Clin Exp Immunol 1996; **104**:208–214

IL-12 protects mice against pulmonary and disseminated infection caused by *Cryptococcus neoformans*

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(Accepted for publication 16 January 1996)

SUMMARY

We examined the role of IL-12 in host resistance to *Cryptococcus neoformans* using a murine model of pulmonary and disseminated infection. In this model, mice were infected intratracheally with viable yeast cells. Mice untreated with IL-12 allowed an uncontrolled multiplication of yeast cells in the lung with infiltrations of few inflammatory cells, and a cryptococcal dissemination to the brain and meningitis by 3 weeks, resulting in death of all animals within 4–6 weeks. IL-12, when administered from the day of tracheal infection for 7 days, induced a marked infiltration of inflammatory cells, consisting mostly of mononuclear cells, and significantly reduced the number of viable yeast cells in the lung. The treatment suppressed brain dissemination, as shown by a marked reduction of yeast cells in the brain and prevention of meningitis. These effects resulted in a significant increase in the survival rate of infected mice. In contrast, late administration of IL-12 commencing on day 7 after instillation of yeast cells failed to protect the mice against infection with *C. neoformans*. In further experiments, early administration of IL-12 markedly induced interferon-gamma (IFN-γ) mRNA in the lungs of infected mice, while no IFN-γ mRNA was detected without this treatment. Our results indicate that IL-12 is effective when administered in the early period of pulmonary cryptococcal infection.

Keywords IL-12 IFN-γ pulmonary cryptococcosis dissemination

INTRODUCTION

Most IL-12 is produced by macrophages in response to infectious pathogens [1]. It stimulates the production of interferon-gamma (IFN-γ) by natural killer (NK) cells [2], an important component of early host defence reaction against infection [3]. Furthermore, IL-12 is important in chronic infection by playing a central role in the generation of Th1 cells [4,5], a prerequisite for protecting the host from infectious agents through the production of IFN-γ [3]. In experimental murine models of infectious diseases, IL-12 also plays a critical role in host resistance to a variety of infectious agents such as *Listeria monocytogenes*, *Toxoplasma gondii*, *Leishmania major*, and *Candida albicans* [6–9].

Cryptococcus neoformans produces a life-threatening infection in patients with impaired cell-mediated immunity, such as AIDS [10]. Host defence mechanisms against infection with this pathogen involve a cell-mediated immunity [11], with $CD4^+$ T cells playing an important role in the elimination of microorganisms [12,13]. Recent studies have demonstrated that IFN-γ is particularly involved in protecting mice against infection with *C. neoformans* by inducing macrophage anti-cryptococcal activity [14,15]. This process suggests that IL-12 may be effective in protecting mice against cryptococcal infection. Recently, Clemons *et al*. demonstrated that treatment with IL-12 reduced the number of viable microorganisms in the brain, but was ineffective against microorganisms present in the spleen and lung in mice infected with *C. neoformans* through the intravenous route [16].

Since *C. neoformans* is acquired through the pulmonary route [17], initial host defence in cryptococcal pulmonary infection is thought to be at the primary site of infection, i.e. the airspace of the lung. In the present study, therefore, we examined the effect of IL-12 on host resistance to cryptococcal infection using a murine model of pulmonary infection. In this model, mice were infected through intratracheal instillation of *C. neoformans*. Our results demonstrated that treatment with IL-12 eliminated the microorganisms from the lung, preventing dissemination to the brain and improving the survival rate of infected mice.

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MATERIALS AND METHODS

Animals

Female (BALB/c $\times$ DBA/2)F_1 mice were purchased from SLC Japan (Hamamatsu, Japan) and used in the present experiments at the age of 7–10 weeks. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of our institution. The anti-cryptococcal activity of macrophages derived from this strain of mice is not detected without stimulation, and is

markedly induced by IFN-γ [18]. All mice were housed in a pathogen-free environment and received sterilized food and water at the Laboratory Animal Centre for Biomedical Science of University of the Ryukyus.

Cryptococcus neoformans

A serotype A-encapsulated strain of *C. neoformans*, YC-11, was established from a patient with pulmonary cryptococcosis. The yeast cells were cultured on potato dextrose agar (PDA) plates for 3–4 days before use.

Intratracheal instillation of microorganisms

Mice were anaesthetized by an i.p. injection of 70 mg/kg of pentobarbital (Abbott Labs, North Chicago, IL) and restrained on a small board. Live *C. neoformans* (1×10^5) were inoculated at 50 μl/mouse by inserting a blunted 25 G needle into and parallel to the trachea.

IL-12

Recombinant murine IL-12 was kindly provided by Hoffman-La Roche Inc. (Nutley, NJ). IL-12 (0·1 μg/mouse) was administered daily by i.p. injection. Two treatment schedules were used. In the first schedule, IL-12 was administered for 7 days starting from the day of cryptococcal instillation. In the second schedule, IL-12 was administered 7 days after infection and continued for a period of 7 days.

