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Different Roles for Interleukin-4 during the Course of *Toxoplasma gondii* Infection

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The course of *Toxoplasma gondii* infection from initiation of disease perorally until day 28 postinfection was compared between interleukin-4 (IL-4) gene knockout (IL-4−/−) mice and their wild-type (IL-4+/+) counterparts on a disease-susceptible genetic background. The rate of mortality was significantly greater in mice deficient in IL-4 than in the immunocompetent controls. Although levels of *T. gondii*-specific spleen cell proliferation measured in vitro were similar between groups at all time points examined throughout infection, the quantities of cytokines released into the culture supernatant differed. Culture supernatants from spleen cells derived from IL-4-deficient mice contained significantly more gamma interferon than those derived from IL-4+/+ mice at day 7 postinfection. Conversely, IL-10 production was significantly greater from the spleen cells derived from wild-type mice at day 28 postinfection. Splenocytes from both groups of mice had a marked inhibition of proliferation in response to soluble tachyzoite antigen as well as reduced proliferation in response to concanavalin A between days 7 and 14 postinfection and marked proliferation on days 21 and 28 postinfection. At day 28 postinfection, histological examination of the brains indicated that IL-4+/+ mice had more severe pathological changes and more cysts than IL-4−/− mice. In addition, although many nonencysted single organisms were present in IL-4+/+ mice within both necrotic lesions and microglial nodules, few nonencysted parasites were found, and no necrotic lesions were present in IL-4-deficient animals. These results suggest that the observed reduction in mortality during the early acute phases of infection may be due to the down-regulatory effects of IL-4 or associated Th2-derived products on proinflammatory cytokines such as gamma interferon. However, the long-term effects of IL-4 are detrimental, possibly because of the ability of this cytokine to inhibit proinflammatory antiparasitic products. This may explain the increased parasite multiplication with cysts observed in the brains of IL-4+/+ mice.

Toxoplasma gondii is a protozoan parasite of widespread medical and veterinary importance. Humans can become infected perorally either through the ingestion of sporulated oocysts derived from cat feces or from the tissue cyst stage found in meat products (30). If a woman is pregnant when she is infected for the first time, congenital transmission frequently occurs (14). Irrespective of which of these life cycle stages initiates infection, the parasite undergoes an initial period of rapid multiplication as the tachyzoite stage, in almost every tissue of the host. Around 10 to 15 days postinfection, probably as a result of a developing immune response, preventing further tachyzoite multiplication, parasites transform into bradyzoites, which form tissue cysts predominately in the central nervous system and skeletal muscle. It is the tissue cyst, which is assumed to be present in infected individuals for life, that gives rise to a severe reactivated infection, frequently resulting in encephalitis in immunosuppressed or immunocompromised individuals (38).

In mice as in humans, the course of infection can be divided into an early phase associated with tachyzoite multiplication and a late phase associated with the presence of bradyzoites in tissue cysts. At least five genes, one of which has been mapped to the major histocompatibility complex, have been demonstrated to be of importance in the early stages of infection (1, 40, 57). Control of the late cyst-stage phase of disease has been comprehensively characterized, and genes within the major histocompatibility complex coding for class I molecules are of paramount importance in determining the number of cysts that develop in the brain and whether the mouse develops encephalitis (1, 5, 52). Consistent with a role for class I molecules, cytotoxic $CD8^+$ T cells have been demonstrated to be of major importance in preventing high cyst burdens and toxoplasmic encephalitis (5, 43, 54). Indeed, adoptive transfer and in vivo depletion studies confirm the importance of $CD8^+$ T cells in mediating immunity to both acute infection and the chronic phase of disease (21, 43, 54).

Nevertheless, many other aspects of the immune system, including macrophage and natural killer cell interactions (24, 29), $CD4^+$ Th1 cells (21), and antibody (19), are believed to contribute to resistance against toxoplasmosis. Many of these cells collectively exert their protective effects through the production of proinflammatory cytokines such as interleukin-1 (IL-1) (9, 16), IL-2 (48), IL-12 (34), gamma interferon (IFN-γ) (37, 53), and tumor necrosis factor alpha (2, 31, 49). Conversely, Th2 cells and their products, which down regulate Th1 cell responses and antagonize the production and effects of many Th1 cell products (13, 18, 20, 42), have generally been implicated in promoting disease progression (22, 27, 28). Work done in this laboratory has demonstrated the presence of mRNA transcripts for the Th2-associated cytokine IL-4 in the brains of mice with progressive toxoplasmic encephalitis (28).

