Brief communication

Lack of IL-6 increases blood–brain barrier permeability in fungal meningitis

XIANG LI*, GUIYANG LIU, JIANLI MA, LIANG ZHOU, QINGZHE ZHANG and LEI GAO

Department of Pharmacy, First Affiliated Hospital of Chinese PLA General Hospital, Beijing 100048, China

**Corresponding author (Fax, +861066867082; Email, lxbj@hotmail.com)*

The pathogenesis of increased blood–brain barrier permeability during *Cryptococcus* meningitis is still largely unknown. Interleukin (IL-6) is a multifunctional cytokine, and numerous studies have shown that IL-6 influences the integrity of the blood–brain barrier. In this study we investigated the role of IL-6 in *Cryptococcus* meningitis. First, wild-type or IL-$6^{-/-}$ mice were injected with *Cryptococcus neoformans* (*C. neoformans*) and the survival time in both groups was recorded. Second, the number of fungi was measured in the brains of IL-$6^{-/-}$ wild-type mice. Finally, the blood–brain barrier permeability index was detected in infected IL-$6^{-/-}$ mice treated with recombinant human IL-6. The blood–brain barrier permeability index was measured in infected wild-type mice treated with anti-IL-6 antibodies as well. The survival of IL-$6^{-/-}$ mice injected with *C. neoformans* was significantly lower than that of identically challenged wild-type mice. The infected IL-$6^{-/-}$ mice had significantly larger brain fungal burdens than wild-type mice. Furthermore, increased blood–brain barrier index was found in infected IL-$6^{-/-}$ mice when compared with that in infected control mice. Similar results were obtained when mice challenged with *C. neoformans* were treated systemically with neutralizing anti-IL-6 antibodies, resulting in an elevation of vascular permeability. Our data revealed that IL-6 reduced the blood–brain barrier permeability during *Cryptococcus* meningitis, and it might provide an explanation for the significantly lower survival of infected IL-$6^{-/-}$ mice.

[Li X, Liu G, Ma J, Zhou L, Zhang Q and Gao L 2015 Lack of IL-6 increases blood–brain barrier permeability in fungal meningitis. *J. Biosci.* **40** 7–12] **DOI** 10.1007/s12038-014-9496-y

1. Introduction

C. neoformans is a ubiquitous encapsulated fungus that causes the third most common opportunistic infection of the CNS in patients with AIDS (Mwaba *et al.* 2001; Bicanic *et al.* 2009; Esposito *et al.* 2009). *C. neoformans* disseminates to the central nervous system in the immunosuppressed patient and occasionally in the normal host, and leads to a potentially life-threatening meningoencephalitis. Studies conducted largely in the United States suggest that between 6% and 10% of patients with AIDS develop *Cryptococcus* meningitis (Powderly 1992; Moosa and Coovadia 1997), which remains a leading cause of death in cohorts of HIV-infected individuals in Africa and Asia (French *et al.* 2002). Traversal of *C. neoformans* across the blood–brain barrier (BBB) is a crucial step in the pathogenesis of *C. neoformans* (Kim *et al.* 2012). The intact BBB enables the brain endothelium to exclude proteins, ions and microorganisms. Disruption of BBB leads to increased intracranial pressure and brain edema. Several mediators of the path physiological pathway contributing to damaged BBB permeability have been identified, including reactive oxygen species and matrix metalloproteinase (Schreibelt *et al.* 2007; Barr *et al.* 2010).

