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Toxoplasma Encephalitis in Congenic B10 and BALB Mice: Impact of Genetic Factors on the Immune Response

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Factors which determine the pathogenesis and course of *Toxoplasma* encephalitis are poorly understood. In the present study, the influence of genetic factors in congenic B10 and BALB mice of H-2^q, H-2^k, and H-2^b haplotypes was examined following oral infection with a low-virulence strain of *Toxoplasma gondii* (DX). There were striking differences among these strains. Whereas B10 mice were highly susceptible, BALB mice had a less severe and more protracted disease. In all animals with a fatal outcome, *Toxoplasma* encephalitis was the cause of death. Within the two congenic groups, the major histocompatibility complex haplotype had a strong impact on the disease. The H-2^k haplotype was associated with early death in B10 mice but with a favorable outcome in BALB mice, whereas the reverse was observed for the H-2^q haplotype. These findings indicate that genetically determined factors are critically involved in determining the intracerebral immune response and the course of murine toxoplasmosis. Some of these factors appear to be associated with the major histocompatibility complex haplotype, but significant differences between B10 and BALB mice point to a modulating role of additional genetic loci. Immunohistochemical studies and cytokine analyses of cerebrospinal fluid and serum revealed significant differences in the intracerebral immune response between susceptible and resistant strains. A poor outcome was associated with a large number of intracerebral parasites, significant tissue necrosis, a reduced number of intracerebral CD4$^+$ T cells, low intrathecal tumor necrosis factor levels, and, to a lesser extent, a reduced number of intracerebral CD8$^+$ T cells.

Toxoplasma encephalitis (TE) is a major central nervous system manifestation of AIDS and contributes significantly to morbidity and mortality caused by human immunodeficiency virus infection. It is generally thought that opportunistic TE results from reactivation of the dormant parasite in the brain. However, epidemiologic studies revealed that only 30% of *Toxoplasma gondii*-seropositive human immunodeficiency virus-infected patients will develop TE during their disease (21). It was therefore concluded that not all AIDS patients are at equal risk of developing TE. Since it is known that the risk does not exclusively depend on the patient's immune status, genetic factors of the host immune system may also regulate susceptibility to TE. This hypothesis is supported by experimental studies on toxoplasmosis in inbred strains of mice, which show marked differences in survival depending on the mouse strain (2, 23). In addition, there is evidence that survival may depend on the action of several genes. Candidates are genes of the major histocompatibility complex (MHC), in particular those of the H-2 locus, as well as non-MHC-linked genes (24, 40). To gain more insight into the genetic regulation of susceptibility to toxoplasmosis, we have conducted a comparative study with congenic B10 and BALB mice of H-2^k, H-2^q, and H-2^b haplotypes which were orally infected with a low-virulence strain of *T. gondii* (DX). The distribution of the parasite, the nature of cellular infiltrates, and the production of cytokines in these animals were systematically analyzed. Our results provide strong evidence for an important role of both MHC-encoded and non-MHC-linked genes in the pathogenesis of TE.

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MATERIALS AND METHODS

Experimental procedure. Six- to eight-week-old female congenic mice of B10 and BALB strains were used (Table 1). The animals were kept under specific-pathogen-free conditions before and throughout the study. Mice were orally infected with a low-virulence strain of *T. gondii* (DX) as described previously (29). The parasites were harvested from the brains of mice chronically infected with *T. gondii*. Brain tissue was dispersed in phosphate-buffered saline (PBS). The final concentration of the infectious agents was adjusted to a dose of 10 cysts per 0.5 ml.

Animals were studied in the acute (11 and 21 days postinfection [p.i.]) and chronic (100 and 180 days p.i.) phases of toxoplasmosis. On the indicated days, mice were anesthetized and perfused intracardially with a 0.9% saline solution containing 25,000 IU of heparin per liter. The brain, heart, lung, liver, kidney, and spleen were dissected out. For immunohistochemical studies, blocks of tissue were mounted on thick filter paper with Tissue-Tek O.T.C. compound (Miles Scientific, Naperville, Ill.), snap frozen at −80°C in isopentane (Merck, Darmstadt, Federal Republic of Germany) precooled with dry ice, and stored at −80°C until used for immunohistochemistry.

For conventional histology, organs were fixed in phosphate-buffered 4% paraformaldehyde (pH 7.4) and embedded in paraffin 24 h later. Paraffin sections were stained with hematoxylin and eosin (Merck), periodic acid-Schiff stain, or Giemsa solution.

TABLE 1. Congenic strains of mice used in this study[a]

MHC haplotype	B10 strain	BALB strain
H-2^k	B10.BR	BALB.K
H-2^b	C57BL10/ScSN	BALB.B
H-2^q	B10.G	BALB.G

[a] All mice were obtained from Harlan-Olac, Blackthorne, United Kingdom, except for BALB.G mice, which were a generous gift from the Basel Institute for Immunology, Basel, Switzerland.

In addition, blood was collected from the retroorbital plexus, and cerebrospinal fluid (CSF) (mean volume, 7 μl) was obtained by suboccipital puncture from exsanguinated mice. CSF samples were suspended in 25 μl of PBS (pH 7.6) containing 10% fetal calf serum. Serum and CSF samples were frozen immediately and stored at −80°C until used in cytokine assays. Repeated freeze-thaw cycles were avoided.