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In addition, a recent report demonstrates that mice susceptible to toxoplasmic encephalitis have more IL-10 in their lymph nodes and central nervous systems than their resistant counterparts (27). Furthermore, IL-10 has been shown to inhibit macrophage L-arginine-dependent killing of intracellular tachyzoites (22). Nevertheless, 100% mortality has resulted when IL-10-deficient mice have been infected with *T. gondii* (48a), and recent evidence also suggests that Th2 products such as IL-10 may be essential in limiting the pathology induced in the brain by proinflammatory mediators (6).

The foregoing apparently contradictory observations collectively indicate that the subtle interplay of cytokine products during *T. gondii* infection awaits clarification. The following study was therefore undertaken to establish the role of IL-4 during the early phases of *T. gondii* infection by comparing disease progression in mice genetically deficient in IL-4, and unable to develop a Th2 response (36), with that in their wild-type immunocompetent counterparts.

MATERIALS AND METHODS

Mice. IL-4−/− (129/Sv × C57BL/6)F_2 mice, generated as previously described (36), were bred and maintained at the University of Strathclyde from stock obtained from Hoffmann-La Roche, Basel, Switzerland. Age-matched wild-type mice (IL-4+/+) of the same strain combination obtained from the same source were used as controls in all the experiments. These mice are of the *H-2*^{b} haplotype. Initial experiments compared the survival of both male and female IL-4−/− and IL-4+/+ mice. All other experiments used the less susceptible male mice.

***T. gondii* and infections.** The moderately virulent RRA (Beverly) strain of *T. gondii* maintained in this laboratory by continual passage of infective brain homogenate in outbred Strathclyde Albino mice was used for all experimental infections. Brains were harvested from mice infected 17 to 21 weeks previously and homogenized in phosphate-buffered saline (PBS; pH 7.2) by six passages through a 21-gauge needle, after which cyst concentrations were calculated as previously described (43). Experimental mice were infected with 20 cysts by gavage.

Monitoring infections. Mice were monitored daily for mortality, and at various time points they were killed by terminal anesthesia, their brains removed for histopathological processing, and their spleens were removed aseptically for in vitro T-cell proliferation assays and cytokine analysis.

Histopathological and immunocytochemical analyses. The brain of each mouse was removed immediately after terminal anesthesia and placed in 0.1 M phosphate buffer (pH 7.2) containing 4% formaldehyde. Sagittal sections were cut from both hemispheres of wax-embedded tissue and stained with hematoxylin and eosin (H&E) or immunostained with monoclonal antibody L42 (supplied by E. Petterson), which recognizes both bradyzoite and tachyzoite life cycle stages as previously described. The sections were pretreated with either pronase XXIIV or microwaving prior to testing by the standard two-stage indirect technique using diaminobenzidine as the chromogen.

Preparation of STAg. Soluble tachyzoite antigen (STAg) was prepared from tachyzoites of the RH strain grown in the peritoneums of cotton rats as previously described (45), and the protein concentration was determined by the method of Bradford (4).

Measurement of anti-*T. gondii* specific IgG1 and IgG2a by ELISA. Blood samples were obtained by cardiac puncture preinfection and on days 4, 7, 14, 21, and 28 postinfection. The plasma portion was collected by centrifugation and stored at −20°C until assayed. The enzyme-linked immunosorbent assay (ELISA) was performed by a modification of the procedure described by Voller et al. (56), described fully by Roberts and Alexander (45). Samples were assayed for STAg-specific immunoglobulin G1 (IgG1) and IgG2a in duplicate twofold serial dilutions from 1:100 to 1:204,800. Anti-mouse IgG1 and IgG2a horseradish peroxidase conjugates (Southern Biotechnology Associates, Birmingham, Ala.) were used at dilutions of 1:800 and 1:8,000, respectively. The results obtained are expressed as means ± standard errors (SE) (reciprocal endpoint titers).

T-cell proliferation assays and quantification of *T. gondii*-specific IFN-γ and IL-10 production. T-cell proliferation assays were carried out as previously described (46) on groups of three IL-4+/+ and IL-4−/− mice preinfection and on days 4, 7, 14, 21, and 28 postinfection. After the removal of erythrocytes, 5×10^5 spleen cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 0.05 mM β-mercaptoethanol (Gibco, Paisley, United Kingdom). They were then either left unstimulated or stimulated with concanavalin A (5 μg/ml) or STAg (100 μg/ml). After 60 h of incubation at 37°C, each well was pulsed with 0.25 μCi of tritiated thymidine (specific activity, 35 Ci/mmol; ICN/Flow, High Wycombe, United Kingdom). Supernatants were collected from parallel cultures at this time for quantification of cytokine production. After a further 12-h incubation, cells were harvested onto filter paper (ICN/Flow) by using a cell harvester (Skatronis, Lier, Norway), and thymidine incorporation was measured by liquid scintillation on a β counter (Pharmacia LKB Biotech, Milton Keynes, United Kingdom). The stimulation index was calculated for each individual mouse as mean counts per minute for triplicate stimulated cultures divided by mean counts per minute for triplicate unstimulated cultures. The mean stimulation index for each group of three animals at each time point ± SE was then calculated.