IL-6 is a multifunctional cytokine with diverse actions, such as regulation of inflammation (Wang *et al.* 2000). Increased cerebral expression of IL-6 has been demonstrated in many CNS diseases, such as HIV encephalopathy,

Keywords. Blood–brain barrier; *Cryptococcus* infection; *Cryptococcus* meningitis; IL-6; IL-$6^{-/-}$ mice

Supplementary materials pertaining to this article are available on the *Journal of Biosciences* Website at *http://www.ias.ac.in/jbiosci/mar2015/supp/Li.pdf*

Published online: 4 February 2015

multiple sclerosis and Alzheimer's disease (Gadient and Otten 1997). Some researchers found anti-IL-6 monoclonal antibodies protected against lethal *Escherichia coli* infection (Starnes *et al.* 1990). In addition, infection with HIV was associated with elevated IL-6 levels and production (Breen *et al.* 1990). Numerous researches have documented important roles for IL-6 in the integrity of BBB. Mice overexpressing IL-6 in astrocytes showed neuropathological abnormalities with a breakdown of the BBB (Brett *et al.* 1995). Therefore, whether IL-6 was involved in the pathogenesis of *Cryptococcus* meningitis needs to be investigated.

The aim of this study was to investigate the impact of IL-6 on the BBB permeability during *Cryptococcus* meningitis using IL-$6^{-/-}$ mice as well as neutralizing anti-IL-6 antibodies in a mice model.

2. Materials and methods

2.1 *Animals*

Male C57/BL6 mice were provided by the Animal Center of Second Military Medical University (Shanghai, China). The IL-$6^{-/-}$ mice were purchased from the Jackson Laboratory. All the animals were housed under controlled temperature (23–25°C) and lighting (8:00–20:00 light, 20:00–8:00 dark) with free access to standard mouse chow and drinking water. All the animals used in the study received humane care in compliance with the institutional guidelines for health and care of experimental animals.

2.2 *Experimental groups*

The survival percentage study consisted of two groups: normal control mice and IL-$6^{-/-}$ mice (n=40 in each group). The research studying culture of *C. neoformans* from various organs consisted of two groups: normal control mice and IL-$6^{-/-}$ mice (n=8 in each group). There were two BBB permeability studies. One consisted of two groups: wild-type mice and IL-$6^{-/-}$ mice (n=8 in each group). The other one consisted of four groups: wild-type mice, IL-$6^{-/-}$ mice, wild-type mice treated with anti-IL-6 antibody (intraperitoneally at the time of infection) and IL-$6^{-/-}$ mice treated with IL-6 (intraperitoneally at the time of infection, n=8 in each group).

2.3 *Experimental infection with C. neoformans.*

C. neoformans (*Cryptococcus neoformans* variety *neoformans*) was cultured on agar slants containing 2.1% YM broth for 4 days at 27°C. All the *Cryptococcus* used in this research was from the same strain. Fungi were enumerated with a haemocytometer, and the viable number determined in c.f.u. For pulmonary infection, wild-type C57BL/6 and IL-$6^{-/-}$ mice were intranasally challenged with 0.04 mL fungal suspension. Thirty minutes later, the lungs of two wild-type and two IL-$6^{-/-}$ mice were removed aseptically and homogenized in 1 mL sterile saline to determine the initial number of organisms. At various time points after the challenge (7, 14, 28, 56 and 120 days after the infection), selected organs such as brains were harvested aseptically and homogenized in sterile saline in the presence of protease inhibitors (Complete Protease Inhibitor Cocktail Tablets; Roche). Appropriate dilutions of the homogenates were plated in duplicate onto trypticase soy agar plates (Eiken Chemical). After 2 days incubation at 37°C, the number of viable organisms was determined in c.f.u. Data were recorded as the mean log 10 (c.f.u.) per brain.

For the determination of c.f.u. in blood, the blood sample was harvested aseptically at various time points after the challenge (7, 14, 28, 56 and 120 days after the infection). Appropriate dilutions of the sample (1:10) were plated in duplicate onto trypticase soy agar plates (Eiken Chemical). After 2 days incubation at 37°C, the number of viable organisms was determined in c.f.u. Data were recorded as the mean log 10 (c.f.u.) per brain.