Histological examination

Mice were killed 3 weeks after instillation with *C. neoformans*. The lung and brain specimens were fixed in 10% neutral formalin, dehydrated, and embedded in paraffin. Sections were cut and stained with haematoxylin and eosin (H–E) or periodic acid-Schiff (PAS) using a standard staining procedure, and examined under a light microscope.

Enumeration of viable C. neoformans

Mice were killed 1 or 3 weeks after infection, and both lungs and brains were excised carefully, then homogenized in 2 ml of distilled water with Polytron (Kinematica AG, Littau, Switzerland) at 13 000 rev/min for 20 s at room temperature. The homogenates, appropriately diluted with distilled water, were inoculated at 100 μl on PDA plates, cultured for 3–4 days followed by counting the numbers of colonies. In some experiments, to ascertain the actual number of disseminated yeast cells, the whole brain homogenates were inoculated at 200 μl each on 10 PDA plates.

Effect of IL-12 on survival

To elucidate the clinical effect of IL-12 on the outcome of infection with *C. neoformans*, the survival of mice receiving early or late treatment with IL-12 was examined over a period of 60 days.

Extraction of RNA and reverse transcription polymerase chain reaction

Total RNA was extracted from the lungs of mice at various time points after instillation of *C. neoformans* by an acid guanidinium thiocyanate-phenol-chloroform method as described by Chomczynski & Sacchi [19]. Usually 30–70 μg of RNA were obtained from one set of lungs and resuspended in 50 μl diethylpyrocarbonate (DEPC)-treated distilled water (dH_2O).

Subsequently, reverse transcription (RT) 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. Then 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 U/ml RNase inhibitor (GIBCO BRL), 3 μl of 100 mM dithiothreitol and 2·5 μl of 10 mM dNTP were added, and the tubes were incubated for 2 min at 37°C. One microlitre of 200 000 U/ml M-MLV reverse transcriptase (GIBCO BRL) was added and incubated 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 DEPC-treated dH_2O. The samples were stored at −20°C until use. This reaction was always performed simultaneously for parallel samples from one experiment.

The polymerase chain reaction (PCR) was carried out in an automatic DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT). For amplification of the desired cDNA, the following gene-specific primers were used as shown by Montgomery & Dallman [20]: IFN-γ sense (5′-AACGCTACACACTGCATCT-3′), IFN-γ antisense (5′-TGCTCATTGTAATGCTTGG-3′), β-actin sense (5′-ATGGATGACGATATCGCT-3′), β-actin antisense (5′-ATGAGGTAGTCTGTCA-GGT-3′). One microlitre of sample cDNA solution was added to 49 μl of the reaction mixture, which contained the following concentrations: 10 mM Tris–HCl pH 8·3, 50 mM KCl, 1·5 mM $MgCl_2$, 10 μg/ml gelatin, dNTP (each at a concentration of 200 μM), 1 μM sense and antisense primer, 1·25 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus). The preparations in the microtubes were amplified by the three-temperature PCR system consisting of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and extention at 72°C for 1·5 min for a total of 35 cycles. The PCR products were electrophoresed on 2% agarose gels, stained with 0·5 μg/ml ethidium bromide and observed with a UV transilluminator.

Statistical analysis

Values were expressed as mean ± s.d. The unpaired Student's *t*-test was used to compare differences between groups. Survival data were analysed using the generalized Wilcoxon test. $P < 0{\cdot}05$ was considered significant.

RESULTS

Effect of IL-12 on the number of viable C. neoformans

The number of viable *C. neoformans* in the lung increased progressively within the first 3 weeks of infection (Fig. 1a). All untreated infected mice died 4–6 weeks after intratracheal instillation of yeast cells. Viable microorganisms were not detected in the brain on day 7 after infection (Fig. 1b). However, a large number of *C. neoformans* disseminated to the brain on week 3 after infection. Therefore, the effect of IL-12 on elimination of *C. neoformans* in the lung and its dissemination to the brain was examined at 3 weeks. As shown in Fig. 2a, treatment with IL-12 significantly reduced the number of viable colonies. This was only true in mice provided with the first treatment schedule (i.e. IL-12 treatment commenced after instillation of the microorganisms, and lasted for 7 days). In contrast, mice treated 7 days after instillation of *C. neoformans* (late treatment), showed no protective effect for IL-12 in the lung. In mice receiving no treatment or late treatment,

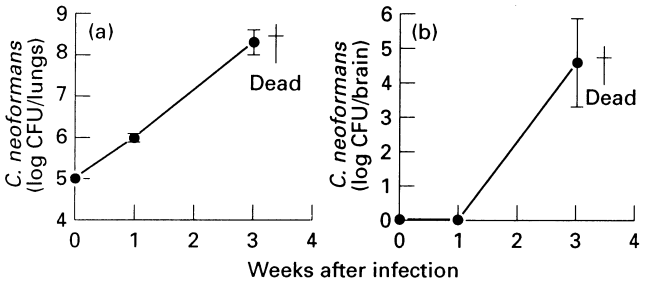

Fig. 1. Experimental model of lung and brain infection with *Cryptococcus neoformans*. Mice were infected intratracheally with 1×10^5 cells of *C. neoformans*, and the numbers of viable colonies in the both lungs (a) and brain (b) were counted 1 and 3 weeks after tracheal instillation. All infected mice died within 4–6 weeks. Each data-point represents the mean ± s.d. of three mice. CFU, Colony-forming units.

