Predominant Interferon- γ -Mediated Expression of CXCL9, CXCL10, and CCL5 Proteins in the Brain During Chronic Infection with *Toxoplasma gondii* in BALB/c Mice Resistant to Development of Toxoplasmic Encephalitis

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We examined the role of interferon- γ (IFN- γ) in expression of chemokine mRNA and proteins in the brain during chronic infection with *Toxoplasma gondii* using BALB/c and BALB/c-background IFN- γ knockout (IFN- $\gamma^{-/-}$) mice. BALB/c mice are genetically resistant to development of toxoplasmic encephalitis and establish a latent, chronic infection in the brain through IFN- γ -mediated immune responses. Amounts of mRNA for CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CCL2/MCP-1, CCL3/MIP-1 α , and CCL5/RANTES significantly increased in the brains of wild-type mice after infection. CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES mRNA were most abundant among these chemokines. An increase in amounts of mRNA for CXCL10/IP-10, CCL2/MCP-1, CCL3/MIP-1 α , and CCL5/RANTES was also observed in the brains of IFN- $\gamma^{-/-}$ mice after infection, although CXCL10/IP-10 and CCL5/RANTES mRNA levels in infected IFN- $\gamma^{-/-}$ mice were significantly lower than those of infected wild-type animals. Amounts of mRNA for CXCL9/MIG and CXCL11/I-TAC remained at the basal levels in infected IFN- $\gamma^{-/-}$ mice. When amounts of the chemokine proteins were examined in the brain homogenates of uninfected and infected mice of both strains, large amounts of CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES were detected only in infected wild-type animals. These results indicate that CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES are the chemokines predominantly induced in the brains of genetically resistant BALB/c mice during chronic infection with *T. gondii*, and their expression is dependent on IFN- γ .

Introduction

TOXOPLASMA GONDII is an obligate intracellular protozoan parasite that can establish a chronic infection in animals and humans. Chronic infection is characterized by formation of tissue cysts primarily in the brain, and chronic infection with *T. gondii* is one of the most common parasitic infections in humans. It is estimated that 5×10^8 people worldwide are chronically infected with this parasite (Denkers and Gazzinelli 1998). The tissue cysts remain largely quiescent for the life of the host, but can reactivate and cause life-threatening toxoplasmic encephalitis (TE) in immunocompromised patients, such as those with AIDS, neoplastic diseases, and organ transplants (Israelski and Remington 1993; Wong and Remington

1994). Although *T. gondii* has 3 predominant genotypes (I, II and III) and infection with all genotypes occurs in humans, type II is predominant in the strains isolated from patients with TE in North America and Europe (Howe and others 1997; Honore and others 2000; Ajzenberg and others 2002). Therefore, for investigating the mechanisms by which the immune system maintains the latency of chronic *T. gondii* infection and prevent TE, strains of mouse that establish a latent, chronic infection with type II parasite in their brains provide an excellent model. BALB/c is one of those strains.

Interferon- γ (IFN- γ) is the absolute requirement for resistance of BALB/c mice against TE. Both T cells (Wang and others 2004) and non-T cells, most likely microglia/blood-derived macrophages (Kang and Suzuki 2001; Suzuki and

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others 2005), need to produce this cytokine to prevent the disease. IFN- γ activates microglia and astrocytes to prevent proliferation of tachyzoites (Chao and others 1993; Halonen and others 2001). In addition, we recently demonstrated that IFN- γ mediates expression of vascular cell adhesion molecule-1 (VCAM-1) on cerebrovascular endothelial cells during chronic infection with *T. gondii*, and the binding of the endothelial VCAM-1 to $\alpha 4\beta 1$ integrin on CD8⁺ T cells is critical for recruitment of the T cells into the brain (Wang and others 2007). Chemokines, in addition to adhesion molecules, are crucial for T cell entry into various organs (Baggiolini 1998). It is possible that IFN- γ plays an important role in inducing expression of not only VCAM-1 but also chemokines for regulating T cell migration into the brain during *T. gondii* infection. Although an importance of IFN- γ for induction of cerebral expression of mRNA for various chemokines has been demonstrated in acute systemic infection (Strack and others 2002), it is unknown whether IFN- γ is crucial for regulation of chemokine expression in the brain during later stage of infection. In addition, there is no information available on whether *T. gondii* infection induces expression of chemokine proteins in the brain. Thus, in this study, we examined the role of IFN- γ in expression of chemokine mRNA and proteins in the brain during chronic infection with type II *T. gondii* using wild-type BALB/c and IFN- γ -deficient (IFN- $\gamma^{-/-}$) BALB/c mice. We found that large amounts of CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES mRNA and proteins are expressed in the brains of mice during chronic infection, and that their expression requires IFN- γ .