2.4 *Determination of BBB permeability in mouse model*

In this experiment, a total of 16 mice were involved in the study, with 8 in each group, and BBB permeability was determined as described previously. Mice were injected intravenously with 0.1 mL of 3% (w/v) Evan's Blue (EB). After 1 h the brains were perfused with 10 mL of ice-cold PBS and removed. The brains were weighed and tissue samples were extracted for 3 days in formamide (5 μL/mg tissues) and the extract centrifuged for 5 min at 500*g*. EB concentration in the supernatants was determined by measuring the absorbance at 650 nm. The BBB permeability index was calculated by dividing the value for each sample by the mean value of the control animals.

The permeability of blood–brain barrier was detected 56 days after infection, when none of the IL-$6^{-/-}$ mice died after they were intranasally challenged with fungal suspension. In other words, 56 days was the longest period when all the IL-$6^{-/-}$ mice were still alive after infection.

2.5 *IL-6 treatment*

Determination of the effect of IL-6 on cerebral vascular permeability was performed as previously described. Recombinant human IL-6 (Biosource, Cammarillo, CA, USA) was injected into the tail vein of C57BL/6 mice. PBS was injected as a control. Six hours after injection, the mice were euthanized to evaluate the effect on BBB permeability.

2.6 *Anti-IL-6 antibody*

For neutralization of IL-6 bioactivity in vivo a polyclonal goat anti-murine IL-6 antibody (R&D Systems GmbH, Wiesbaden, Germany) (<10 ng of endotoxin/mg of protein) was diluted to a concentration of 500 µg/kg body weight in 0.5 mL of PBS and injected i.p. The ability of this antibody to react with mouse IL-6 and to neutralize IL-6 bioactivity was demonstrated *in vivo* and *in vitro* in previous studies. Six hours after injection, the mice were euthanized to evaluate the effect on BBB permeability.

2.7 *Statistical analysis*

Survival curses were analysed by Kaplan-Meier log-rank test. The values were expressed as mean±SEM. Data sets of two groups were compared using the unpaired Student's *t*-test. Data sets of four groups were compared using one-way ANOVA and Dunnett t3 test. Differences were considered significant at $P<0.05$.

3. Results

3.1 *Survival rates significantly reduced in infected IL-6 $^{-/-}$ mice*

There was a 90% survival rate among infected wild-type mice, and most of them remained viable after 3 months (figure 1). In contrast, there was a rapid death in IL-6$^{-/-}$ mice infected with *C. neoformans*, with a 100% death rate on day 124. The difference in survival rates between wild-type and IL-6 $^{-/-}$ mice was highly significant ($P<0.001$).

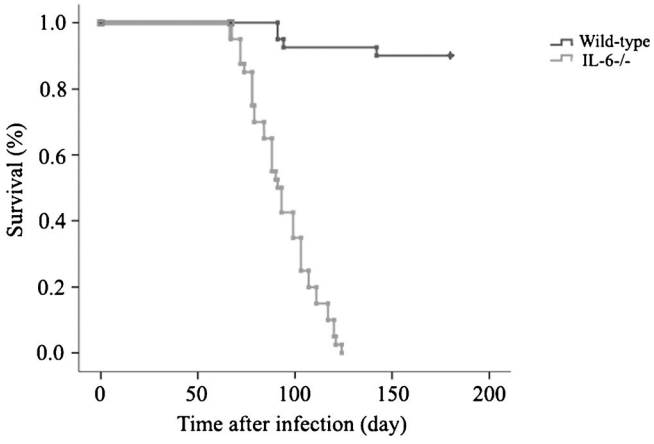

Figure 1. Survival of wild-type and IL-6$^{-/-}$ mice following infection with *C. neoformans*. Wild-type (n=40) and IL-6$^{-/-}$(n=40) were intranasally infected with 3.6×10^6c.f.u. *C. neoformans*, and survival was observed over the course of 180 days. $P<0.001$ (IL-6$^{-/-}$ *vs* wild-type mice).