The following hybridomas were obtained from the American Type Culture Collection (Rockville, Md.) and kept under standard hybridoma culture conditions: M1/42.3.9.8 HLK (immunoglobulin G2a [IgG2a], H-2, all haplotypes) (31), M5/114.15.2 (I-A; b, d, q haplotypes; IgG2b (4), M1/70.15 (Mac1, CR3, IgG2b) (32), F4/80 (F4/80 antigen, IgG2b) (3), and RA3-3A1/6.1 (B220 antigen, IgM) (7). Supernatants of these hybridomas were collected and stored in aliquots at −80°C. A rabbit antiserum to *T. gondii* was prepared at our institution. The following primary and secondary antibodies or antisera were obtained from commercial sources: CD45 (T200; Boehringer, Mannheim, Federal Republic of Germany), CD5 (Lyt1; Becton-Dickinson, Heidelberg, Federal Republic of Germany), CD4 (L3T4; Becton-Dickinson), CD8 (Lyt2; Becton-Dickinson); I-A^k (Becton-Dickinson), peroxidase-linked sheep anti-rat immunoglobulin F(ab')$_2$ fragments (Amersham-Buchler, Braunschweig, Federal Republic of Germany), biotinylated mouse anti-rat IgG F(ab')$_2$ fragments (Jackson-Dianova, Hamburg, Federal Republic of Germany), rabbit anti-cow glial fibrillary acidic protein (Dakopatts, Hamburg, Federal Republic of Germany), goat anti-rabbit IgG F(ab')$_2$ fragments (Jackson-Dianova), and peroxidase-linked streptavidin-biotin complex (Dakopatts).

Immunohistochemistry. Ten-micrometer cryostat sections of each tissue were fixed in acetone and chloroform for 10 and 7 min, respectively.

For the detection of CD45, CD5, CD4, CD8, B220, I-A, and H-2 antigens, an indirect immunoperoxidase method was employed. Incubation with the appropriately diluted primary antibody for 1 h was followed by application of peroxidase-conjugated sheep anti-rat IgG F(ab')$_2$ fragments (1:60) for 1 h. The same method was used for the detection of *T. gondii*. As secondary antibody, goat anti-rabbit IgG F(ab')$_2$ fragments (1:50, 1 h) were used. Immunostaining of Mac1 (CR3) and F4/80 antigens was performed by using the avidin-biotin complex technique (15). To block endogenous biotin, sections were pretreated with 0.1% streptavidin (Sigma, Deisenhofen, Federal Republic of Germany) and 0.1% D-biotin (Sigma) for 30 min each. They were covered with hybridoma supernatants (1 h), incubated with biotinylated mouse anti-rat IgG F(ab')$_2$ fragments (1:200, 45 min), and finally incubated with peroxidase-linked streptavidin-biotin complex for 30 min.

The peroxidase reaction product was visualized according to the protocol of Graham and Karnovsky (13) with 3,3'-diaminobenzidine tetrahydrochloride as the substrate. Sections were in part lightly counterstained with hemalum and mounted with D.P.X. (Serva, Heidelberg, Federal Republic of Germany).

As a control reaction to exclude unspecific staining in the various immunohistochemical reactions, either an irrelevant antibody of the same immunoglobulin isotype was applied or the primary antibody was omitted in the first step of incubation.

Cytokine assays. Tumor necrosis factor (TNF) and gamma interferon (IFN-γ) were measured in serum and/or CSF of infected mice. Sera of four or five individual animals and pooled CSF samples from five mice of each experimental group were assayed. For detection of these cytokines, commercially available enzyme-linked immunosorbent assays (Genzyme, Boston, Mass.) were used according to the manufacturer's instructions. The detection limits were 100 and 125 pg/ml for TNF and IFN-γ, respectively. All samples were assayed in duplicate, and laboratory standards were included on each plate.

Statistical evaluation. The numbers of CD4$^+$ and CD8$^+$ T cells, the extent of tissue necrosis, and the numbers of tachyzoites and toxoplasma cysts were evaluated quantitatively on immunostained sections in acute (11 and 21 days p.i.) and chronic (100 and 180 days p.i.) TE. Generally, 100 fields were analyzed at high-power magnification. Five mice of each group were evaluated, and the mean and standard error of the mean were calculated. Levels of significance between groups of mice were determined by using Student's *t* test. Statistical significance was defined as $P < 0.05$. Data from cytokine analyses are expressed as mean concentrations ± standard error of the mean, except for pooled CSF, for which data are presented as the mean of two pooled samples.

RESULTS

Following oral infection, all animals developed toxoplasmosis, irrespective of the strain. However, we observed significant differences in the course of the disease and the survival rates. In general, B10 mice were more susceptible to infection with *T. gondii* than BALB mice, which had a more protracted and less severe course of disease.

Mortality. Survival was significantly different for susceptible and resistant strains. B10.BR (H-2^k) mice were most susceptible (Fig. 1a). All animals of this strain developed a severe wasting syndrome, with a mortality rate of 70% at 11 days p.i. C57BL10/ScSN mice (H-2^b) showed an intermediate degree of susceptibility, with mortality rates of 10, 16, and 100% at 11, 21, and 100 days p.i., respectively. Among congenic B10 mice, the B10.G (H-2^q) strain was less susceptible, with mortality rates of 6, 17, and 42% at days 11, 21, and 100 p.i., respectively.

