IL-12.** Recently, we demonstrated that administration of IL-12 promoted the clearance of *C. neoformans* from the lungs and prevented dissemination of the fungal organism into the brain, resulting in reduced mortality (23). To elucidate the mechanism of the effect of IL-12, we investigated whether IL-12 treatment influences the synthesis of cytokines and iNOS in infected lungs. As shown in Fig. 2A, treatment caused an increase in the expression of IL-2 mRNA on days 7 and 14 and of LT and IFN-γ mRNAs through the course of infection. In contrast, IL-12 had little effect on the production of IL-4 and IL-10 during the early period of infection (days 1 and 3). Surprisingly, IL-12 treatment enhanced the production of these cytokines during the late stage of infection (days 7 and 14). The expression of IL-6 mRNA was shifted to the later time in the IL-12-treated mice compared to PBS-treated mice. IL-12 treatment has been reported recently to suppress the development of a primary Th2 response to a protein-hapten conjugate (27) and IL-4 production in mice during infection caused by *Leishimania major* (49). Among the macrophage-derived cytokines, IL-12 treatment caused an increase in the expression of TNF-α from day 3 and of IL-12p40 and IGIF mRNAs through the course of infection (Fig. 2B). Furthermore, it also increased TGF-β mRNA expression in the late period of infection (days 7 and 14), similar to that observed with Th2 cytokines. The expression of IL-1β mRNA was enhanced through the course of infection compared with infected PBS-treated mice (Fig. 2B). In addition, iNOS mRNA was considerably induced by this treatment on days 3, 7, and 14 postinfection (Fig. 2C).

**Detection of cytokines in lung homogenates by ELISA.** To confirm the results obtained by RT-PCR, we measured the concentrations of IFN-γ, TNF-α, IL-4, and IL-10 by ELISA using lung homogenates obtained from infected PBS- and IL-12-treated mice. No IFN-γ and small amounts of TNF-α were detected in lung homogenates on days 7 and 14 postinfection. In contrast, IL-12 treatment caused an increase in the production of these cytokines (Table 2). On the other hand, small but significant amounts of IL-4 and IL-10 were detected during natural infection, and the production of these Th2 cytokines was significantly augmented by IL-12 treatment (Table 2). These cytokines were not detected in the lung homogenates obtained from uninfected animals (data not shown).

## DISCUSSION

In this study, we examined the production of Th1 and Th2 cytokines in the lungs of mice throughout the course of pul-

TABLE 1. Sequences of the oligonucleotide primers used for PCR amplification of cytokine and iNOS mRNA, product size predicted, and number of cycles

| mRNA | Primer sequence[a] | Product (bp) | No. of cycles |
|---|---|---|---|
| HPRT | | | |
| Sense | 5′-GTTGGATACAGGCCAAGACTTTGTTG-3′ | 165 | 32 |
| Antisense | 5′-GATTCAACTTGCGCTCATCTTAGGC-3′ | | |
| IL-1β | | | |
| Sense | 5′-GCAACTGTTCCTGAACTCA-3′ | 382 | 35 |
| Antisense | 5′-CTCGGAGCCTGTAGTGCAG-3′ | | |
| IL-2 | | | |
| Sense | 5′-AACAGCGCACCCACTTCAA-3′ | 442 | 35 |
| Antisense | 5′-TTGAGATGATGCTTTGACA-3′ | | |
| IL-4 | | | |
| Sense | 5′-TAGTTGTCATCCTGCTCTT-3′ | 404 | 35 |
| Antisense | 5′-CTACGAGTAATCCATTTGC-3′ | | |
| IL-6 | | | |
| Sense | 5′-TTCCTCTCTGCAAGAGACT-3′ | 532 | 35 |
| Antisense | 5′-TGTATCTCTCTGAAGGACT-3′ | | |
| IL-10 | | | |
| Sense | 5′-TCCTTAATGCAGGACTTTAAGGGTTACTTG-3′ | 256 | 35 |
| Antisense | 5′-GACACCTTGGTCTTGGAGCTTATTAAAATC-3′ | | |
| IL-12p40 | | | |
| Sense | 5′-CAGAAGCTAACCATCTCCTGGTTTG-3′ | 394 | 35 |
| Antisense | 5′-TCCGGAGTAATTTGGTGCTTCACAC-3′ | | |
| IFN-γ | | | |
| Sense | 5′-AACGCTACACACTGCATCT-3′ | 342 | 35 |
| Antisense | 5′-TGCTCATTGTAATGCTTGG-3′ | | |
| TNF-α | | | |
| Sense | 5′-GGCAGGTCTACTTTGGAGTCATTGC-3′ | 308 | 35 |
| Antisense | 5′-ACATTCGAGGCTCCAGTGAATTCGG-3′ | | |
| LT | | | |
| Sense | 5′-TCAGAAGCACTTGACCCAT-3′ | 322 | 35 |
| Antisense | 5′-AAGTCCCGGATACACAGACT-3′ | | |
| TGF-β | | | |
| Sense | 5′-TGGACCGCAACAACGCCATCTATGAGAAAACC-3′ | 525 | 32 |
| Antisense | 5′-TGGAGCTGAAGCAATAGTTGGTATCCAGGGCT-3′ | | |
| IGIF | | | |
| Sense | 5′-ACTGTACAACCGCAGTAATACGG-3′ | 434 | 35 |
| Antisense | 5′-AGTGAACATTACAGATTTATCCC-3′ | | |
| iNOS | | | |
| Sense | 5′-CATGGCTTGCCCCTGGAAGTTTCTCTTCAAAG-3′ | 754 | 35 |
| Antisense | 5′-GCAGCATCCCCTCTGATGGTGCCATCG-3′ | | |