Cytokine assays. Cytokines were measured in the supernatants of STAg-stimulated splenocyte cultures by capture ELISA using the antibody pairs at predetermined concentrations. Microtiter plates were coated overnight at 4°C with capture antibody (2 μg/ml) in PBS (pH 9.0) (rat anti-mouse IL-10 [clone JES5-2A5] or rat anti-mouse IFN-γ [clone R4-6A2]). Following three washes in PBS (pH 7.0) containing 0.05% Tween (Sigma, Poole, United Kingdom), plates were blocked for 1 h at 37°C with PBS (pH 7.0) containing 10% fetal calf serum. Samples were applied in duplicate along with standards consisting of recombinant cytokine (IL-10 [0 to 10 pg/ml] or IFN-γ [0 to 7,000 pg/ml; Cambridge Bioscience, Cambridge, United Kingdom) and incubated for 2 h at 37°C. After a further three washes, biotinylated detecting antibody (rat anti-mouse IL-10 [clone SXC-1] or rat anti-mouse IFN-γ [clone XMG1.2]) was added at a concentration of 1 μg/ml in PBS (pH 7.0) containing 10% fetal calf serum. The plates were incubated for 45 min at 37°C before washing. Streptavidin-alkaline phosphatase (Southern Biotechnology Associates) was added to each well (0.5 μg/ml) for 30 min, after which the wells were washed three times. Binding was visualized with substrate consisting of *p*-nitrophenyl phosphate in glycine buffer. The A_{405} was measured on a Titertek Multiscan plate reader after a 2-h incubation. Cytokine concentrations were determined from the appropriate standard curve.

Statistical analyses. Statistical analyses were performed with the Mann-Whitney U test for comparison of stimulation indices, cytokine production, antibody titers, and brain cyst numbers. The survival data were analyzed by the Mantzel-Haentzel test.

RESULTS

Mortality. In a series of five experiments, IL-4-deficient mice had significantly ($P < 0.025$) poorer survival rates than their wild-type counterparts. This pattern was observed in both male and female mice, though male mice consistently had lower mortality rates than females, as previously reported (47). In a representative experiment (Fig. 1A) using male mice, 100% of IL-4+/+ mice survived following *T. gondii* infection for the 20-day study, while only 83.3% survival was recorded for IL-4−/− mice. Similarly, 50% of female IL-4+/+ mice survived infection, compared with only 23.1% of female IL-4−/− mice (Fig. 1B).

Comparison of *T. gondii*-specific IgG2a responses in IL-4+/+ and IL-4−/− mice infected orally with *T. gondii*. *T. gondii*-specific IgG2a was detectable in the plasma of IL-4+/+ and IL-4−/− mice from 14 days postinfection onward. At all time points, the plasma from IL-4−/− mice contained significantly more *T. gondii*-specific IgG2a than that of the IL-4+/+ mice (days 14, 21, and 28; $P < 0.05$) (Fig. 2). In comparison, IgG1 was undetectable in the plasma of all mice irrespective of whether they were IL-4−/− or IL-4+/+ (results not shown).

Comparison of *T. gondii*-specific T-cell proliferative responses from IL-4+/+ and IL-4−/− mice infected orally with *T. gondii*. A slight increase in the stimulation indices was noted in the spleen cell cultures from both IL-4−/− and IL-4+/+ mice on day 4 postinfection (1.38 ± 0.43 and 1.86 ± 0.63, respectively), but they had decreased significantly below background levels by day 7 postinfection (0.1 ± 0.003 and 0.47 ± 0.18, respectively), indicating that STAg inhibited splenocyte multiplication at this time point (Fig. 3). This marked inhibition of proliferation in response to STAg was again observed at day 14 postinfection in cultures from both IL-4−/− and IL-4+/+ mice (0.033 ± 0.012 and 0.064 ± 0.022, respectively). This period of lymphocyte unresponsiveness to STAg coincided with mitogen unresponsiveness. In contrast, by 21 days postinfection, marked proliferation was observed in the STAg-stimulated splenocyte cultures from both IL-4−/− and IL-4+/+ mice (6.7 ± 2.71 and 4.25 ± 3.93, respectively), although