Of three congenic BALB strains (Fig. 1b), the BALB.G (H-2^q) strain was most susceptible, BALB.B (H-2^b) mice showed a moderate susceptibility, and none of the BALB.K (H-2^k) animals succumbed to *T. gondii* infection. Among the BALB mice, only animals of the BALB.G strain died during acute toxoplasma infection. The mortality rates were 3 and 8% at days 11 and 21 p.i., respectively. All surviving BALB.G animals were critically ill at 3 months p.i., with a mortality rate of 68% at 100 days p.i. In contrast to the case with the resistant BALB.K strain, 37.5% of the BALB.B animals died between days 21 and 100 p.i. At later stages, no additional fatalities were seen in this group.

These mortality data demonstrate that the MHC haplotype had a strong influence on outcome within each congenic strain. However, susceptibility to toxoplasmosis was appar-

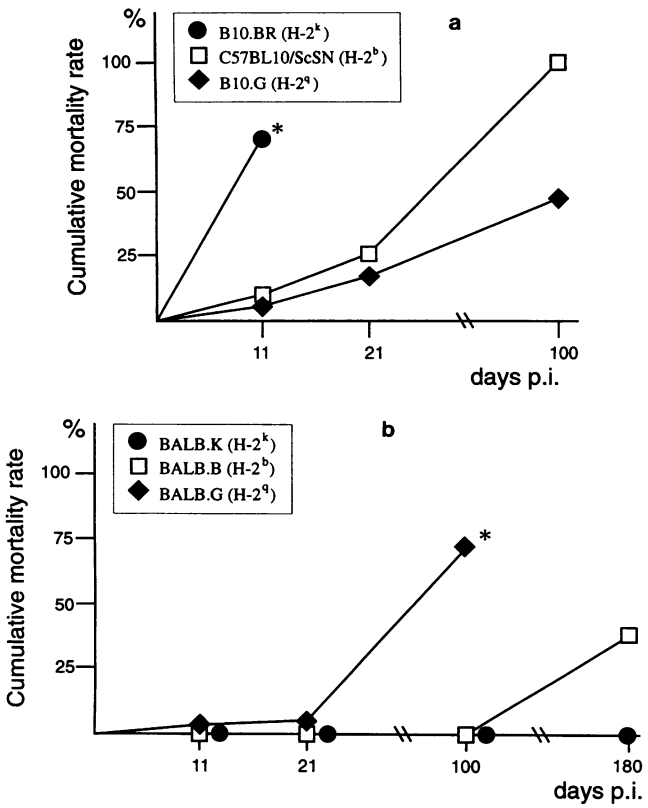

FIG. 1. Cumulative mortality rates for congenic B10 (a) and BALB (b) mice. *, surviving mice were critically ill and were sacrificed.

ently not exclusively linked to the MHC haplotype, since the H-2^k and H-2^q haplotypes showed a significantly different impact on survival in B10 as compared with BALB mice.

Tachyzoites and toxoplasma cysts. There was a close correlation between the parasite load in the brain, disease activity, and mortality (Table 2 and Fig. 2). The highly susceptible B10.BR strain had the largest amounts of free tachyzoites and cysts in their brains at an early stage (11 days p.i.) as evidenced by immunohistochemistry and periodic acid-Schiff staining (Fig. 2a), while at the same stage the moderately and slightly affected C57BL10/ScSN and B10.G mice had only a few extracellular and encysted parasites in the brain ($P < 0.00005$ for both parameters). In addition, intracerebral tachyzoites did not increase further in the less severely affected C57BL10/ScSN and B10.G mice during later stages of disease. Whereas mildly diseased B10.G mice could resist further intracerebral cyst formation, the number of cysts increased in the brains of moderately susceptible C57BL10/ScSN mice in parallel to disease activity ($P < 0.05$) between days 11 and 21 p.i.

Although these differences were less prominent among BALB strains, BALB.G mice exhibited the highest concentrations of tachyzoites and cysts in the brain. Consistent with the more protracted course of disease, during which a full-blown encephalitis had not yet developed at 11 days p.i., differences in parasite numbers between BALB strains reached statistical significance later (21 days p.i.) than for B10 mice. At 21 days p.i., the number of intracerebral cysts paralleled the severity of disease in all BALB strains, and susceptible BALB.G mice had significantly more cysts in their brains than did the resistant BALB.K strain ($P < 0.00005$). Prior to death (100 days p.i.), BALB.G mice displayed both intracerebral tachyzoites and cysts in large amounts ($P < 0.00005$ versus BALB.K and BALB.B) (Fig. 2b and c).

Histopathology. In acute toxoplasmosis, the heart and lung were equally affected in all B10 and BALB strains. In addition, BALB mice developed acute hepatitis and nephritis. The chronic stages of toxoplasmosis manifested predominantly in the brain, whereas pathological changes in other organs resolved. In B10.G mice, the liver and kidney were infiltrated by $CD4^+$ and $CD8^+$ T lymphocytes at 100 days p.i., but *T. gondii* could not be detected in these organs immunohistochemically.

Histopathology revealed striking differences in the intracerebral immune response among the various strains of mice. Cellular and humoral parameters of the host immune reaction were found to strongly correlate with disease susceptibility and outcome. Particularly strong was the impact of intracerebral necrosis and of the amounts of $CD4^+$ and $CD8^+$ T cells on the course of TE.