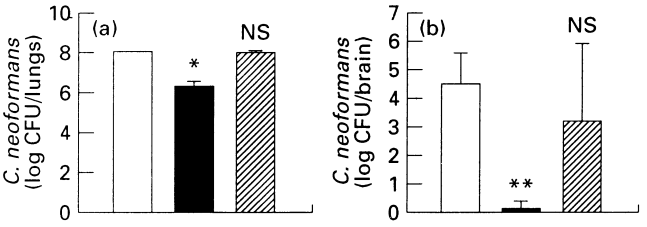

Fig. 2. Effect of IL-12 treatment on the number of viable *Cryptococcus neoformans* in the lung and brain. Mice received no treatment (□) or daily i.p. injections of 0·1 μg IL-12 for 7 days from the day of (■) or 7 days after intratracheal instillation of 1×10^5 cells of *C. neoformans* (▨). The numbers of viable colonies in both lungs (a) and brain (b) were counted 3 weeks after infection. Each column represents the mean ± s.d. of four mice. $*P < 0{\cdot}05$; $**P < 0{\cdot}005$. NS, not significant, compared with untreated mice. CFU, Colony-forming units.

dissemination of *C. neoformans* to the brain occurred in all four mice in each group. In contrast, dissemination to the brain was observed in only two of the four mice receiving early treatment. As shown in Fig. 2b, the number of viable colonies in the brain was significantly lower in mice receiving early treatment than in those of the untreated group, while mice receiving late treatment were not protected against dissemination of the microorganisms to the brain.

Induction of cellular inflammatory response by early, but not late, treatment

Early treatment with IL-12 produced clear histological changes compared with control mice. As shown in Fig. 3a,d, alveolar spaces in the untreated mice were filled with a large number of multiplying yeast cells together with evidence of alveolar wall destruction (arrows), although only few inflammatory cells infiltrated the lung parenchyma. In contrast, early treatment with IL-12 caused a massive accumulation of inflammatory cells, consisting mostly of mononuclear cells infiltrating the peribronchial and perivascular areas, in the interalveolar septa and alveolar spaces (Fig. 3b). Some granulomatous lesions were also found, as shown in Fig. 3e (arrowhead). In lung sections stained with PAS, the number of yeast cells was clearly reduced compared with untreated mice (data not shown). Interestingly, histological features similar to those in the untreated group were observed in the late treatment group. The inflammatory reaction was very little and alveolar spaces were filled with a large number of multiplying yeast cells (Fig. 3c,f).

Suppression of cryptococcal meningitis

There were clear histological differences in brain sections of control and mice treated early with IL-12. As shown in Fig. 4a, a large number of *C. neoformans* (arrowheads) was found invading the submeningeal areas and infiltrations of inflammatory cells, consisting mostly of mononuclear cells, were observed. In contrast, no meningeal lesion was found in the brains of mice receiving early treatment with IL-12 (Fig. 4b). In the late treatment group, both cryptococcal invasions and infiltrations of mononuclear cells in the submeningeal areas were clearly observed, although the degree was milder than that of the untreated group (Fig. 4c).

Effect of IL-12 on survival rate

All untreated mice died 4–6 weeks after intratracheal instillation of *C. neoformans* due to severe pulmonary and meningeal cryptococcal infection. In contrast, early treatment with IL-12 significantly improved the survival rate of infected mice (Fig. 5), while late treatment failed to improve the survival rate.

Induction of IFN-γ mRNA in lungs by early treatment with IL-12

To understand the mechanism by which early treatment with IL-12 protects mice against infection with *C. neoformans*, the expression of IFN-γ mRNA in the lungs of infected mice was examined. As shown in Fig. 6, IFN-γ mRNA was not detected at any time point examined in untreated mice, while early administration of IL-12 markedly induced mRNA of this cytokine in lungs with time of infection. In contrast, the expression of β-actin mRNA remained constant in mice both treated and untreated with IL-12.

DISCUSSION

The major finding of the present study was that early treatment with IL-12 protected mice from pulmonary infection with *C. neoformans*. In this model, mice infected intratracheally with a strongly virulent strain of *C. neoformans* died within 4–6 weeks of severe pulmonary infection and dissemination to the brain. IL-12 treatment enhanced the elimination of the microorganism from the lung, the primary site of infection, and prevented its dissemination to the brain.