[a] Primer sequences were obtained from previous reports by Montgomery and Dallman (30) for IL-1β, IL-2, IL-4, IL-6, IFN-γ, and LT, by Romani et al. (35) for IL-10, by Yoshida et al. (51) for IL-12p40, by Murray et al. (32) for TNF-α, by Chang et al. (4) for TGF-β, by Okamura et al. (34) for IGIF, and by Gazzinelli et al. (11) for HPRT and iNOS.

monary and disseminated infection with *C. neoformans* and found little or no production of Th1 cytokines (IL-2, LT, and IFN-γ) but considerable formation of Th2 cytokines (IL-4, IL-6, and IL-10). Furthermore, there was little or no production of macrophage-derived cytokines (TNF-α, IL-12p40, and IGIF), while IL-1β and TGF-β mRNAs were detected in considerable amounts during infection. Our results also demonstrated a lack of iNOS mRNA synthesis in infected lungs, consistent with the impaired generation of IFN-γ and TNF-α. On the other hand, the pulmonary production of Th1 cytokines, TNF-α, IL-12p40, IGIF, and iNOS was augmented following administration of IL-12. Interestingly, similar results were obtained regarding the effect of IL-12 treatment on synthesis of IL-4, IL-10, and TGF-β.

Several studies emphasized the importance of Th1 cytokines in host defense mechanisms against infection caused by various microbial pathogens (reviewed by Scott and Kaufmann [40]). In particular, IFN-γ is considered to play a critical role in the activation of macrophages through induction of NO, a principal mediator for macrophage killing activity in mice (33). In

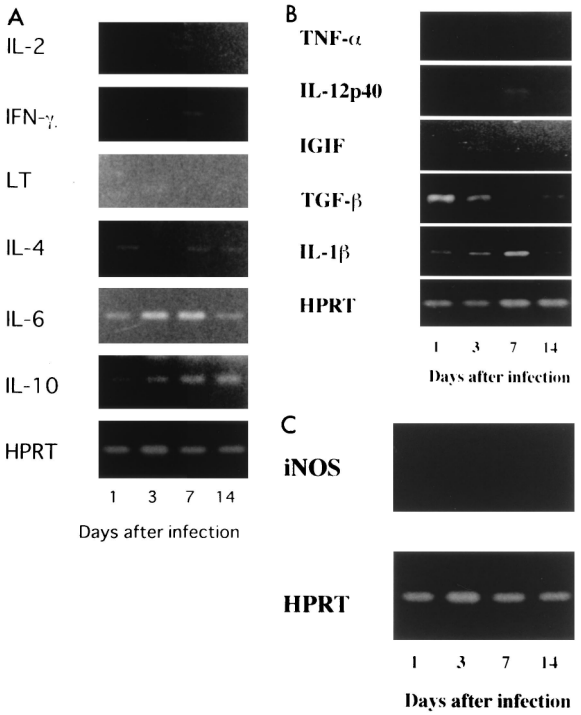


FIG. 1. Expression of cytokines and iNOS mRNA in the lungs of mice infected with *C. neoformans*. Mice received daily intraperitoneal injections of 200 μl of PBS for 7 days from the day of intratracheal instillation of $10^5$ cells of *C. neoformans*. On days 1, 3, 7, and 14 of instillation, mice were sacrificed and total RNA was extracted from their lungs. Subsequently, RT-PCR was carried out for Th1 and Th2 cytokines (A), macrophage-derived cytokines (B), and iNOS (C). HPRT was used as an internal control. The PCR products were electrophoresed on 2% agarose gels, stained with 0.5 μg of ethidium bromide per ml, and observed with a UV transilluminator. The RNA samples were obtained from three mice in each group, and the results are representative of three separate samples.