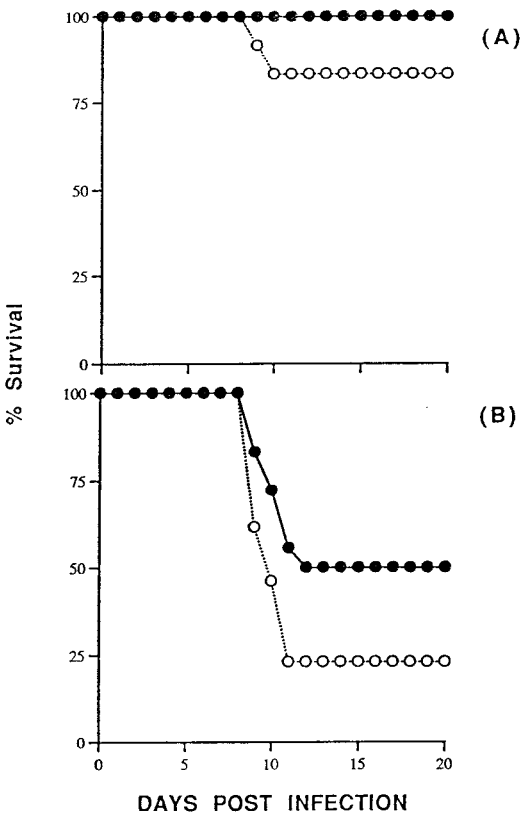

FIG. 1. Percent survival of male (A) and female (B) IL-4+/+ (closed circles) and IL-4−/− (open circles) mice infected orally with 20 *T. gondii* cysts (RRA strain). IL-4+/+ males, $n = 10$; IL-4+/+ females, $n = 18$; IL-4−/− males, $n = 12$; IL-4−/− females, $n = 13$. Data are from a representative experiment of five in which IL-4−/− mice had significantly poorer survival than IL-4+/+ mice ($P < 0.025$).

no significant difference in the magnitude of response was observed between groups at this time point or at day 28, when stimulation indices of 7.72 ± 6.85 and 5.61 ± 2.29 were recorded for IL-4−/− and IL-4+/+ mice, respectively. Mitogen responsiveness was also restored to splenocyte cultures by day 21 postinfection.

Comparison of IL-10 and IFN-γ production by STAg-stimulated spleen cells from IL-4+/+ and IL-4−/− mice infected orally with *T. gondii*. Examination of splenocyte culture supernatants from IL-4−/− and IL-4+/+ mice revealed significant differences in cytokine production in response to in vitro stimulation with STAg (Fig. 4). Splenocytes cultured from spleens of IL-4−/− mice produced more IFN-γ in the early stage of infection than did splenocytes cultured from spleens of IL-4+/+ mice (day 4, 2.1 ± 0.82 and 0.15 ± 0.086 ng/ml, respectively [$P < 0.05$]; day 7, 114.4 ± 30.7 and 25 ± 0.82 ng/ml, respectively [$P < 0.05$]). IFN-γ levels had diminished by day 14 coincident with the inhibited T-cell proliferation observed in parallel cultures at this time. IFN-γ production had increased in IL-4−/− and IL-4+/+ mice by day 21 postinfection (44.3 ± 20.1 and 32.2 ± 11.8 ng/ml, respectively), although not to the same levels as observed in the cultures established 7 days postinfection. By day 28 postinfection, IFN-γ production had decreased to similar levels in both strains of mice (20.1 ± 10.6 and 20.3 ± 5.7 ng/ml, respectively).

Small quantities of IL-10 were present in the supernatants of both IL-4−/− and IL-4+/+ mice at days 4, 7, 14, and 21 postinfection. However, splenocytes isolated 28 days postinfection from IL-4+/+ mice produced significantly greater quantities of IL-10 than those isolated at the same time from IL-4−/− mice (22.15 ± 9.38 and 3.31 ± 2.64 ng/ml, respectively).

Comparison of pathology and development of encephalitis in IL-4+/+ and IL-4−/− mice infected orally with *T. gondii*.

(i) Day 7 postinfection. At day 7 postinfection, the brains of both IL-4+/+ and IL-4−/− mice appeared normal and similar to those of uninfected control mice. There was no evidence of microglial nodules or inflammatory cell infiltration. Careful examination of multiple sections failed to reveal any parasites except for a single small cyst in one of the IL-4−/− mice.