3.2 *Number of C. neoformans increased significantly in infected IL-6 $^{-/-}$ mice*

We reasoned that the most likely explanation for the increased mortality in IL-6$^{-/-}$mice were the increased BBB permeability. To assess this possibility, we compared the brain fungal burden in IL-6$^{-/-}$ mice to that of wild-type controls, and the blood fungal burden was compared as well. As shown in figure 2, although the blood fungal burden of the IL-6$^{-/-}$ mice was equivalent to that in wild-type mice, the number of *C. neoformans* in the brain increased gradually and significantly in IL-6$^{-/-}$ mice. Consequently, by day 60, IL-6$^{-/-}$ mice contained nearly 50-fold more fungi than the infected control mice ($P<0.05$). It peaked on day 120.

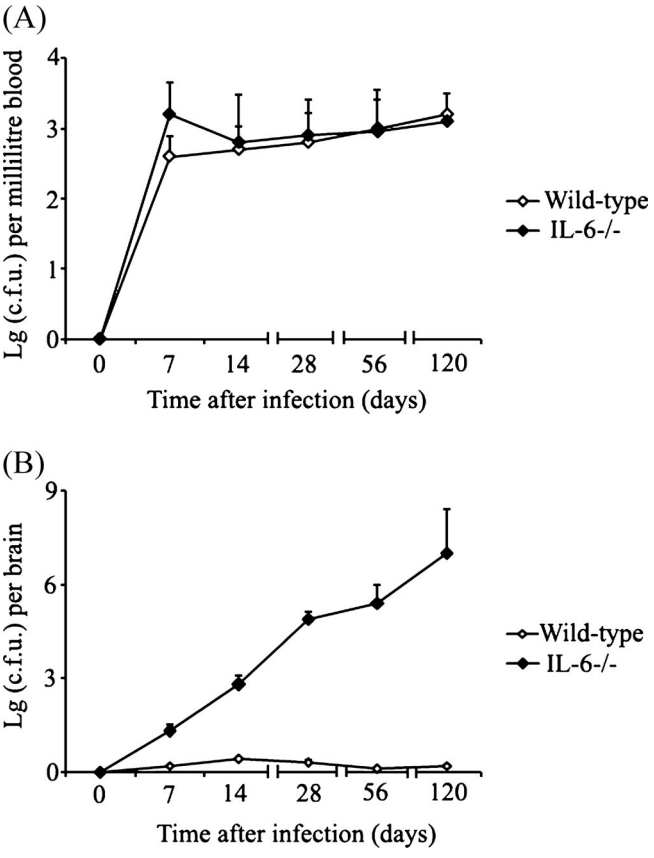

Figure 2. Culture of *C. neoformans* from blood (**A**) and brain (**B**) after intranasal infection with mice. Wild-type and IL-6$^{-/-}$ mice were inoculated intranasally with 3.6×10^6c.f.u. *C. neoformans* per mouse and analysed on days 0, 7, 14, 28, 56 and 120. Aliquots of homogenized organs were plated on agar plates, and total c.f.u. per organ was determined. Data were recorded as the mean log 10 (c.f.u.) per organ.$^*P<0.05$ *vs* wild-type infected mice.

3.3 *IL-6 decreased BBB permeability*

BBB permeability in mice was also assessed by measuring the permeability index using EB as a marker of extravasations. In infected IL-$6^{-/-}$ mice, the permeability index was significantly increased ($P<0.05$), indicating that vascular leak significantly elevated in these mice (figure 3).

As shown in figure 4A, treatment with neutralizing anti-IL-6 antibody in infected wild-type mice, significantly increased the BBB permeability. To confirm the impact of IL-6 on cerebral vessel permeability, recombinant human IL-6 was injected into the tail vein of infected IL-$6^{-/-}$ mice. The rise in BBB index was significantly attenuated to almost basal levels ($P<0.05$), indicating that vascular leak was prevented in these mice (figure 4A). In accordance with the change in BBB index, the brain fungal burden increased when the BBB permeability was destroyed. Lack of IL-6 led to more severe fungal infection in IL-6 knock-out mice when compared with their wild-type counterparts. Inhibiting the action of IL-6 in wild-type mice could worsen the fungal infection while pre-treating IL-6 knock-out mice with recombinant human IL-6 could ameliorate the infection to some extent (as indicated in figure 4B). These data indicated thatIL-6 played an important role in preventing breakdown of the BBB.