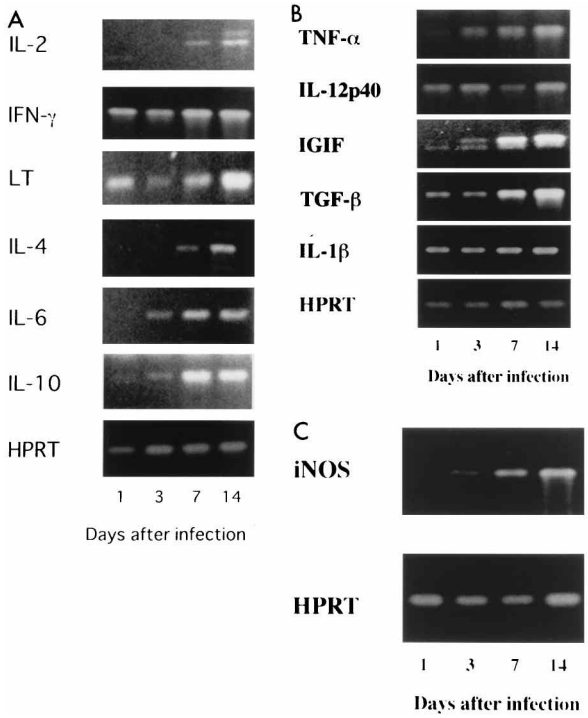


FIG. 2. Modification of cytokines and iNOS mRNA expression by IL-12. Mice received daily intraperitoneal injections of recombinant murine IL-12 (0.1 μg/mouse) for 7 days from the day of intratracheal instillation of $10^5$ cells of *C. neoformans*. On days 1, 3, 7, and 14 of instillation, mice were sacrificed and total RNA was extracted from their lungs. Subsequently, RT-PCR was carried out for Th1 and Th2 cytokines (A), macrophage-derived cytokines (B), and iNOS (C). HPRT was used as an internal control. The PCR products were electrophoresed on 2% agarose gels, stained with 0.5 μg of ethidium bromide per ml, and observed with a UV transilluminator. The RNA samples were obtained from three mice in each group, and the results are representative of three separate samples.

addition, IFN-γ enhances the antigen-presenting activity of macrophages, resulting in the expansion of Th1 cell population (21). The activated Th1 cells in turn produce large amounts of IFN-γ and effectively augment macrophage antimicrobial activity. These biological responses may establish a positive feedback circuit to promote host resistance to microbial infection. In our model of pulmonary and disseminated infection with *C. neoformans*, all mice died within 4 to 6 weeks due to the rapid multiplication of cryptococci in the lungs and its dissemination to the brain (23). Therefore, the present results indicate that insufficient production of Th1 cytokines and iNOS in the infected lungs may account for the uncontrolled infection with *C. neoformans.*

In contrast, induction of TGF-β and Th2 cytokines, including IL-4 and IL-10, became evident during the early stages of infection. Previous studies showed that constitutive production of IL-4 in transgenic mice resulted in increased susceptibility to infection with *L. major* (25) and that resistance to infection occurs when the endogenously synthesized IL-4 is neutralized in the susceptible mice (5). IL-10 is reported to inhibit Th1 cell-mediated responses mostly by downregulating expression of major histocompatibility complex class II and CD80 on antigen-presenting macrophages (8, 9). The resistance of mice to infection with various microorganisms is also impaired by administration of recombinant IL-10 (10, 42). Furthermore, administration of a neutralizing antibody enhances the resistance to microbial infection (7). Similarly, treatment with TGF-β results in failure of animals to protect themselves against infection caused by *Toxoplasma gondii*, probably due to suppression of IFN-γ production (19). These observations indicate that production of greater amounts of Th2 than of Th1 cytokines and the generation of TGF-β, as shown in the present study, may be closely correlated with the lethal pulmonary and disseminated infection with *C. neoformans* in our