Materials and Methods

Mice and infection with *T. gondii*

Female BALB/c and BALB/c-background IFN- $\gamma^{-/-}$ mice (The Jackson Laboratories, Bar Harbor, ME) were given 10 cysts of the ME49 (type II) strain perorally by gavage (Wang and others 2004). To control the proliferation of tachyzoites in IFN- $\gamma^{-/-}$ mice so that a chronic infection could be established, sulfadiazine-supplemented drinking water (400 mg/L) was given to these animals for 3 weeks beginning 4 days after infection (Wang and others 2007). In one experiment, wild-type BALB/c mice received sulfadiazine beginning 14 days after infection. There were 3–6 mice in each experimental group.

Semiquantitative reverse transcriptase-polymerase chain reaction for detection of chemokine mRNA

At 25 days after infection, RNA was isolated from brains of BALB/c and IFN- $\gamma^{-/-}$ mice and cDNA was synthesized (Kang and Suzuki 2001). Polymerase chain reaction (PCR) for a housekeeping gene (β -actin) (Suzuki and others 2005) and chemokines (CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CCL2/MCP-1, CCL3/MIP-1 α , CCL4/MIP-1 β , and CCL5/RANTES) (Hamilton and others 2002; Strack and others 2002) was performed with 5 μ L of undiluted cDNA reaction mixture with a Geneamp 9700 thermocycler (Applied Biosystems, Foster City, CA) as described previously (Kang and Suzuki 2001; Suzuki and others 2005). Reverse transcriptase (RT)-PCR products were viewed with gel electrophoresis, and homology of PCR products for each chemokine, except for CXCL11/I-TAC, to the predicted transcript sequence was examined by Southern blot analysis (Strack and others 2002; Suzuki and

others 2005). PCR for CXCL10/IP-10 and CCL5/RANTES was also performed with 5 μ L of 1:10 diluted cDNA. The quantification of chemokine mRNA was normalized to the β -actin level.

Real-time RT-PCR for CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES and for IFN- γ and tumor necrosis factor- α

To quantify the amounts of mRNA for CXCL9/MIG, CXCL10/IP-10, CCL5/RANTES, IFN- γ , and tumor necrosis factor- α (TNF- α) in the brains of infected BALB/c and IFN- $\gamma^{-/-}$ mice, cDNA prepared from their brains was applied for real-time PCR. The reactions for CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES were performed using StepOne Plus thermalcycler (Applied Biosystems) and AmpliTaq Gold PCR Master Mix (Applied Biosystems) with SYBR Green (Fine and others 2003; Komatsu and others 2008). To confirm amplification specificity, PCR products were subjected to a melting-curve analysis. Real-time RT-PCR for IFN- γ (Couper and others 2009) and TNF- α was performed using the same machine but with 6-FAM-labeled probes. The primers and probe for TNF- α were 5' GACCCTCACACTCAGATCATCT TCT 3' (sense), 5' GCGCTGGCTCAGCCACTC 3' (antisense), and 5' TAGCCACGTCGTAGCAAACCAACCA 3' (probe). The quantification of mRNA was normalized to the β -actin level, which was measured using a commercial kit (Applied Biosystems).