Recently, Clemons *et al*. [16] showed that IL-12 had a considerable effect in eliminating *C. neoformans* from the brain in systemically infected mice. The effect of IL-12 on the brain in their study, however, was weaker than that observed in our study. Since in the study of Clemons *et al.* mice were infected through administration of microorganisms intravenously, the yeast cells were probably eliminated to a large extent by a local host defence system in the brain independent of that in the lung. In our model, a reduction in *C. neoformans* invading the brain was dependent on local host defence systems not only in the brain but also in the lung. While IL-12 treatment reduced the number of viable yeast cells in the lung significantly, IL-12 in Clemons *et al*. [16] failed to eliminate *C. neoformans* in the lung. Differences in the route of infection, strain or dose of yeast cells and strain of mice, used in both studies may account for this inconsistency in the results, although the exact mechanism is not fully understood.

Remarkable differences in the outcome of treatment were observed between early and late treatment schedules. Early treatment reduced the number of viable yeast cells both in the lung and brain and improved the survival rate of infected mice, while late treatment was ineffective in protecting mice. These results suggest

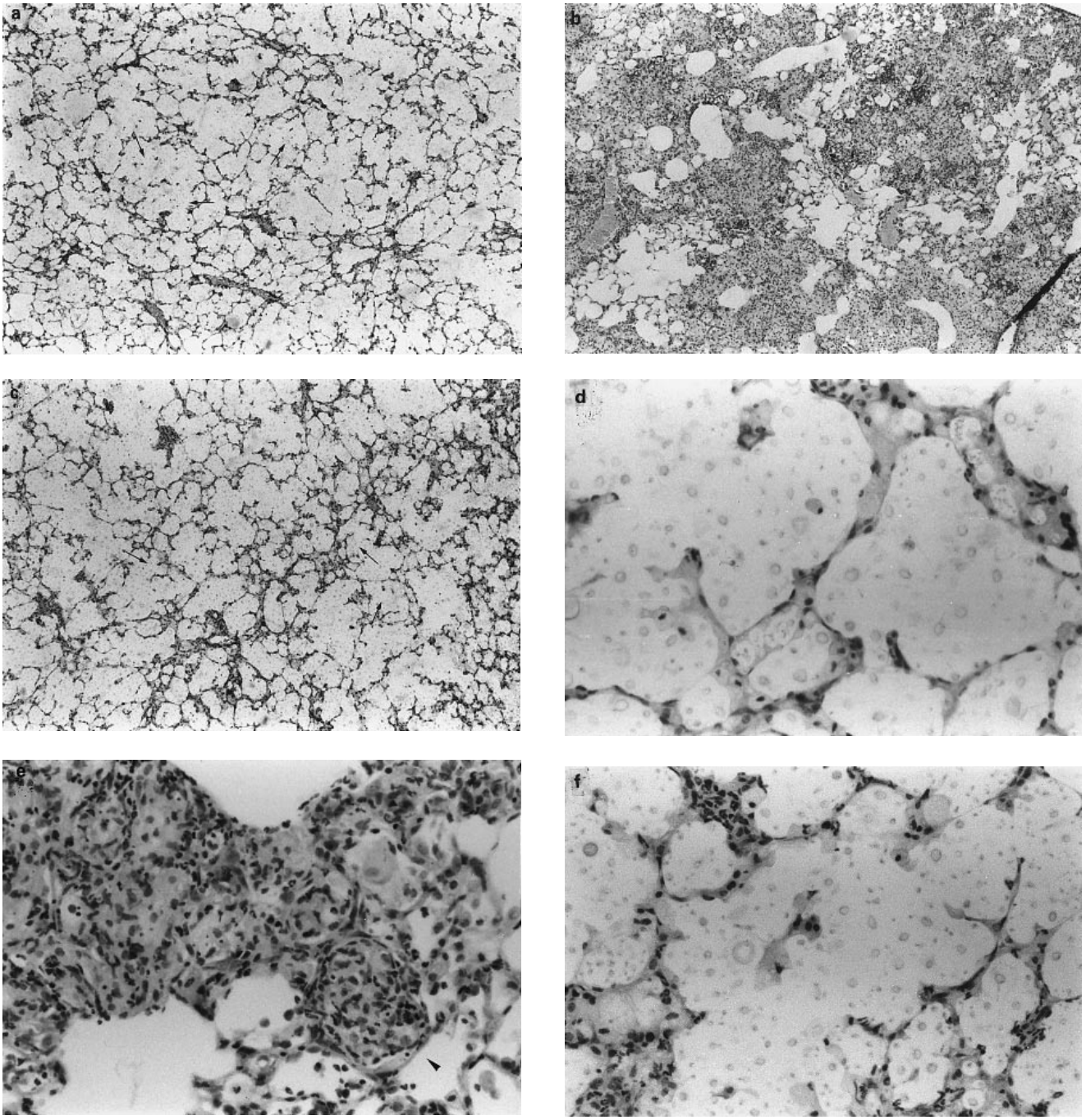

Fig. 3. Histological examination of lung tissue demonstrating the effect of IL-12 treatment. Mice received daily i.p. injections of 0·1 μg IL-12 for 7 days from the day of or 7 days after intratracheal instillation of 1×10^5 cells of *Cryptococcus neoformans*. Paraffin sections of the lung were prepared from mice killed 3 weeks after instillation of the microorganisms, stained with haematoxylin and eosin (H–E), and examined at $\times$40 (a,b,c) and $\times$200 (d,e,f) under a light microscope. Arrowhead (e): granulomatous lesion; arrows: destruction of alveolar structure. a,d, untreated group; b,e, early treated group; c,f, late treated group.

that IL-12 plays a more important role in early host resistance to infections compared with late resistance mechanisms. This finding is consistent with that of Gazzinelli *et al*. [6], who demonstrated that IL-12 plays an essential role in acute infection, but not in chronic infection caused by *T. gondii*.