During acute TE, all strains developed both scattered parenchymal and perivascular inflammatory infiltrates in the brain. These inflammatory foci were composed of $CD4^+$ and $CD8^+$ T cells, B lymphocytes, and macrophages and were commonly associated with toxoplasma antigen. There was a strong and widespread activation of microglia in the brains of all animals, irrespective of the strain and the stage of TE. These cells showed upregulation of Mac1 (CR3) and F4/80

TABLE 2. Numbers of tachyzoites and toxoplasma cysts in the brains of congenic B10 and BALB mice

Strain	No.[a] (mean ± SEM) on day p.i.:							
	11		21		100		180	
	Tachyzoites	Cysts	Tachyzoites	Cysts	Tachyzoites	Cysts	Tachyzoites	Cysts
B10 mice								
B10.BR (H-2^k)	21 ± 1	12 ± 2	—[b]	—	—	—	—	—
C57B110 (H-2^b)	12 ± 1	1 ± 0.5	13 ± 1	4 ± 1	—	—	—	—
B10.G (H-2^q)	15 ± 2	1 ± 0.9	12 ± 1	1 ± 0.7	0 ± 0	0.3 ± 0.2	—	—
BALB mice								
BALB.K (H-2^k)	2 ± 0.3	0 ± 0	1 ± 1	3 ± 0.3	0 ± 0	2 ± 0.5	0 ± 0	1 ± 0.5
BALB.B (H-2^b)	2 ± 0.8	3 ± 0.2	2 ± 0.5	7 ± 1.4	0 ± 0	3 ± 0.9	0 ± 0	2 ± 0.7
BALB.G (H-2^q)	4 ± 0.8	0 ± 0	3 ± 0.9	11 ± 0.3	3 ± 1	59 ± 2	—	—

[a] Numbers of tachyzoites and toxoplasma cysts in 100 high-power fields. Five mice of each group were analyzed by use of immunostained sections.
[b] —, deceased.