(ii) Day 14 postinfection. At day 14 postinfection, the brains of IL-4−/− mice contained many cysts which were often associated with small microglial nodules containing a few single organisms or small groups of organisms (Fig. 5a and b). There was some cuffing of the blood vessels by inflammatory cells and a moderate inflammatory infiltrate present in the meninges and brain tissue of these animals (Table 1).

At this time point, no significant differences were observed in the number of tissue cysts present in the brains of IL-4−/− and IL-4+/+ mice, although cysts were generally larger in the IL-4-deficient animals (Table 1). A number of microglial nodules containing early cyst structures or single organisms were observed in the brains of all three IL-4+/+ mice (Fig. 5c). Two of the three IL-4+/+ mice examined at this time point had necrotic lesions consisting of a central area of necrosis with cysts and nonencysted organisms toward the periphery. Such lesions were not observed in any of the IL-4−/− mice. There was moderate cuffing of blood vessels by inflammatory cells and a moderate meningitis evident in IL-4+/+ mice (Table 1).

(iii) Day 28 postinfection. At day 28 postinfection, the brains of the IL-4+/+ mice showed severe pathological changes (Fig. 5). All brains contained extremely large numbers of cysts of various sizes which were often present in groups (Fig. 5e). Many of the clusters of cysts were associated with nonencysted organisms. There were large numbers of inflammatory nodules, many of which contained single organisms or parasite debris. Large necrotic lesions containing large masses of free organisms, particularly around the periphery, occupied a significant area of the brain (Fig. 5e to g). There was marked

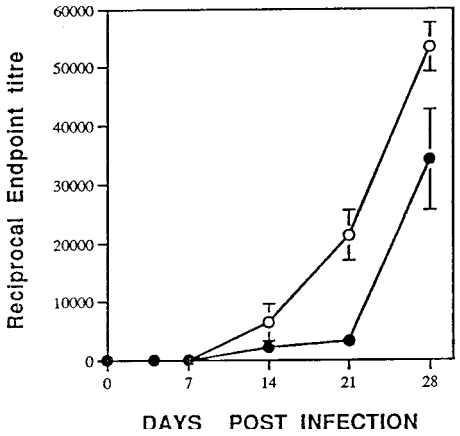

FIG. 2. Comparison of the development of STAg-specific IgG2a in the plasma of IL-4+/+ (closed circles) and IL-4−/− (open circles) mice over 28 days. Mice were infected with 20 *T. gondii* cysts (RRA strain) by the oral route. Results from a representative experiment of two are expressed as mean reciprocal endpoint titer ± SE from three mice of each strain at each time point. IgG2a titers were significantly greater in the plasma of IL-4−/− mice than in that of the IL-4+/+ mice on days 14, 21, and 28 postinfection ($P < 0.05$).

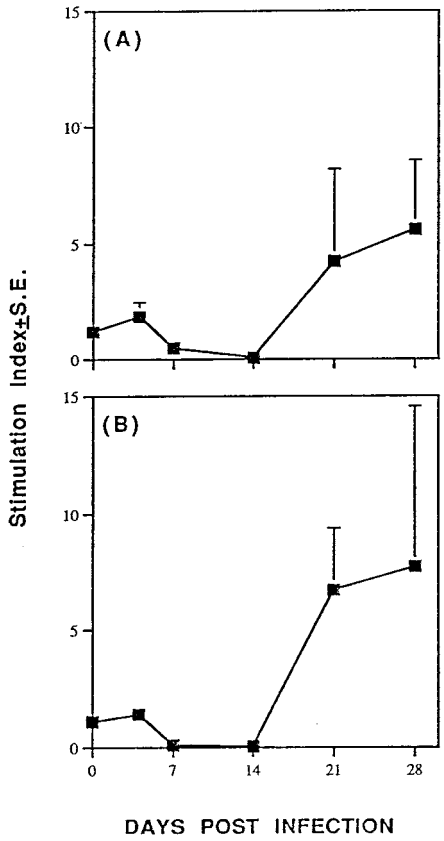

FIG. 3. Comparison of STAg-specific proliferation of spleen cells cultured from IL-4+/+ (A) and IL-4−/− (B) mice at various times postinfection. Mice were infected with 20 *T. gondii* cysts (RRA strain) by the oral route. Results from a representative experiment of three are expressed as mean stimulation indices ± SE from three mice of each strain at each time point. There were no significant differences in the magnitude of stimulation indices at any time point examined.

infiltration of inflammatory cells into the brain tissue, marked meningitis, and perivascular cuffing.