ELISA for detecting chemokine proteins in brain homogenates

At 23–26 days after infection, a half of each brain collected from BALB/c and IFN- $\gamma^{-/-}$ mice was homogenized in 0.5 mL of RPMI1640 medium (Sigma Chemical Co., St. Louis, MO) containing 10% fetal bovine serum (Sigma Chemical Co.). The homogenates were then sonicated on ice for 15 s six times. Concentrations of CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CCL2/MCP-1, CCL3/MIP-1 α , and CCL5/RANTES in the brain sonicates were measured using ELISA kits (R&D Systems, Minneapolis, MN).

Statistical analysis

Numerical data are presented as mean $\pm$ standard deviation. Student's *t*-test or Alternative Welch *t*-tests were used to evaluate differences between treatment groups.

Results

Expression of mRNA for chemokines in the brains of BALB/c and IFN- $\gamma^{-/-}$ mice during chronic infection with *T. gondii*

We first examined the amounts of mRNA for CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CCL2/MCP-1, CCL3/MIP-1 α , CCL4/MIP-1 β , and CCL5/RANTES in the brains of IFN- $\gamma^{-/-}$ and wild-type BALB/c mice using semiquantitative RT-PCR at 25 days after infection. IFN- $\gamma^{-/-}$ mice were treated with sulfadiazine for 3 weeks from day 4 after infection to establish a chronic infection (Kang and Suzuki 2001; Kang and others 2003; Wang and others 2005); unless treated, these animals die within 2 weeks after infection with uncontrolled proliferation of tachyzoites (Scharton-Kersten and others 1996, 1997). A group of wild-type mice

also received sulfadiazine beginning 14 days after infection. Uninfected animals of both strains were used as controls. None or only trace amounts of mRNA for most of the chemokines were detected in the brains of uninfected IFN- $\gamma^{-/-}$ and wild-type mice (Fig. 1A). Infection with *T. gondii* caused a marked increase in amounts of mRNA for CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CCL2/MCP-1, CCL3/MIP-1 α , and CCL5/RANTES in the brains of wild-type mice ($P < 0.05$) (Fig. 1A). mRNA levels for CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES were relatively more abundant than those for CXCL11/I-TAC, CCL2/MCP-1, and CCL3/MIP-1 α (Fig. 1A). Amounts of CCL4/MIP-1 β mRNA slightly increased in these animals after infection, but such increase was not significant when compared to its expression levels in uninfected animals (Fig. 1A). Treatment with sulfadiazine did not alter the expression level of the chemokines in infected wild-type mice (Fig. 1).

In IFN- $\gamma^{-/-}$ mice, expression of mRNA for CXCL10/IP-10, CCL2/MCP-1, CCL3/MIP-1 α , and CCL5/RANTES significantly increased in their brains after infection, as observed in wild-type animals (Fig. 1A; $P < 0.05$). However, mRNA levels for CXCL10/IP-10 and CCL5/RANTES in infected IFN- $\gamma^{-/-}$ mice were lower than those in infected wild-type mice (Fig. 1A, B). The difference in mRNA levels for CCL5/RANTES between the strains of mice was not detectable in PCRs with undiluted cDNA (Fig. 1A), but the difference became detectable in the reaction with 1:10 diluted cDNA (Fig. 1B; $P < 0.005$). The difference in mRNA levels for CXCL10/IP-10 was also more clearly visible in reactions

with 1:10 diluted cDNA than those with undiluted cDNA ($P < 0.05$ with undiluted cDNA [Fig. 1A] and $P < 0.0001$ with 1:10 diluted cDNA [Fig. 1B]). Expression of mRNA for CXCL9/MIG and CXCL11/I-TAC stayed in the basal levels after infection in IFN- $\gamma^{-/-}$ mice (Fig. 1), and the amounts of mRNA for these 2 chemokines in infected IFN- $\gamma^{-/-}$ mice were significantly less than those of infected wild-type mice (Fig. 1; $P < 0.05$). These results indicate that CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES are major chemokine mRNA induced in the brain of TE-resistant BALB/c mice during chronic infection with *T. gondii*, and among these 3 chemokines, CXCL9/MIG mRNA expression requires IFN- γ . mRNA for CXCL10/IP-10 and CCL5/RANTES can be induced in the absence of IFN- γ after infection, but IFN- γ is essential for their optimum expression.