What determines the outcome of treatment of cryptococcal infection? Several IL-12 biological activities are thought to be mediated by IFN-γ [3]. In chronic infection, Th1 cells are particularly important as producers of IFN-γ [2], and IL-12 is a major inducer of Th1 cells [4,5]. Conversely, Th2 cytokines, such as IL-4 and IL-10, suppress Th1 response, a process disadvantageous in protecting the host from infections [21–23]. Our results indicate that the favourable effects of early treatment with IL-12 are mediated through the enhancement of the Th1 response, as

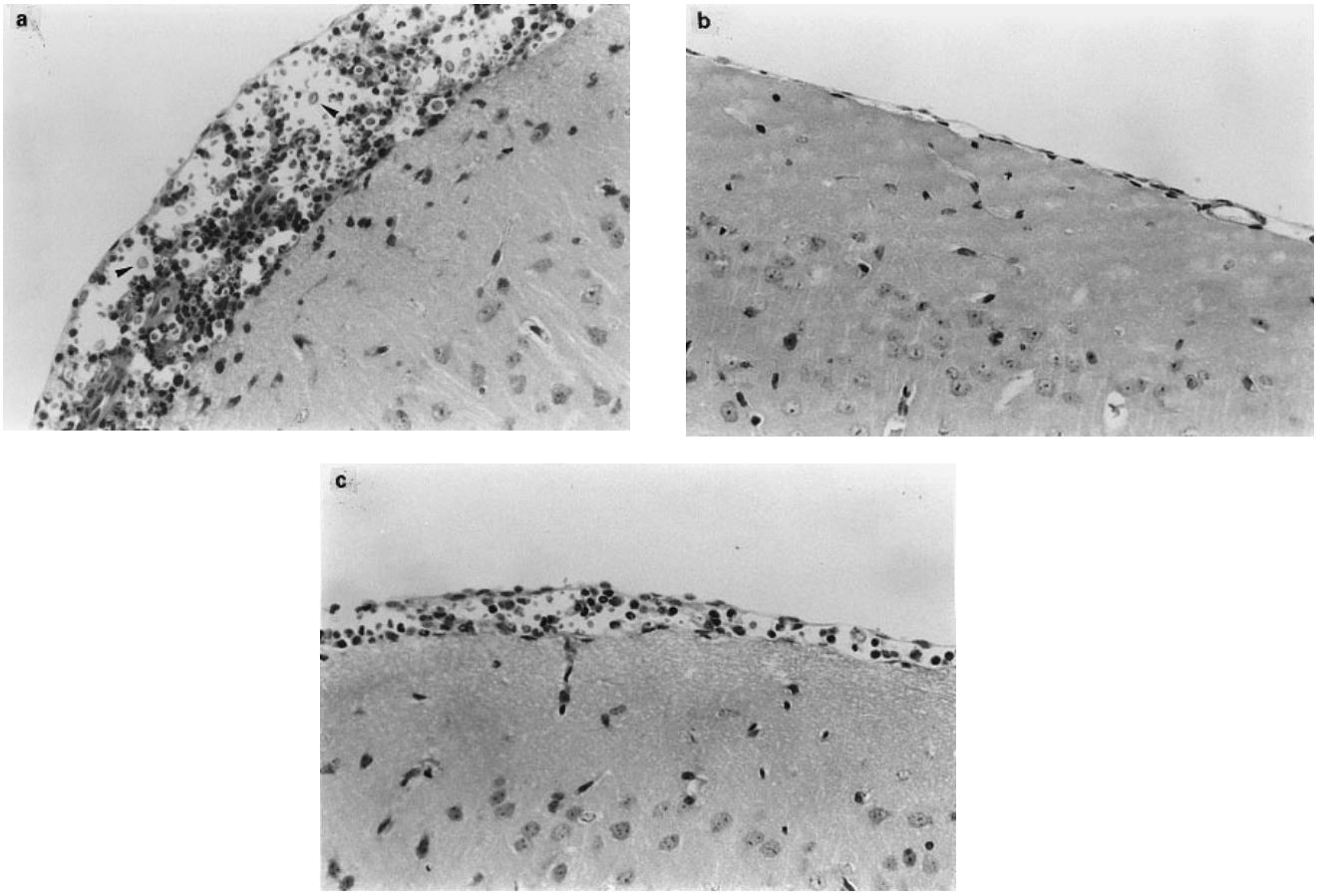

Fig. 4. Histological examination of mice brain in disseminated infection. The paraffin sections of the brain prepared from mice were treated as shown in Fig. 3, 3 weeks after instillation, stained with periodic acid-Shiff (PAS), and examined at ×200 under a light microscope. Arrowhead (a): *Cryptococcus neoformans.* a, untreated group; b, early treated group; c, late treated group.

shown by the inducation of IFN-γ mRNA in lungs and probably through the suppression of the Th2 response. In the late phase of infection, helper T cells may already complete the differentiation process towards Th2 cells. Thus, administration of IL-12 at that stage may not be effective in generating Th1 response due to the suppressive effect of Th2 cytokines. Further studies are underway in our laboratory to examine these differences.