The pathological changes in the brains of the IL-4−/− mice were less severe than those described above for the IL-4+/+ mice. While the cuffing of the blood vessels and variable meningitis were similar to those observed in the IL-4+/+ mice, both the number and size of the microglial nodules were reduced in the brains of IL-4−/− mice. The brains of IL-4−/− mice had no evidence of the large necrotic lesions with numerous tachyzoites that were observed in the IL-4+/+ mice (Table 1). The brains of the IL-4−/− mice had significantly fewer cysts than those of the IL-4+/+ mice (177 ± 78 and 427 ± 101, respectively; $P < 0.05$). Although cysts were often found in groups in the IL-4−/− mice (Fig. 5h and i), these were not associated with a central area of necrosis or free tachyzoites as observed in the IL-4+/+ mice (Fig. 5e to g).

DISCUSSION

The potential multifarious roles of a cytokine such as IL-4 during the course of a parasitic infection are amply demonstrated by these results. During the early phases of a *T. gondii* infection, when the combination of rapidly dividing tachyzoites plus the immune response that this generates can often result in host death, IL-4 plays an ameliorative role and reduces mortality. Conversely, the same cytokine increases parasite multiplication outside cysts, which results in the development of severe necrotic cerebral lesions. These alternating protective and exacerbative effects, which reduce short-term mortality yet increase long-term morbidity, may be attributable to two factors: first, the possible requirement of different immunological conditions to control a parasite with more than one life cycle stage in a single host; and second, the ability of IL-4 directly, and its influence on Th2 expansion indirectly, to inhibit proinflammatory cytokine production and activity (18, 20, 42).

Previous work has indicated that the production of Th2 cytokines such as IL-4 and IL-10 may be a contributory factor leading to death in the acute phases of *T. gondii* infection (27, 28). The evidence from this study does not support these observations and indicates that IL-4, or the ability to generate some form of Th2-type immunity, can significantly reduce mortality. This was observed for both male and female mice, although the latter sex was by far the most susceptible, as previously noted (47). As IL-4 is known to promote humoral rather than classical cellular immunity and antagonize the production of IFN-γ (13), these results may appear somewhat contraintuitive. In fact, results of a recent study using IL-4 neutralizing antibodies appear to contradict the present findings in as far as IL-4-depleted mice exhibited decreased mortality (55). However, antibody subclass analysis by these authors indicated the persistence of a *T. gondii*-specific IgE response, suggesting at best a partial depletion of this cytokine

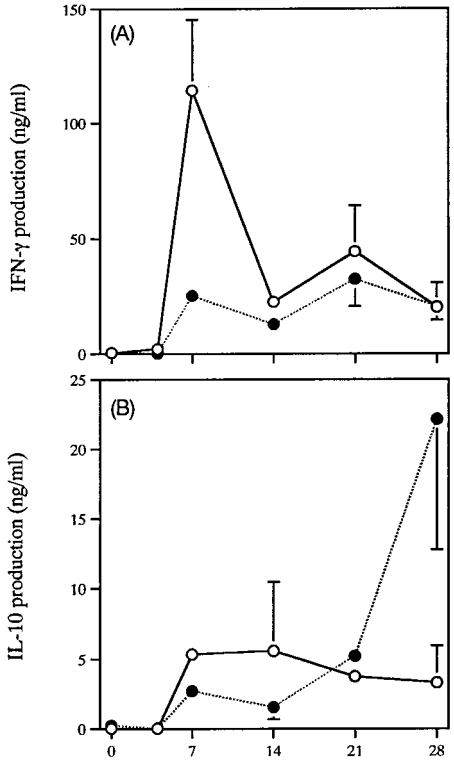

FIG. 4. Comparison of STAg-specific IFN-γ (A) and IL-10 (B) production in the culture supernatants of spleen cells cultured from IL-4+/+ (closed circles) and IL-4−/− (open circles) mice at various time points postinfection. Mice were infected with 20 *T. gondii* cysts (RRA strain) by the oral route. Results from a representative experiment of three are expressed as mean cytokine production ± SE from three mice of each strain at each time point. IFN-γ production was significantly greater in the spleen cell cultures from IL-4−/− mice than in those from the IL-4+/+ mice on days 4 and 7 postinfection ($P < 0.05$). IL-10 production was significantly greater in the spleen cell cultures from IL-4−/− mice than in those from the IL-4+/+ mice on day 28 postinfection ($P < 0.05$).