Quantification of mRNA for CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES in the brains of BALB/c and IFN- $\gamma^{-/-}$ mice during chronic infection with *T. gondii*

To further confirm the differences in the amounts of mRNA for the 3 dominant chemokines, CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES, between the brains of infected wild-type BALB/c and IFN- $\gamma^{-/-}$ mice, we measured the amounts of these chemokine mRNA by real-time RT-PCR. CXCL9/MIG mRNA levels in the brains of infected IFN- $\gamma^{-/-}$ mice were as low as those of uninfected animals: $<0.1\%$ of the amounts detected in infected wild-type mice ($P < 0.0001$,

FIG. 1. Expression of mRNA for chemokines in the brains of IFN- $\gamma^{-/-}$ and wild-type BALB/c mice chronically infected with *Toxoplasma gondii*. IFN- $\gamma^{-/-}$ and wild-type mice were infected with 10 cysts of the ME49 strain perorally. The IFN- $\gamma^{-/-}$ mice were treated with sulfadiazine for 3 weeks beginning 4 days after infection. One group of infected wild-type animals was also treated with sulfadiazine for 11 days beginning 14 days after infection. All mice were sacrificed 25 days after infection, and the brains were analyzed for mRNA for a housekeeping gene (β -actin) and chemokines (CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CCL2/MCP-1, CCL3/MIP-1 α , CCL4/MIP-1 β , and CCL5/RANTES) by semiquantitative reverse transcriptase (RT)-polymerase chain reaction with 5 μ L of undiluted (A) or 1:10 diluted (B) cDNA (see Materials and Methods section). Brains of uninfected animals of each strain were used as controls. Each column represents 1 mouse. IFN- γ , interferon- γ .

FIG. 2. Quantification of mRNA for CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES in the brains of IFN- $\gamma^{-/-}$ and wild-type BALB/c mice chronically infected with *Toxoplasma gondii*. IFN- $\gamma^{-/-}$ and wild-type mice were infected, and cDNA was prepared from the brains of these animals as described in Fig. 1. Amounts of mRNA for CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES were measured by real-time reverse transcriptase (RT)-polymerase chain reaction (see the Materials and Methods section). Brains of uninfected animals of each strain were used as controls. * $P < 0.0001$, ** $P < 0.0005$, and *** $P < 0.005$ when compared to infected BALB/c mice. IFN- γ , interferon- γ .

Fig. 2). In contrast, the amounts of CXCL10/IP-10 and CCL5/RANTES mRNA in infected IFN- $\gamma^{-/-}$ mice were significantly greater than those of uninfected animals ($P < 0.05$; Fig. 3). However, such mRNA levels in infected IFN- $\gamma^{-/-}$ mice were significantly lower than those of infected wild-type animals (Fig. 2): 8.3% ($P < 0.0005$) and 27.1% ($P < 0.005$) of the wild-type animals for CXCL10/IP-10 and CCL5/RANTES, respectively. These results are consistent with our observation in semiquantitative RT-PCR shown in Fig. 1 and clearly indicate that CXCL9/MIG mRNA expression in the brain after infection with *T. gondii* requires IFN- γ , whereas mRNA for CXCL10/IP-10 and CCL5/RANTES can be induced at reduced levels in the absence of IFN- γ after infection.