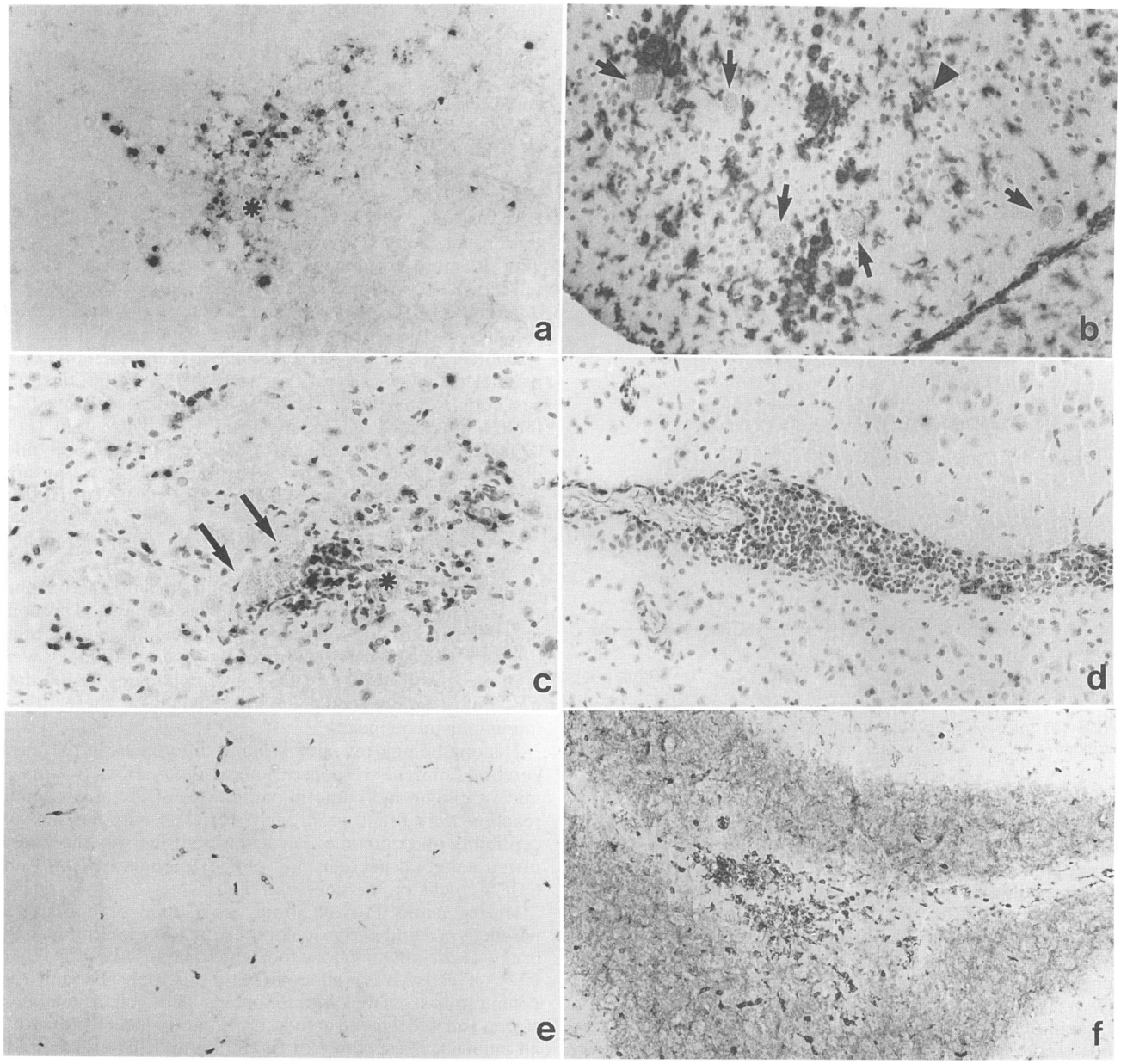

FIG. 2. (a) Brain of a B10.BR (H-2^k) mouse on day 11 of infection. Numerous parasites are associated with a central necrosis (asterisk). Anti-*T. gondii* immunostaining was used. Magnification, ×200. (b) Brain of a BALB.G (H-2^q) mouse on day 100 of infection. Macrophages and activated microglia (arrowhead) are in close contact with multiple toxoplasma cysts (arrows). Mac-1 immunostaining and slight counterstaining with hemalum were used. Magnification, ×150. (c) Brain of a BALB.G (H-2^q) mouse on day 100 of infection. An inflammatory focus with $CD4^+$ T cells surrounds toxoplasma cysts (arrows) and a central necrosis (asterisk). In addition, there are $CD4^+$ T cells which diffusely infiltrate the brain. L3T4 immunostaining and slight counterstaining with hemalum were used. Magnification, ×200. (d) Brain of a BALB.K (H-2^k) mouse on day 100 of infection. Numerous $CD4^+$ T cells in a granuloma. Note the focal character of the immune response, in contrast to panels b and c. L3T4 immunostaining and slight counterstaining with hemalum were used. Magnification, ×200. (e) Brain of B10.BR (H-2^k) mouse on day 11 of infection. A discrete T-cell population is detectable in the brain. Lyt1 immunostaining was used. Magnification, ×100. (f) Cerebellum of a B10.G (H-2^q) mouse on day 11 of infection. T cells contribute to an inflammatory focus. Note the large number of infiltrating T cells in comparison to panel e. Lyt1 immunostaining was used. Magnification, ×150.

antigens as well as prominent expression of MHC class II gene products (Fig. 2b).

The extent of cerebral tissue necrosis (Table 3) correlated with severity of disease and prognosis. At 11 days p.i., large numbers of parasites were associated with significant tissue destruction in susceptible B10.BR mice (Fig. 2a) but not in less susceptible B10.G animals, which were able to resist the parasite-induced tissue destruction. In the moderately affected C57BL10/ScSN strain, necrotic areas increased between days 11 and 21 p.i. and significantly exceeded the changes observed in mildly diseased B10.G mice ($P < 0.005$). At 100 days p.i., all remaining animals exhibited signs of a chronic encephalitis. In resistant B10.G and BALB.K mice, the immune response showed a granulomatous char-

TABLE 3. Numbers of necrotic foci in the brains of congenic B10 and BALB mice

Strain	No.[a] (mean ± SEM) and CD4/CD8 day p.i.:			
	11	21	100	180
B10 mice				
B10.BR (H-2^k)	0.4 ± 0.2	—[b]	—	—
C57B110 (H-2^b)	0.5 ± 0.4	3.0 ± 0.8	—	—
B10.G (H-2^q)	0.0 ± 0.0	0.5 ± 0.3	0.0 ± 0.0	—
BALB mice				
BALB.K (H-2^k)	0.0 ± 0.0	1.0 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
BALB.B (H-2^b)	0.0 ± 0.0	0.7 ± 0.4	0.0 ± 0.0	0.6 ± 0.42
BALB.G (H-2^q)	0.4 ± 0.2	0.5 ± 0.3	4.5 ± 1.0	—

[a] Numbers of necrotic foci in 100 high-power fields. Five mice of each group were analyzed by use of immunostained sections.
[b] —, deceased.

acter (Fig. 2d). These granulomas and perivascular cuffs were composed of T and B cells and macrophages. A granulomatous immune reaction was also evident in moderately affected BALB.B mice, but in this strain, many immune cells were also scattered throughout the brain. In contrast, animals of the severely affected BALB.G strain displayed large numbers of inflammatory cells diffusely infiltrating the brain, whereas granulomas were entirely absent. Although macrophages and CD4$^+$ and CD8$^+$ T cells had established close contact with toxoplasma cysts in these animals, parasites were apparently able to proliferate (Fig. 2b and c). Both tachyzoites and toxoplasma cysts were present. All BALB.G mice showed a severely necrotizing and fatal TE. In BALB.B and BALB.K mice, neither florid tissue destruction nor significant numbers of parasites occurred in the brain at stages later than 21 days p.i. The few intracerebral cysts detectable in the less affected BALB.K, BALB.B, and B10.G strains appeared to reside primarily areactive in the brain, without inducing necrosis or a significant inflammatory reaction. Differences between BALB.G mice and BALB.B and BALB.K mice reached a high level of significance ($P < 0.0005$) at 100 days p.i.

Characterization of T-lymphocyte subsets. (i) CD4$^+$ T cells. At 11 days p.i., significantly lower numbers of CD4$^+$ T cells had invaded the brain in highly susceptible, terminally ill B10.BR mice than in the intermediate- and low-susceptibility C57BL10/ScSN and B10.G strains, respectively ($P < 0.00005$) (Table 4 and Fig. 2e and f). At 21 days p.i., B10.G mice had significantly increased numbers of intracerebral CD4$^+$ T cells, whereas helper T cells dramatically declined in the brains of C57BL10/ScSN mice prior to death and were significantly lower than in B10.G mice ($P < 0.00005$).