4. Discussion

In the majority of hosts, infection with *C. neoformans* is asymptomatic. However, in the patient with severe cell-mediated immunodeficiency, the organism may enter the circulation, leading to disseminated disease. The most common target organs involve the lungs and CNS. *Cryptococcus* meningitis has become the most common lethal fungal infection in patients with AIDS worldwide (Mitchell and Perfect 1995).

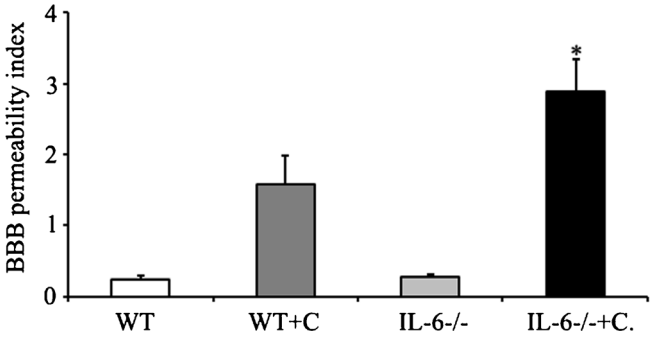

Figure 3. IL-6 decreased BBB permeability during fungal meningitis. Injection of *C. neoformans* increased BBB permeability index in IL-$6^{-/-}$ mice which was significantly reduced in infected wild-type mice. $^{*}P<0.05$ *vs* infected wild-type (C.: *C. neoformans*).