Another mechanism for the observed differences between early and late treatment schedules may involve the pattern and speed of yeast growth *in vivo*. In the present study, yeast cells multiplied to approximately 10 times the initial number during the first 7 days of infection. Such rapid growth may cause a suppression of Th1 cells and activation of Th2 cells as a result of a high antigen load. Previous studies demonstrated that high levels of antigen suppressed the Th1 response and rather stimulated the Th2 response [24,25]. This argument is supported by a recent finding by Nabors *et al*. demonstrating that a reduction in antigen levels by an anti-leishmanial drug resulted in a switch from Th2 to Th1 cells in mice infected with *L. major* [26].

Histological examination in the present study demonstrated the presence of severe inflammatory reaction in early treated mice, but not in late treated or untreated animals. It is well known that adhesion molecules and chemotactic cytokines are involved in inflammatory cell accumulation at the site of infection. A direct effect of IL-12 involves the induction of expression of certain

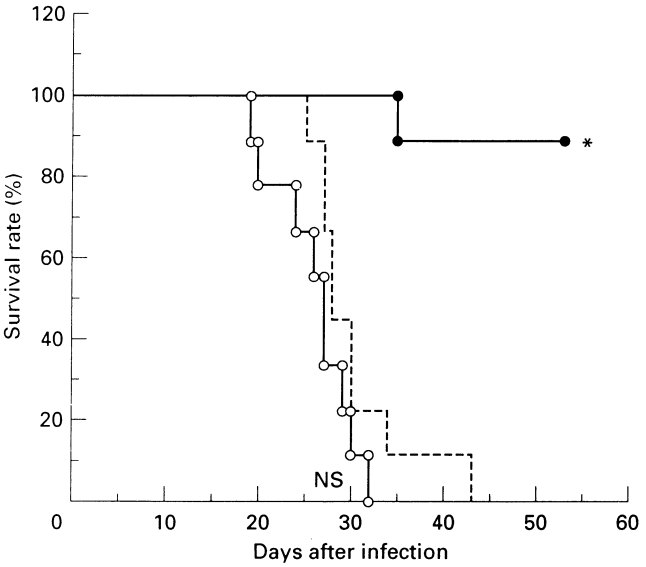

Fig. 5. Effect of IL-12 treatment on the survival rate of mice infected with *Cryptococcus neoformans*. Mice received no treatment (---) or daily i.p. injections of 0·1 μg IL-12 for 7 days from the day of (●, —) or 7 days after intratracheal instillation of 1×10^5 cells of *C. neoformans* (○, —). The numbers of live mice were noted. Each group consisted of nine mice. *$P < 0{\cdot}05$. NS, Not significant compared with untreated mice.

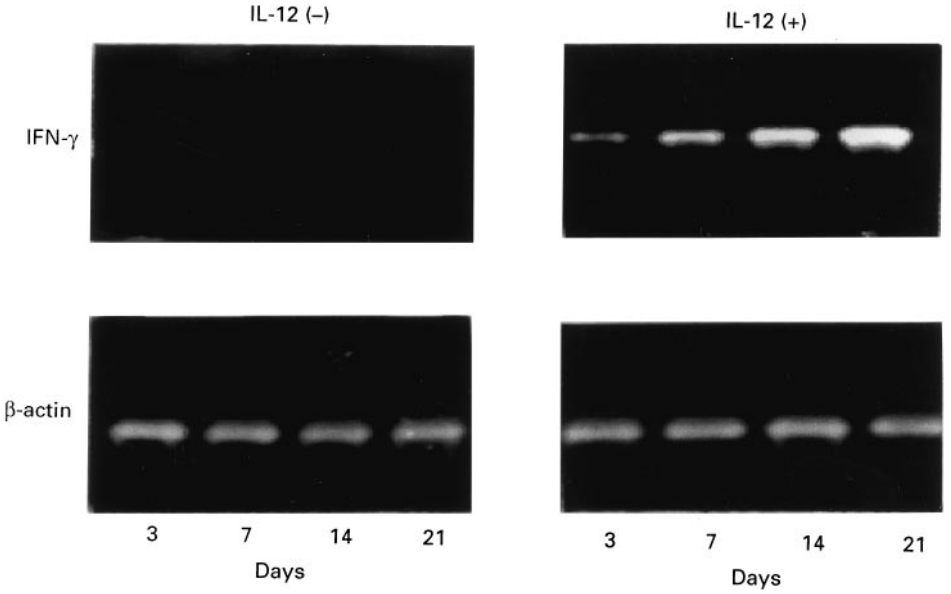

Fig. 6. Induction of IFN-γ mRNA in lungs by early treatment with IL-12. The total lung RNA was extracted 3, 7, 14 or 21 days after infection from mice which received early administration of IL-12 or no treatment, and the expression of IFN-γ and β-actin mRNA was examined. IL-12 (−), Untreated; IL-12 (+), early treated with IL-12.