Quantification of mRNA for IFN- γ and TNF- α in the brains of BALB/c and IFN- $\gamma^{-/-}$ mice during chronic infection with *T. gondii*

It has been shown that CXCL10/IP-10 and CCL5/RANTES expression can be induced by TNF- α in astrocytes *in vitro*

(Barnes and others 1996; Croitoru-Lamourey and others 2003), whereas CXCL9/MIG expression requires IFN- γ (Farber 1997). Since CXCL10/IP-10 and CCL5/RANTES mRNA expression was observed at reduced levels in the brains of infected IFN- $\gamma^{-/-}$ mice, we examined whether TNF- α mRNA is expressed in the brains of IFN- $\gamma^{-/-}$ mice after infection using real-time RT-PCR. As shown in Fig. 3, considerably large amounts of mRNA for TNF- α were detected in the brains of chronically infected IFN- $\gamma^{-/-}$ mice. Their mRNA levels reached to 45% of those detected in infected wild-type mice, although such levels were still significantly lower than those of the wild-type animals ($P < 0.005$; Fig. 3). In contrast, IFN- γ mRNA was detected only in infected wild-type mice, as expected (Fig. 3).

Quantification of chemokine proteins in the brains of BALB/c and IFN- $\gamma^{-/-}$ mice during chronic infection with *T. gondii*

We next examined whether chemokine proteins are expressed in the brains of chronically infected mice, and whether

FIG. 3. Quantification of mRNA for IFN- γ and TNF- α in the brains of IFN- $\gamma^{-/-}$ and wild-type BALB/c mice chronically infected with *Toxoplasma gondii*. IFN- $\gamma^{-/-}$ and wild-type mice were infected, and cDNA was prepared from the brains of these animals as described in Fig. 1. Amounts of mRNA for IFN- γ and TNF- α were measured by real-time reverse transcriptase (RT)-polymerase chain reaction (see the Materials and Methods section). Brains of uninfected animals of each strain were used as controls. * $P < 0.000005$ and ** $P < 0.01$ when compared to infected BALB/c mice. IFN- γ , interferon- γ ; TNF- α , tumor necrosis factor- α .

FIG. 4. Expression of chemokine proteins in the brains of IFN- γ ^{-/-} and wild-type BALB/c mice chronically infected with *Toxoplasma gondii*. IFN- γ ^{-/-} and wild-type mice were infected as described in Fig. 1. At 23–26 days after infection, homogenates of the brains were prepared from the animals and the amounts of CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CCL2/MCP-1, CCL3/MIP-1 α , and CCL5/RANTES proteins in the brain homogenates were measured by ELISA (see Materials and Methods section). Brain homogenates of uninfected animals of each strain were used as controls. There were 3 uninfected and 6 infected mice in each strain, and the data shown are the mean values $\pm$ standard deviations in each group. * P < 0.005, ** P < 0.0005, and *** P < 0.0001 when compared to infected IFN- γ ^{-/-} mice in panel A. * P < 0.001 when compared to infected IFN- γ ^{-/-} mice in panel B. IFN- γ , interferon- γ .

the chemokine protein levels differ between the brains of infected IFN- γ ^{-/-} and wild-type mice. The amounts of CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CCL2/MCP-1, CCL3/MIP-1 α , and CCL5/RANTES proteins were measured by ELISA in the homogenates of the brains of animals at 23–26 days after infection. CXCL9/MIG, CXCL10/IP-10, CCL2/MCP-1, CCL3/MIP-1 α , and CCL5/RANTES protein levels were significantly greater in infected than in unin-

fected wild-type mice (Fig. 4A, B). Consistent with the mRNA expression shown in Fig. 1, CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES proteins were most abundant among these chemokines in the brains of infected wild-type mice (Fig. 4A). Especially, CXCL9/MIG was most dominant and its level was 10 and 5.6 times greater than those of CXCL10/IP-10 and CCL5/RANTES, respectively (Fig. 4A). Small amounts of CCL2/MCP-1 and CCL3/MIP-1 α were

also detected in infected wild-type animals, but the amounts of these chemokines were only 2.0% and 0.18% of the amount of CXCL9/MIG, respectively (Fig. 4A, B).