CD4$^+$ T cells also declined sharply in the terminal phase of disease in susceptible BALB.G mice, following an increase to very high levels in acute TE (21 days p.i.). In contrast, BALB.K and BALB.B mice had a moderate increase in intracerebral CD4$^+$ T cells during acute TE. These levels persisted at 100 days p.i. and significantly outnumbered CD4$^+$ T cells in BALB.G mice ($P < 0.005$ and $P < 0.025$, respectively). At later stages, beyond 100 days p.i., CD4$^+$ T cells declined in both BALB.K and BALB.B mice in parallel to clinical recovery. Even at this late stage the CD4$^+$ T-cell response significantly exceeded numbers of CD4$^+$ T cells during acute TE.

(ii) CD8$^+$ T cells. At 11 days p.i., intracerebral CD8$^+$ T-cell numbers were low in susceptible B10.BR mice, intermediate in moderately susceptible C57BL10/ScSN mice, and high in less susceptible B10.G mice (Table 4). This difference between B10.BR and B10.G strains was highly significant ($P < 0.00005$) (Fig. 2e and f). Animals which survived this early phase of TE showed a further increase in the CD8$^+$ T-cell response.

A steady increase of intracerebral CD8$^+$ T cells occurred in all BALB strains. Whereas at 11 and 21 days p.i., BALB.K mice displayed lower levels of CD8$^+$ T cells than did the highly susceptible BALB.G strain ($P < 0.05$ and $P < 0.00005$ at 11 and 21 days p.i., respectively), there were no significant differences between the congenic BALB strains at 100 days p.i., and thus BALB.G mice had levels of intracerebral CD8$^+$ T cells comparable to those of the moderately susceptible BALB.B and resistant BALB.K strains. Thereafter, in surviving BALB.B and BALB.K mice, CD8$^+$ T cells declined in parallel to the regression of the disease.

Cytokines. (i) TNF. All B10 (Table 5) and BALB (Table 6) strains produced TNF in response to infection with *T. gondii*, and levels of TNF in CSF exceeded those in serum in all strains. Moreover, there was a correlation between the intrathecal TNF response and survival: while resistant B10.G mice had high titers at 11 days p.i., highly susceptible and critically ill B10.BR mice had lower levels of intrathecal TNF. In B10.G mice, high TNF levels persisted during both acute and chronic TE. In contrast, TNF declined sharply between days 11 and 21 p.i. in susceptible C57BL10/ScSN mice prior to death.

In general, BALB strains produced lower levels of intrathecal TNF than did B10 mice. Intrathecal TNF production also was inversely correlated with susceptibility to *T. gondii*

TABLE 4. Numbers of CD4$^+$ and CD8$^+$ T cells and CD4/CD8 ratio in the brains of congenic B10 and BALB mice

Strain	No.[a] (mean ± SEM) and CD4/CD8 ratio at day p.i.:											
	11			21			100			180		
	CD4$^+$	CD8$^+$	CD4/CD8	CD4$^+$	CD8$^+$	CD4/CD8	CD4$^+$	CD8$^+$	CD4/CD8	CD4$^+$	CD8$^+$	CD4/CD8
B10 mice												
B10.BR (H-2^k)	67 ± 14	40 ± 7	1.6	—[b]	—	—	—	—	—	—	—	—
C57B110 (H-2^b)	197 ± 21	58 ± 10	3.4	165 ± 28	621 ± 57	0.3	—	—	—	—	—	—
B10.G (H-2^q)	238 ± 24	140 ± 11	1.7	871 ± 104	569 ± 91	1.5	820 ± 137	477 ± 45	1.7	—	—	—
BALB mice												
BALB.K (H-2^k)	1 ± 1	1 ± 1	1.0	719 ± 76	631 ± 101	1.1	464 ± 46	1,022 ± 84	0.5	215 ± 53	172 ± 42	1.3
BALB.B (H-2^b)	81 ± 16	88 ± 84	0.9	857 ± 3	1,112 ± 88	0.8	402 ± 36	973 ± 103	0.4	280 ± 50	246 ± 62	1.1
BALB.G (H-2^q)	7 ± 2	7 ± 2	1.0	1,281 ± 105	1,267 ± 121	1.0	245 ± 68	1,133 ± 143	0.2	—	—	—

[a] Numbers of CD4$^+$ and CD8$^+$ T lymphocytes in 100 high-power fields. Five animals of each group were analyzed by use of immunostained sections.
[b] —, deceased.

TABLE 5. TNF and IFN-γ in serum and CSF of congenic B10 mice[a]

Strain and day p.i.	TNF (pg/ml)[b] in:		IFN-γ (pg/ml)[c] in serum (mean ± SEM)
	CSF (mean)	Serum (mean ± SEM)	
B10.BR (H-2^k)			
11	720	400 ± 45	835 ± 55
21	—[d]	—	—
100	—	—	—
C57BL10/ScSN (H-2^b)			
11	1,200	800 ± 322	587 ± 290
21	80	140 ± 61	170 ± 50
100	—	—	—
B10.G (H-2^q)			
11	1,600	400 ± 136	1,110 ± 290
21	1,200	180 ± 40	220 ± 60
100	1,200	200 ± 22	88 ± 52

[a] Values for serum were obtained from five individual animals; values for CSF represent the mean value obtained from the pooled CSF of five animals each.
[b] Detection limit, 100 pg/ml.
[c] Detection limit, 125 pg/ml.
[d] —, deceased.

among the congenic BALB strains. Resistant and moderately susceptible BALB.K and BALB.B animals produced higher TNF levels than susceptible BALB.G mice. Intrathecal TNF levels declined below the detection limit of the assay during chronic stages of TE in BALB mice.