adhesion molecules such as lymphocyte function-associated antigen (LFA)-1 [27]. The expression of adhesion molecules on inflammatory and endothelial cells is up-regulated by various proinflammatory cytokines such as IL-1, tumour necrosis factor-alpha (TNF-α) and IFN-γ [28–30]. Furthermore, the production of these cytokines is directly induced by IL-12 [3] and more effectively by IFN-γ [31], which is in turn produced by stimulation with IL-12 [2,3]. In addition, IL-12 induces also the production of IL-8, a prototypic chemokine [3], and IFN-γ is known to stimulate macrophages to produce certain chemotactic cytokines, such as IL-8 [32] and macrophage chemotactic peptide (MCP)-1 [33]. Thus, IL-12 may promote the accumulation of inflammatory cells in pulmonary parenchyma directly or indirectly by up-regulating the expression of a number of adhesion molecules and the production of a variety of chemokines.

Finally, we also demonstrated in the present study a weak cellular inflammatory response in mice receiving late treatment compared with those treated with the early treatment schedule. Recently, Vecchiarelli *et al*. [34] demonstrated that cryptococcal polysaccharide strongly down-regulated the production of IL-1 and TNF-α by macrophages. These two monokines are important in initiating cellular inflammatory responses [28–30,35]. In mice receiving late treatment schedule, the increased number of yeast cells and the accumulated polysaccharide may halt or prevent the infiltration of inflammatory cells in the lung. Other possible mechanisms may involve the suppression of the inflammatory response by Th2 cytokines, such as IL-4 and IL-10 [8].

In conclusion, we demonstrated that IL-12 is a strong immunomodulatory cytokine that potentiates host resistance to cryptococcal infection, but that an effective therapeutic treatment was not achieved successfully in this experimental system. Therefore, additional interventions, such as a combined treatment with an anti-fungal reagent, may be necessary for effective therapy against severe cryptococcal infection in patients with impaired cell-mediated immunity.

ACKNOWLEDGMENTS

We are grateful to Ms Yuko Yamashiro for her excellent technical assistance. We also thank Dr F. G. Issa (Department of Medicine, University of Sydney, Australia) for careful reading and editing of the manuscript.

REFERENCES

1 D'Andrea A, Rengaraju M, Valiante NM *et al*. Production of natural killer cell stimulatory factor (NKSF/IL-12) by peripheral blood mononuclear cells. J Exp Med 1992; **176**;1387–98.
2 Chan SH, Perussia B, Gupta JW *et al*. Induction of IFN-γ production by NK cell stimulatory factor (NKSF): characterization of the responder cells and synergy with other inducers. J Exp Med 1991; **173**:869–79.
3 Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol 1995; **13**:251–76.
4 Hsieh C, Macatonia SE, Trip CS, Wolf SF, O'Garra A, Murphy KM. Listeria-induced Th1 development in $\alpha\beta$-TCR transgenic CD4$^+$ T cells occurs through macrophage production of IL-12. Science 1993; **260**:547–9.
5 Seder RA, Gazzinelli R, Sher A, Paul WE. IL-12 acts directly on CD4$^+$ T cells to enhance priming for IFN-γ production and diminishes IL-4 inhibition of such priming. Proc Natl Acad Sci USA 1993; **90**:10188–92.
6 Gazzinelli RT, Wysocka M, Hayashi S *et al*. Parasite-induced IL-12 stimulates early IFN-γ synthesis and resistance during acute infection with *Toxoplasma gondii*. J Immunol 1994; **153**:2533–43.
7 Heinzel FP, Schoenhaut DS, Rerko RM, Rosser LE, Gately MK. Recombinant interleukin 12 cures mice infected with *Leishmania major*. J Exp Med 1993; **177**:1505–9.
8 Romani L, Mencacci A, Tonnetti L *et al*. IL-12 is both required and prognostic *in vivo* for T helper type 1 differentiation in murine candidiasis. J Immunol 1994; **152**:5167–75.
9 Tripp CS, Gately MK, Hakimi J, Ling P, Unanue ER. Neutralization of IL-12 decreases resistance to *Listeria* in SCID and CB-17 mice. J Immunol 1994; **152**:1883–7.