In the brains of infected IFN- γ ^{-/-} mice, expression of the 3 major chemokines, CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES, was markedly impaired and their amounts were <10% of the amounts detected in the brains of infected wild-type mice (Fig. 4A; $P < 0.005$): 0.91% (0.260 versus 28.7 ng/mL) for CXCL9, 3.65% (0.088 versus 2.41 ng/mL) for CXCL10, and 9.1% (0.452 versus 4.99 ng/mL) for CCL5. CCL2/MCP-1 protein levels were also less in infected IFN- γ ^{-/-} than in wild-type mice (Fig. 4B; $P < 0.001$), although levels of this chemokine in the wild-type animals were low as mentioned above. CCL3/MIP-1 α protein levels were low and did not differ between infected wild-type and IFN- γ ^{-/-} mice (Fig. 2B). CXCL11/I-TAC proteins were detected in uninfected and infected mice of both strains, and amounts of this chemokine protein did not differ between the strains and between uninfected and infected animals of the same strain (Fig. 4A). These results indicate that CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES are the chemokine proteins predominantly induced in the brain of TE-resistant BALB/c mice during chronic infection with *T. gondii*, and their expression is largely dependent on IFN- γ .

Discussion

We investigated the role of IFN- γ in expression of chemokines at both mRNA and protein levels in the brain during chronic infection with type II *T. gondii* in BALB/c and IFN- γ ^{-/-} mice. Using semiquantitative RT-PCR, we observed significant increases of mRNA for CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CCL2/MCP-1, CCL3/MIP-1 α , and CCL5/RANTES in the brains of infected wild-type mice, as compared to uninfected wild-type animals. Among these chemokines, CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES were relatively most abundant. Quantification of the chemokine proteins demonstrated that CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES are those predominantly expressed in the brains of chronically infected wild-type BALB/c mice. To our knowledge, this is the first evidence of expression of chemokine proteins in the brain of *T. gondii* infected hosts.

In contrast to wild-type BALB/c mice, we detected only low levels of all the chemokine proteins in the brains of infected IFN- γ ^{-/-} mice. Levels of CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES proteins, which are most abundant in the brain of infected wild-type animals, were limited in the IFN- γ ^{-/-} mice only at 0.91%, 3.65%, and 9.1% of the levels in the wild-type animals, respectively. These results correlated with mRNA levels of each of these chemokines (<0.1%, 8.3%, and 27.1%, respectively) measured by real-time RT-PCR. Thus, IFN- γ is crucial for inducing expression of these 3 chemokines in both mRNA and proteins in the brain during the chronic stage of infection with *T. gondii*. Because of the time course (the chronic stage of infection) of this study, in addition to IFN- γ itself, mediators induced by IFN- γ could also be involved in expression of these chemokines. However, regardless of a direct or indirect effect, it is clear that IFN- γ plays a critical role in expression of CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES in the brains of mice chronically infected with *T. gondii*. The importance of IFN- γ in chemokine expression in the brain during chronic infection with this parasite was not reported before.

It was previously reported that TNF- α can induce expression of CXCL10/IP-10 and CCL5/RANTES in astrocytes *in vitro* (Barnes and others 1996; Salmaggi and others 2002). We detected TNF- α mRNA in the brains of infected IFN- γ ^{-/-} mice as high as a half of the levels expressed in infected wild-type BALB/c mice. Therefore, this TNF- α expression is most likely a reason for the induction of expression of CXCL10/IP-10 and CCL5/RANTES in the brains of infected IFN- γ ^{-/-} mice, although their expression levels are less than those in infected wild-type animals. In support of this possibility, the amount of TNF- α mRNA in each individual of infected IFN- γ ^{-/-} mice correlated well with the amounts of CXCL10/IP-10 and CCL5/RANTES mRNA expressed in each individual (data not shown).