There were no significant differences in serum TNF levels between congenic B10 and BALB strains, and serum TNF levels were not associated with disease activity.

(ii) IFN-γ. Serum IFN-γ levels correlated with systemic disease activity. They were highest in animals of both the B10 (Table 5) and BALB (Table 6) strains at 11 days p.i. and declined thereafter. This decrease was more pronounced in moderately susceptible C57BL10/ScSN mice at 21 days p.i.

TABLE 6. TNF and IFN-γ levels in Sera and CSF congenic BALB mice[a]

Strain and day p.i.	TNF (pg/ml)[b] in:		IFN-γ (pg/ml)[c] in serum (mean ± SEM)
	CSF (mean)	Serum (mean ± SEM)	
BALB.K (H-2^k)			
11	510	80 ± 5	1,432 ± 688
21	390	90 ± 12	244 ± 61
100	Neg[d]	Neg	180 ± 40
180	Neg	Neg	104 ± 36
BALB.B (H-2^b)			
11	655	100 ± 70	2,172 ± 269
21	415	73 ± 72	190 ± 74
100	Neg	Neg	90 ± 4
180	Neg	Neg	108 ± 81
BALB.G (H-2^q)			
11	252	123 ± 77	8,820 ± 2,028
21	150	65 ± 37	116 ± 8
100	Neg	100 ± 57	58 ± 34
180	—[e]	—	—

[a] Values for serum were obtained from five individual animals; values for CSF represent the mean value obtained from the pooled CSF of five animals each.
[b] Detection limit, 100 pg/ml.
[c] Detection limit, 125 pg/ml.
[d] Neg., TNF concentrations were below the detection limit of the assay in all samples analyzed.
[e] —, deceased.

and in highly susceptible, terminally ill BALB.G mice at 100 days p.i. However, these differences did not reach statistical significance.

DISCUSSION

This study demonstrates that genetic factors are major determinants for susceptibility to infection with *T. gondii*. Two genetically different groups of mice infected with *T. gondii* showed a completely different course of disease: B10 mice were highly susceptible, whereas BALB mice were more resistant. In addition to a more protracted and less severe disease in BALB animals than in B10 mice, different organs were affected in the two groups of mice. Whereas in B10 mice the brain, heart, and lung were predominantly involved, BALB mice also developed hepatitis and nephritis. These differences between B10 and BALB strains indicate the involvement of genetic factors which cannot be linked to the MHC haplotype. However, the MHC haplotype also exerted a decisive influence on the disease within the congenic strains of both B10 and BALB mice. Whereas in B10 mice, the H-2^k haplotype was associated with high susceptibility and early death (11 days p.i.), BALB mice of the H-2^q haplotype exhibited the highest mortality. In a study of congenic B10 mice, Suzuki et al. (35) also attributed susceptibility to TE to the B10 (H-2^b) and B10.BR (H-2^k) haplotypes. The differences in susceptibility between animals with H-2^k and H-2^q haplotypes in genetically distinct B10 and BALB mice point to a multigenic regulation of susceptibility outside the MHC. Indeed, a gene linked to the H-13 locus (40) and several other loci, including a gene controlling resistance to *Ectromelia* virus (24), have been implicated in the regulation of susceptibility to *T. gondii*.

In all animal strains included in this study, outcome was determined by the extent of the immune reaction in the brain, and the intracerebral immune response was strikingly different between susceptible and resistant strains. Despite the difference in the genetic background, common prognostic indicators could be identified for both B10 and BALB strains. In all resistant strains, effective control of TE was associated with elevated levels of intracerebral CD4$^+$ T cells, which points to an important protective role of this cell population. In susceptible strains, CD4$^+$ T cells declined sharply to very low levels prior to death, as reflected by a decrease in the CD4/CD8 ratio followed by massive replication and spread of the parasite and severe tissue necrosis. A major regulatory role for CD4$^+$ T cells in the development of resistance to *T. gondii* has also been demonstrated by Vollmer et al. (39): treatment of mice with an antibody to L3T4 (GK 1.5) resulted in a reduction of both the antibody- and cell-mediated immune responses and, subsequently, in a significantly higher number of parasites in the brains of these animals. The decline of CD4$^+$ T cells in severely affected animals bears an interesting parallel to the situation in human AIDS patients, in whom a profound CD4$^+$ lymphocyte deficiency is associated with reactivation of intracerebral *T. gondii* and development of TE. Our histopathological findings of large areas of necrosis and replicating parasites are reminiscent of the case with opportunistic human TE (26, 27, 33). However, the precise mechanism of reactivation of *T. gondii* in human patients is still unknown. There is evidence for a protective role of both CD4$^+$ and CD8$^+$ T cells in TE; however, their exact role is still controversial (17, 38).