10 Stevens DA. Fungal infections in AIDS patients. Br J Clin Practice 1990; **44**(Suppl.1):11–22.
11 Lim TS, Murphy JW. Transfer of immunity to cryptococcosis by T-enriched splenic lymphocytes from *Cryptococcus neoformans*-sensitized mice. Infect Immun 1980; **30**:5–11.
12 Hill JO, Harmsen AG. Intrapulmonary growth and dissemination of an avirulent strain of *Cryptococcus neoformans* in mice depleted of $CD4^+$ or $CD8^+$ T cells. J Exp Med 1991; **173**:755–8.
13 Mody CH, Lipscomb MF, Street NE, Toews GB. Depletion of $CD4^+$ ($L3T4^+$) lymphocytes *in vivo* impairs murine host defense to *Cryptococcus neoformans*. J Immunol 1990; 1**44**:1472–7.
14 Kawakami K, Kohno S, Kadota J *et al*. T cell-dependent activation of macrophages and enhancement of their phagocytic activity in the lungs of mice inoculated with heat-killed *Cryptococcus neoformans*: involvement of IFN-γ and its protective effect against cryptococcal infection. Microbiol Immunol 1995; **39**:135–43.
15 Salkowski CA, Balish E. A monoclonal antibody to gamma interferon blocks augmentation of natural killer cell activity induced during systemic cryptococcosis. Infect Immun 1991; **59**:486–93.
16 Clemons KV, Brummer E, Stevens DA. Cytokine treatment of central nervous system infection: efficacy of interleukin-12 alone and synergy with conventional antifungal therapy in experimental cryptococcosis. Antimicrob Agents Chemother 1994; **38**:460–4.
17 Kozel TR. Cryptococcosis. In: Murphy JW, Friedman H, Bendinelli M, eds. Fungal infection and immune responses. New York: Plenum Press, 1993:277–302.
18 Brummer E, Stevens D. Anticryptococcal activity of macrophages: role of mouse strain, C5, contact, and L-arginine. Cell Immunol 1994; **157**:1–10.
19 Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; **162**:156–9.
20 Montgomery RA, Dallman MJ. Analysis of cytokine gene expression during fetal thymic ontogeny using the polymerase chain reaction. J Immunol 1991; **147**:554–60.
21 Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 1989; **170**:2081–95.
22 Peleman R, Wu J, Fargeas C, Delespesse G. Recombinant interleukin 4 suppresses the production of interferon γ by human mononuclear cells. J Exp Med 1989; **170**:1751–6.
23 Sher A, Gazzinelli RT, Oswald IP *et al*. Role of T-cell derived cytokines in the downregulation of immune responses in parasitic and retroviral infection. Immunol Rev 1992; **127**:183–204.
24 Bretscher PA, Wei G, Menon JN, Bielefeldt-Ohmann H. Establishment of stable, cell-mediated immunity that makes ''susceptible'' mice resistant to *Leishmania major*. Science 1992; **257**:539–42.
25 Burstein HJ, Abbas AK. *In vivo* role of interleukin 4 in T cell tolerance induced by aqueous protein antigen. J Exp Med 1993; **177**:457–63.
26 Nabors GS, Afonso LCC, Farrell JP, Scott P. Switch from a type 2 to a type 1 T helper cell response and cure of established *Leishmania major* infection in mice is induced by combined therapy with interleukin 12 and Pentostam. Proc Natl Acad Sci USA 1995; **92**:3142–6.
27 Rabinowich H, Herberman RB, Whiteside TL. Differential effects of IL-12 and IL-12 on expression and function of cellular adhesion molecules of purified human natural killer cells. Cell Immunol 1993; **152**:481–98.
28 Sica A, Wang JM, Colotta *et al*. Monocyte chemotactic and activating factor gene expression induced in endothelial cells by IL-1 and tumor necrosis factor. J Immunol 1990; **144**:3034–8.
29 Springer TA. Adhesion receptors of the immune system. Nature 1990; **346**:425–34.
30 Strieter RM, Kunkel SL, Showell HJ, Marks RM. Endothelial cell gene expression of a neutrophil chemotactic factor by TNF, IL-1 and LPS. Science 1989; **243**:1467–9.
31 Green SJ, Turpin JA, Nacy CA. Cytotoxic effector activities of macrophages. In: Rich RR, Fleisher TA, Schwartz BD, Shearer WT, Strober W, eds. Clinical immunology: principles and practice. St Louis: Mosby-Year Book, Inc., 1996:290–8.
32 Yoshimura T, Matsushima K, Tanaka S, Robinson EA, Appella E, Oppenheim JJ, Leonard EJ. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. Proc Natl Acad Sci USA 1987; **84**:9233–7.
33 Colotta F, Borre A, Wang JM *et al*. Expression of a monocyte chemotactic cytokine by human mononuclear phagocytes. J Immunol 1991; **148**:760–5.
34 Vecchiarelli A, Retini C, Pietrella D, Monari C, Tascini C, Beccari T, Kozel TR. Downregulation by cryptococcal polysaccharide of tumor necrosis factor alpha and interleukin-1β secretion from human monocytes. Infect Immun 1995; **63**:2919–23.
35 Ramilo O, Saez-Llorens Z, Mertsola J *et al*. Tumor necrosis factor α/cachectin and interleukin β initiate meningeal inflammation. J Exp Med 1990; **172**:497–507.