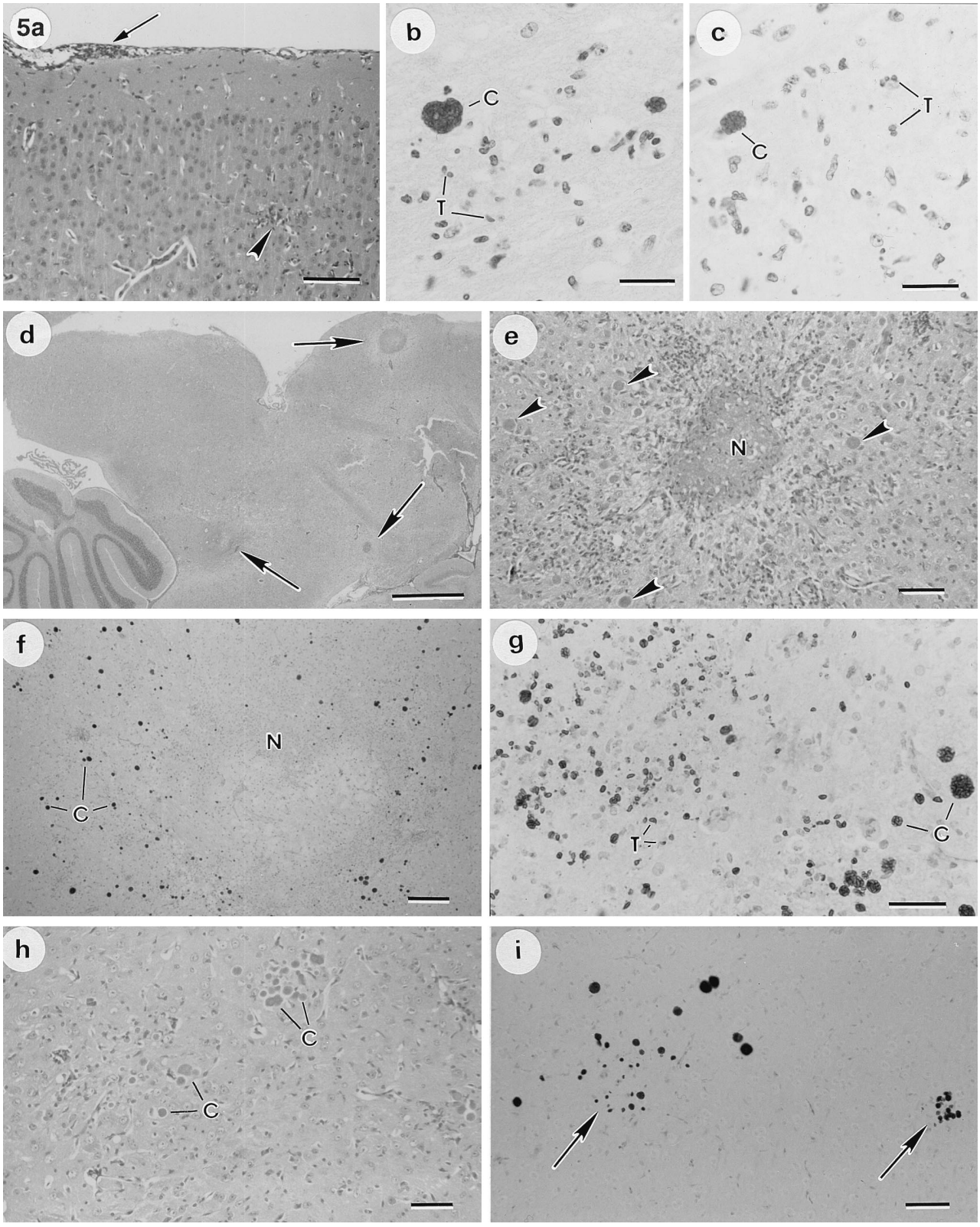

FIG. 5. (a) Section through an IL-4−/− mouse brain at 14 days postinfection showing a small microglial nodule (arrowhead) and a few inflammatory cells within the meninges (arrow). H&E stained; bar = 100 μm. (b and c) Details of immunostained sections of the brains of IL-4−/− (b) and IL-4+/+ (c) mice at 14 days postinfection showing a number of tachyzoites (T) plus small tissue cysts (C). Bar = 10 μm. (d) Low-power micrograph of the brain of an IL-4+/+ mouse at 28 days postinfection showing a number of large necrotic lesions (arrows). H&E stained; bar = 1 mm. (e) Details of a lesion in the brain of an IL-4+/+ mouse at 28 days postinfection showing the area of central necrosis (N) with a number of small tissue cysts (arrowheads) present in the surrounding tissue. H&E stained; bar = 100 μm. (f) Immunostaining of a necrotic lesion showing numerous tissue cysts (C) surrounding the area of central necrosis (N). Bar = 200 μm. (g) Detail from the periphery of a necrotic lesion in an IL-4+/+ mouse at 28 days postinfection showing large numbers of tachyzoite-like organisms (T) and a number of small cysts (C). Immunostained; bar = 10 μm. (h) Section through the brain of an IL-4−/− mouse at 28 days postinfection showing groups of tissue cysts (C) but no evidence of necrosis. H&E stained; bar = 100 μm. (i) Immunostained section of an IL-4−/− mouse at 28 days postinfection showing the presence of numerous tissue cysts often present in clumps (arrows). Bar = 100 μm.