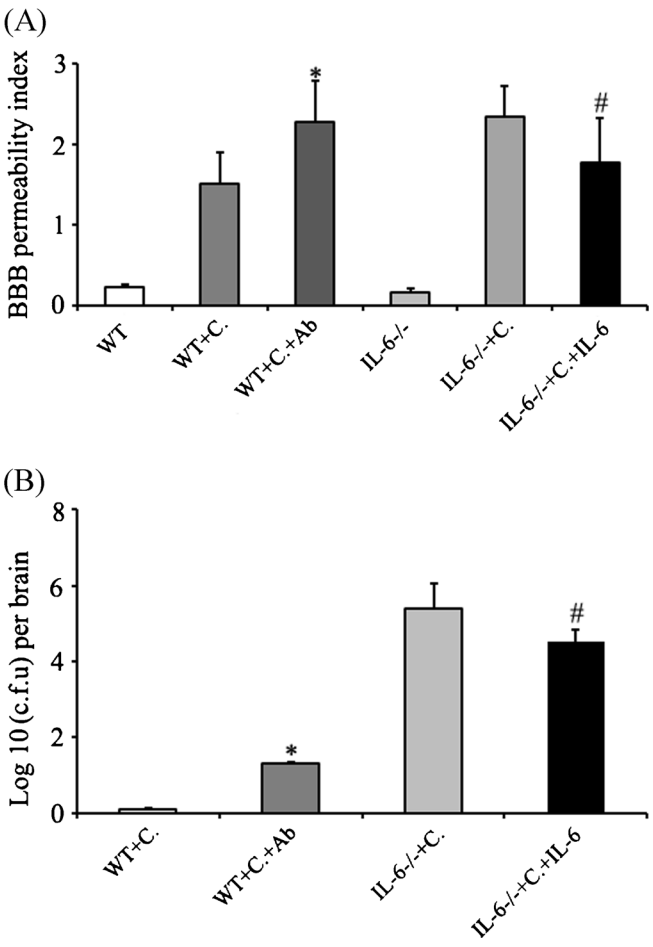

Figure 4. Effect of IL-6 on BBB permeability and brain fungal burden. As shown in (**A**), increased BBB permeability was found in infected wild-type mice pre-treated with anti-IL-6 antibodies. Direct injection of recombinant human IL-6 into the serum of infected IL-$6^{-/-}$ mice decreased BBB permeability index, which was significantly increased in infected IL-$6^{-/-}$mice. The number of *C. neoformans* in brains of infected mice was illustrated in (**B**). 56 days after inoculation with *C. neoformans* organisms, infected wild-type mice and IL-$6^{-/-}$ mice were treated with anti-IL-6 antibody and recombinant human IL-6 for a period of 7 days, respectively. Aliquots of homogenized brains were plated on agar plates, and total c.f.u. per brain was determined. Data were recorded as the mean log 10 (c.f.u.) per brain. $^{*}P<0.05$ *vs* wild-type infected mice. $^{\#}P<0.05$ *vs* IL-$6^{-/-}$ infected mice.

Here we found that fungal meningitis in IL-$6^{-/-}$ mice caused rapid death, with a 100% death rate 124 days after infection, while there was a 90% survival rate among infected wild-type mice. The results were in accordance with the previous study. Beenhouwer *et al*. (2001) found that IL-6-deficient mice were more susceptible to cryptococcal infection than C57BL/6J mice. In that study, all IL-$6^{-/-}$ mice died 20 days after intravenous infection of fungus, while

about 40% of the C57BL/6J mice were still alive at that time. The longer survival time in our study was probably related to the intranasal administration route.

Then we reasoned that the most likely explanation for increased mortality in infected IL-$6^{-/-}$ mice was the increased BBB permeability. To assess this possibility, we compared the brain fungal burden in IL-$6^{-/-}$ mice to that of wild-type controls. The number of *C. neoformans* in the brain increased gradually and significantly in IL-$6^{-/-}$ mice although the blood fungal burden of the IL-$6^{-/-}$ mice was equivalent to that in wild-type mice. These results demonstrated IL-6 played an important role in the host defence against *C. neoformans.*

IL-6 is traditionally considered an activator of acute phase responses and a lymphocyte stimulatory factor. However, there were several studies that showed that IL-6 influences the integrity of the BBB. For example, IL-6 was demonstrated to reduce the transendothelial electrical resistance and to induce changes in the morphology and permeability of endothelial cells in an *in vitro* model of the BBB (de Vries *et al*. 1996; Duchini *et al*. 1996). Moreover, the systemic administration of IL-6 increased the permeability of the BBB in rats (Saija *et al*. 1995). However, we found that lack of IL-6 contributed to the disruption of BBB. Following experiments using IL-6-antibody-treated mice revealed that the BBB permeability increased during *Cryptococcus* meningitis. When directly injecting recombinant human IL-6 into the blood of IL-$6^{-/-}$ mice, the BBB permeability decreased significantly. To know whether the changed BBB permeability showed any effects on the number of *C. neoformans* in brain, we measured the colony forming units of fungus in the brain of infected mice. In accordance with the BBB permeability results, the c.f.u. increased when the BBB permeability elevated while it decreased when the BBB permeability decreased. Taken together, these results indicated that IL-6 decreased the BBB permeability during *Cryptococcus* meningitis. Since all these experiments were performed in mice infected with *C. neoformans* 56 days later, we also detected the permeability of BBB in the beginning of the infection. We found the BBB permeability significantly increased 72 h after the infection, indicating the BBB became more permeable in the very early of the infection (supplementary figure 1).

So far, IL-6 deficiency is not known to be associated with human cryptococcosis. Although previous results found that IL-6 disrupted BBB permeability, our results using IL-6 $^{-/-}$ mice revealed that lack of IL-6 in mice infected with *C. neoformans* allowed a more severe infection in the brain.