In relation to our finding on the importance of IFN- γ in expression of chemokine mRNA and proteins in the brains during chronic infection with *T. gondii*, Strack and others (2002) previously reported that IFN- γ ^{-/-} mice failed to express mRNA for CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES and expressed reduced levels of mRNA for CCL2/MCP-1, CCL3/MIP-1 α , and CCL4/MIP- β in their brains during acute systemic toxoplasmosis (10 days after infection). Thus, along with the finding in this study, IFN- γ plays an important role in inducing chemokine expression during both the acute and chronic stages of *T. gondii* infection, whereas its effects on the expression seems to differ between the acute and chronic stages of infection.

In an acute systemic toxoplasmosis model, CXCL10/IP-10 expression was observed in astrocytes, whereas CXCL9/MIG and CCL5/RANTES expression was in microglia (Strack and others 2002). These glial cells could be the source of these chemokines during the chronic stage of infection as well. *In vitro* studies demonstrated that human and simian astrocytes express CXCL9/MIG and CCL5, in addition to CXCL10/IP-10, following stimulation with IFN- γ plus TNF- α (Croitoru-Lamoury and others 2003). Since both IFN- γ and TNF- α are expressed in the brains of mice chronically infected with *T. gondii*, it is possible that astrocytes, in addition to microglia, produce these chemokines in their brain. Active proliferation of tachyzoites does not occur in the brains of genetically resistant BALB/c mice during the chronic stage of infection. In addition, these animals have only small numbers (less than a few hundred in the entire brain) of tissue cysts, the dormant stage of the parasite, in their brains (Suzuki and others 1993; Brown and others 1995). Therefore, a majority of the cells producing the chemokines in the brains of chronically infected BALB/c mice would probably not be those infected with the parasite.

Our previous study demonstrated an importance of IFN- γ for recruiting CD8⁺ immune T cells into the brains of chronically infected BALB/c mice (Wang and others 2007). CXCR3, the receptor for CXCL9/MIG and CXCL10/IP-10, is expressed predominantly on activated T cells, and this chemokine receptor is considered to play the primary role in recruitment of effector T cells into the site of a type I immune response. Since IFN- γ production by T cells, especially CD8⁺ T cells, that migrated into the brain is required for maintaining the latency of chronic *T. gondii* infection in the brain and genetic resistance of BALB/c mice to development of TE (Wang and others 2004), IFN- γ -mediated expression of CXCL9/MIG and CXCL10/IP-10 in the brains of infected BALB/c mice, as revealed in the present study, could play a crucial role in recruitment of IFN- γ -producing effector T cells into the brain for prevention of TE during the chronic stage of infection.

CCL5/RANTES, another chemokine abundantly expressed in the brain of chronically infected BALB/c mice, is 1 of 3 ligands that bind to CCR5. In addition to CXCR3, CCR5 is preferentially expressed on T_H1 cells and plays an important role in infiltration of these T cells in T_H1-type reactions (Moser and Loetscher 2001). CCR5 is also expressed on macrophages, which are important effector cells activated by IFN- γ to prevent proliferation of *T. gondii* tachyzoites (Suzuki and others 1988). Therefore, expression of CCL5/RANTES in combination with CXCL9/MIG and CXCL10/IP-10 could play an important role in recruiting T cells to maintain a type I immune response and facilitating migration of effector macrophages into the brain to control the parasite.

CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES play nonredundant roles in resistance against viral infection in the brain (Liu and others 2000, 2001; Ure and others 2005). During the acute stage of *T. gondii* infection, CXCL10/IP-10 plays an important role in inducing massive influx of T cells into the lungs and livers and controlling the parasite in these organs (Khan and others 2000). CCR5 is important for natural killer (NK) cell trafficking into the spleen and liver and host survival after acute acquired infection (Khan and others 2006), whereas NK cells do not appear to be required for prevention of TE during the later stage of infection (Kang and Suzuki 2001). Therefore, CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES could induce migration of immune cells into different organs depending on the stages of infection, and the cell populations that migrate into the organs and control the parasite could be different between the stages. During the chronic stage of infection with *T. gondii*, CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES may play important roles in recruiting immune T cells and macrophages into the brain to maintain the latency of infection and to prevent TE.

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Author Disclosure Statement

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