TABLE 2. Cytokine levels in lung homogenates

| Cytokine | Level[a] | | | |
|---|---|---|---|---|
| | 7 days | | 14 days | |
| | PBS/infected[b] | IL-12/infected[c] | PBS/infected | IL-12/infected |
| IFN-γ | ND[d] | 526 ± 73.1 | ND | 861.3 ± 347.0 |
| TNF-α | 91.5 ± 30.8 | 1,623 ± 574.6 | 74.3 ± 19.2 | 1,306.4 ± 225.8 |
| IL-4 | 8.2 ± 1.7 | 13.8 ± 7.5 | 6.1 ± 0.5 | 15.2 ± 4.1 |
| IL-10 | 4.9 ± 1.1 | 16.2 ± 3.8 | 5.3 ± 2.4 | 31.5 ± 8.1 |

[a] The concentration of each cytokine in lung homogenate was measured by ELISA. Levels of IFN-γ, TNF-α, and IL-4 are in picograms/milliliter; levels of IL-10 are in units/milliliter. Each value is the mean ± standard deviation for three mice.

[b] Mice were infected intratracheally with *C. neoformans* but not treated with IL-12.

[c] Mice infected with *C. neoformans* received daily intraperitoneal injections of recombinant IL-12 (0.1 μg/mouse/day) for 7 days.

[d] ND, not detected.

model. To confirm this possibility, further studies with neutralizing antibodies against these cytokines will be necessary.

IL-12 enhances host defense against infection caused by microbial pathogens (12, 14, 23, 47). Studies with cultured cells have demonstrated that IL-12 induces the production of IFN-γ by NK cells, γδT cells, and conventional T cells (43, 46) and potentiates the development of Th1 cells from naive cells (46). In addition, it suppresses the generation of Th2 cytokines and their biological activities through the induction of IFN-γ (46). These observations suggest that IL-12 treatment may suppress the in vivo generation of Th2 cytokines in our experimental murine model, thus protecting these animals from death. However, the results showed that the expression of Th2 cytokine mRNA was markedly enhanced, particularly in the late stage of infection. In addition, the same treatment enhanced the expression of TGF-β mRNA, a potent immunosuppressive cytokine (36, 39, 41). The mechanism by which IL-12 augments the generation of Th2 cytokines and TGF-β in the lungs remains to be elucidated. Recently, several investigators (20, 28, 38, 50) have reported similar observations, showing that IL-12 enhanced the production of IL-4 and IL-10 by Th0 and Th2 cells.

We demonstrated in our recent study (23) that IL-12 treatment induced marked cellular inflammatory changes in the lungs of mice during the early stage of pulmonary and disseminated cryptococcosis. Our unpublished results further showed that no live *C. neoformans* was detected and the inflammatory changes subsided in the lungs 9 weeks after infection in those mice that survived the infection. These observations suggest that some mechanism operates to attenuate persistent inflammatory reactions, which may be harmful to host. In previous studies (13, 24), systemic injection of endotoxin was demonstrated to induce the fatal hyperinflammatory responses through overproduction of cytokines such as TNF-α and IFN-γ. In this endotoxemia model, administration of IL-10 protected animals from the lethal endotoxin shock, probably through suppressing the production of TNF-α and IFN-γ (26). Similarly to their findings, in our model of cryptococcal infection, persistent activation of Th1 cell-mediated responses induced by IL-12 could result in the fatal outcome due to hyperinflammatory responses. However, no mice died because of this mechanism. Considered together, these findings indicate there may be a certain negative feedback mechanism operating through Th2 cytokines and TGF-β to prevent uncontrolled Th1 responses, as suggested by Meyaard et al. (28). Further studies are necessary to examine this possibility.

IGIF induces the production of IFN-γ, proliferation of T cells, and activation of NK cells, effects that are similar to those of IL-12 (34). Interestingly, the effects of IGIF on the induction of IFN-γ production paralleled those of IL-12 (34). At present, the biological significance of this newly identified cytokine remains unclear. Combining the data in the present study with those from our previous study (23), we conclude that IGIF synthesis appears to be correlated with protection of mice from cryptococcal infection. This may suggest that IGIF plays a role in host defense to pathogenic organisms. Furthermore, the induction of both IL-12 and IGIF synthesis following treatment with IL-12 may support a possible positive feedback mechanism involved in the generation of IFN-γ and resulting in the potentiation of the protective effects.

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