Cryptococcus infection was associated with limited inflammation, but low concentrations of proinflammatory cytokines could still be detected (Lortholary *et al.* 2001).We measured the level of pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α in infected mice, and we found that the level of these cytokines increased gradually after the infection (supplementary figure 2). It is normally assumed that pro-inflammatory cytokines may work hand-in-hand to increase inflammation. IL-6, as one of the most important cytokines in inflammation, was involved in the regulation of other cytokines. For example, the level of pro-inflammatory cytokines such as IL-1 and TNF-α failed to increase significantly in IL-6-knock-out mice after turpentine administration (Fantuzzi and Dinarello 1996). The likely mechanism for the loss of response to turpentine observed in IL-$6^{-/-}$ mice is that IL-6 is the critical mediator in the induction of the acute-phase response in the sterile inflammation. As observed with other inflammatory stimulus such as LPS, the level of inflammation-associated cytokines in IL-$6^{-/-}$ mice were unchanged when compared with that in wild-type counterparts, suggesting the production of inflammatory cytokines was mediated by other factors rather than IL-6 in the LPS-induced inflammation. Thus, the production of inflammatory cytokines was not totally dependent on the expression of IL-6. It could depend on various kinds of inflammatory stimuli as well. As shown in our experiments, the production of IL-1 and TNF-α induced by *Cryptococcus* infection was unrelated to the expression of IL-6.

Astrocytes, the macrophages in the brain, are the major source of IL-6 in CNS injury and inflammation (Ma *et al.* 2010). Depletion of tissue macrophages prior to infection enhanced the susceptibility to infection (Monga 1981). Numerous studies confirmed that tissue macrophages played an important role in fungal dissemination (Kechichian *et al.* 2007). In addition, either over-expression of IL-6 or lack of IL-6 is generally detrimental, adding to the pathophysiology associated with CNS disorders, which was probably why infection with *C. neoformans* in IL-$6^{-/-}$ mice was fatal. Dissecting of the downstream signalling pathways of IL-6 affecting vascular permeability will be helpful to better understand the pathophysiology of fungal meningitis.

References

Barr TL, Latour LL, Lee KY, Schaewe TJ, Luby M, Chang GS, El-Zammar Z, Alam S, *et al*. 2010 Blood-brain barrier disruption in humans is independently associated with increased matrix metalloproteinase-9. *Stroke* **41** e123–e128

Beenhouwer DO, Shapiro S, Feldmesser M, Casadevall A and Scharff MD 2001 Both Th1 and Th2 Cytokines Affect the Ability of Monoclonal Antibodies To Protect Mice against Cryptococcus neoformans. *Infect. Immun.* **69** 6445–6455

Bicanic T, Muzoora C, Brouwer AE, Meintjes G, Longley N, Taseera K, Rebe K, Loyse A, *et al*. 2009 Independent association between rate of clearance of infection and clinical outcome

of HIV-associated cryptococcal meningitis: analysis of a combined cohortof 262 patients. *Clin. Infect. Dis.* **49** 702–709

Breen EC, Rezai AR, Nakajima K, Beall GN, Mitsuyasu RT, Hirano T, Kishimoto T and Martinez-Maza O 1990 Infection with HIV is associated with elevated IL-6 levels and production. *J. Immunol.* **144** 480–484

Brett FM, Mizisin AP, Powell HC and Campbell IL 1995 Evolution of neuropathologic abnormalities associated with blood-brain barrier breakdown in transgenic mice expressing interleukin-6 in astrocytes. *J. Neuropathol. Exp. Neurol.* **54** 766–775

de Vries HE, Blom-Roosemalen MC, van Oosten M, de Boer AG, van Berkel TJ, Breimer DD and Kuiper J 1996 The influence of cytokines on the integrity of the blood-brain barrier in vitro. *J. Neuroimmunol.* **64** 37–43

Duchini A, Govindarajan S, Santucci M, Zampi G and Hofman FM 1996 Effects of tumor necrosis factor-alpha and interleukin-6 on fluid-phase permeability and ammonia diffusion in CNS-derived endothelial cells. *J. Investig. Med.* **44** 474–482

Esposito V, Viglietti R, Gargiulo M, Parrella R, Onofrio M, Sangiovanni V, Ambrosino D and Chirianni A 2009 Successful treatment of Cryptococcal meningitis with a combination of liposomal amphotericin B, flucytosine and posaconazole: two case reports. *In Vivo* **23** 465–468