In the B10 animals, low levels of CD8$^+$ T cells during acute TE were associated with a poor prognosis. This may

reflect reduced killing of the invading organisms, since $CD8^+$ T cells are cytotoxic for *T. gondii* in vitro (14, 34). During the early stage of TE, the number of intracerebral tachyozites was high in susceptible strains, and there soon was a substantial increase in toxoplasma cysts. In addition, the extent of toxoplasma-induced brain tissue necrosis was closely correlated with susceptibility. These data are in accordance with the findings of Brown and McLeod (5), who demonstrated an association between the number of toxoplasma cysts and MHC class I and II genes in *T. gondii* infection. These authors were able to significantly increase the number of cysts in originally resistant mice by in vivo ablation of $CD8^+$ T cells. In addition, they have demonstrated large increases in cyst number in mice carrying a mutation in Ia, thus providing further support for an important role of the combined action of MHC-restricted $CD4^+$ T cells with $CD8^+$ T cells.

Low numbers of intracerebral $CD8^+$ T cells were associated with a poor prognosis in B10 mice. However, a different situation exists in BALB mice. A potential explanation for this difference is that probably the simultaneous reduction of $CD4^+$ and $CD8^+$ T cells in susceptible B10 mice results in early death, whereas the selective decline of $CD4^+$ T cells in BALB mice may be compensated for temporarily, resulting in a fatal outcome but not before the chronic stage of TE. These findings stress the importance of both $CD4^+$ and $CD8^+$ T cells for a successful immune response to *T. gondii* and are in accordance with data reported by Suzuki and Remington (38) and Gazzinelli et al. (12).

In the present study, a strong association between elevated intrathecal TNF levels and resistance to *T. gondii* was generally observed. Since the gene for TNF resides within the MHC locus (25), these data appear to further support the importance of genetic factors in determining susceptibility to *T. gondii*. The existence of a protective role of TNF in toxoplasmosis is controversial. Freund et al. (11) have correlated resistance to TE with specific restriction length polymorphisms and microsatellite variants in the TNF-α gene and have demonstrated elevated levels of TNF-α mRNA in the brains of susceptible mice. In contrast, resistant animals showed no detectable TNF-α mRNA expression in the brain during chronic TE. On the other hand, there are several recent studies which point to a protective role of TNF-α in TE. Application of TNF-α was able to protect mice against toxoplasmosis (6), while neutralization of TNF-α in murine toxoplasmosis led to death of acutely infected animals (18). TNF-α also mediates toxoplasmastatic activity in murine macrophages in the presence of small amounts of IFN-γ (20). Furthermore, the correlation between intrathecal TNF levels and resistance in the present study favors a protective role of this cytokine in TE.

Experimental studies have shown a unique capacity of TNF-α to orchestrate granuloma formation (30). Amiri et al. (1) have identified an essential role of TNF-α in mediating granuloma formation in schistosomiasis. In vivo depletion of TNF-α has the potential to prevent the formation of granulomas elicited by *Mycobacterium bovis* (19). A granuloma-inducing activity of TNF-α may also operate in our model of TE, since resistant strains with high TNF-α levels displayed locally confined intracerebral granulomas in association with toxoplasma antigen. This reaction was virtually absent in susceptible strains with low intrathecal TNF production. The fact that only intrathecal, but not serum, TNF levels were correlated with outcome stresses the prognostic significance of TE for murine toxoplasmosis. The increased concentration of TNF in the CSF suggests local TNF production in the brain. Primary candidates for the cell of origin are microglial cells which are able to produce TNF in vitro (10). Microglial cells, which are the main target for human immunodeficiency virus in the brain in humans, play a central role in the immune defense against *T. gondii*, and they are strongly activated during both acute and chronic TE as demonstrated by their pronounced upregulation of the CR3 receptor as well as the expression of I-A, ICAM-1, and LFA-1 (9, 28, 29).

Different levels of intracerebral TNF may also reflect differences in factors required for stimulation of macrophages, which are the main source of TNF. However, the concentration of IFN-γ, the primary TNF-α-inducing cytokine with potent macrophage inducing activity, was not correlated with TNF levels in the congenic B10 and BALB strains, and IFN-γ levels did not differ significantly among the various strains. Moreover, activation of macrophages and microglia, the resident macrophage population of the central nervous system, was identical in all strains analyzed, as evidenced immunohistochemically by comparable MHC class II antigen induction and Mac-1 (CD11b/CD18) upregulation. Thus, it appears more likely that distinct genetic regulation of TNF production accounts for the observed differences in susceptibility to TE among the different strains.

IFN-γ, which is derived from $CD8^+$ and also $CD4^+$ T cells, is the major protective cytokine in immunity to *T. gondii* (12, 36). There was a rough correlation between serum IFN-γ levels and susceptibility to TE. However, these differences did not reach statistical significance. Suzuki et al. (37) could not detect differences in the IFN-γ concentration in serum between BALB/c and CBA/Ca mice, which differ significantly in susceptibility during acute and chronic TE. In contrast, McLeod et al. (22) reported significant differences in serum IFN-γ levels between susceptible C57BL/6 and resistant A/J mice. The issue of whether reduced IFN-γ levels contribute to susceptibility in different strains of mice is therefore not yet resolved. Mice with targeted mutations of the IFN-γ or IFN-γ receptor genes, which have recently been generated (8, 16), may provide a potent tool to address this question.

In conclusion, this study demonstrates the critical importance of genetic factors as determinants of susceptibility to toxoplasmosis. A prognostic parameter is the intracerebral immune response, in which the numbers of intracerebral $CD4^+$ and $CD8^+$ T cells, the intrathecal TNF levels, the number of intracerebral parasites, and the extent of brain tissue necrosis appear to play decisive roles. The nature of the genes possibly involved and the potential significance of genetic traits for TE in human AIDS patients remain to be addressed in future studies.

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