Fantuzzi G and Dinarello CA 1996 The inflammatory response in interleukin-1 beta-deficient mice: comparison with other cytokine-related knock-out mice. *J. Leukoc. Biol.* **59** 489–493

French N, Gray K, Watera C, Nakiyingi J, Lugada E, Moore M, Lalloo D, Whitworth JA, *et al*. 2002 Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS* **16** 1031–1038

Gadient RA and Otten UH 1997 Interleukin-6 (IL-6)–a molecule with both beneficial and destructive potentials. *Prog. Neurobiol.* **52** 379–390

Kechichian TB, Shea J and Del PM 2007 Depletion of alveolar macrophages decreases the dissemination of a glucosylceramide-deficient mutant of *Cryptococcus neoformans* in immunodeficient mice. *Infect. Immun.* **75** 4792–4798

Kim JC, Crary B, Chang YC, Kwon-Chung KJ and Kim KJ 2012 *Cryptococcus neoformans* activates RhoGTPase proteins followed by protein kinase C, focal adhesion kinase, and ezrin to promote traversal across the blood-brain barrier. *J. Biol. Chem.* **287** 36147–36157

Lortholary O, Dromer F, Mathoulin-Pelissier S, Fitting C, Improvisi L, Cavaillon JM and Dupont B 2001 Immune mediators in cerebrospinal fluid during cryptococcosis are influenced by meningeal involvement and human immunodeficiency virus serostatus. *J. Infect. Dis.* **183** 294–302

Ma X, Reynolds SL, Baker BJ, Li X, Benveniste EN and Qin H 2010 IL-17 enhancement of the IL-6 signaling cascade in astrocytes. *J. Immunol.* **184** 4898–4906

Mitchell TG and Perfect JR 1995 Cryptococcosis in the era of AIDS–100 years after the discovery of *Cryptococcus neoformans*. *Clin. Microbiol. Rev.* **8** 515–548

Monga DP 1981 Role of macrophages in resistance of mice to experimental cryptococcosis. *Infect. Immun.* **32** 975–978

Moosa MY and Coovadia YM 1997 Cryptococcal meningitis in Durban, South Africa: a comparison of clinical features, laboratory findings, and outcome for human immunodeficiency virus (HIV)-positive and HIV-negative patients. *Clin. Infect. Dis.* **24** 131–134

Mwaba P, Mwansa J, Chintu C, Pobee J, Scarborough M, Portsmouth S and Zumla A 2001 Clinical presentation, natural history, and cumulative death rates of 230 adults with primary cryptococcal meningitis in Zambian AIDS patients treated under local conditions. *Postgrad. Med. J.* **77** 769–773

Powderly WG 1992 Therapy for cryptococcal meningitis in patients with AIDS. *Clin. Infect. Dis.* **14** S54–S59

Saija A, Princi P, Lanza M, Scalese M, Aramnejad E and De Sarro A 1995 Systemic cytokine administration can affect blood-brain barrier permeability in the rat. *Life Sci.* **56** 775–784

Schreibelt G, Kooij G, Reijerkerk A, van Doorn R, Gringhuis SI, van der Pol S, Weksler BB, Romero IA, *et al*. 2007 Reactive oxygen species alter brain endothelial tight junction dynamics via RhoA, PI3 kinase, and PKB signaling. *FASEB J.* **21** 3666–3676

Starnes HJ, Pearce MK, Tewari A, Yim JH, Zou JC and Abrams JS 1990 Anti-IL-6 monoclonal antibodies protect against lethal *Escherichia coli* infection and lethal tumor necrosis factor-alpha challenge in mice. *J. Immunol.* **145** 4185–4191

Wang J, Homer RJ, Chen Q and Elias JA 2000 Endogenous and exogenous IL-6 inhibit aeroallergen-induced Th2 inflammation. *J. Immunol.* **165** 4051–4061

MS received 16 June 2014; accepted 10 November 2014

Corresponding editor: Satyajit Rath