TABLE 1. Summary of histopathological examination of brain sections from IL-4+/+ and IL-4−/− mice 14 and 28 days postinfection[a]

Days postinfection	Mouse strain	No. of:			Score[b]		
		Cysts	Microglial nodules	Necrotic lesions	Perivascular cuffing	Meningitis	Encephalitis
14	IL-4+/+	22	16	2	+++	++	++
	IL-4+/+	26	3	0	++	++	++
	IL-4+/+	27	9	2	+	+	++
	IL-4−/−	41	4	0	++	++	++
	IL-4−/−	26	2	0	++	++	++
	IL-4−/−	50	9	0	+	+	+
28	IL-4+/+	318	15	3	+++	+++	++
	IL-4+/+	335	20	4	+++	+++	++
	IL-4+/+	628	20	2	+++	+++	++
	IL-4−/−	172	10	0	+++	+++	+++
	IL-4−/−	314	12	0	+++	++	++
	IL-4−/−	45	6	0	+++	++	+++

[a] At day 7 postinfection, brains from IL-4−/− and IL-4+/+ mice showed no inflammatory changes, and with the exception of one IL-4−/− mouse, no parasites were observed.

[b] Two sagittal sections, one from each hemisphere, were immunostained for *T. gondii* antigen and counterstained with H&E. The sections from each mouse were examined and scored for perivascular cuffing, meningitis, and encephalitis. +, mild; ++, moderate; +++, severe.

(11). In contrast, IL-4 production has been totally ablated in the gene-disrupted mice used in the present study, and their immune systems have also developed in the absence of this cytokine (36).

While IFN-γ has been shown to have a protective role throughout all phases of *T. gondii* infection (51, 53), it is significant that those mouse strains with the highest mortality rates during acute infection are those producing the most IFN-γ (39). Similarly, in this study we find that the IL-4−/− mice, which produce more IFN-γ early in infection as suggested by enhanced IgG2a production (50) and the ability of their splenocytes to produce more of this cytokine ex vivo, are more susceptible, as measured by mortality in acute toxoplasmosis, than their immunocompetent counterparts. That overproduction of IFN-γ may be detrimental is suggested not only in these studies by the splenocytes of IL-4-deficient mice being able to produce significantly more IFN-γ than their wild-type counterparts but also by the recent observation that IL-10-deficient mice are exquisitely susceptible to disease compared with their immunocompetent counterparts (48a).

IL-4 has been shown to suppress TNF-α production directly (18) and indirectly through down regulation of IFN-γ and IL-2, both of which can induce macrophages to produce TNF-α (17). Furthermore IFN-γ acting in synergy with TNF-α and/or TNF-β may activate macrophages to produce reactive oxygen intermediates (25) and reactive nitrogen intermediates (49), both of which have been shown to be effective in the control of *T. gondii* infection (25, 32, 37, 44). However, these molecules have been shown to be toxic to the host if produced in significantly large quantities (12). It is perhaps for this reason that those mice expressing the highest levels of Vβ5 and responsive to *T. gondii* superantigen-driven IFN-γ production are those most susceptible to toxoplasmosis as measured by mortality (15). IL-4 and Th2-associated cytokines thus may play a crucial role in controlling an excessive inflammatory response.

As in previous studies described by other groups (7, 23), mice infected with *T. gondii* in this study exhibited a period of T-cell nonresponsiveness as measured by lack of proliferation when stimulated with *T. gondii* antigen or mitogen from about days 7 to 14 postinfection. This nonresponsiveness was not affected by either the presence or absence of IL-4 in these mice. Other workers have attributed this unresponsiveness to the presence of nitric oxide (7), IFN-γ (8), IL-10 (35), or IL-12 (26) or the absence of IL-2 (23). We find that T-cell unresponsiveness precedes and accompanies early IFN-γ production in these splenocyte cultures.

With regard to the growth of *T. gondii* in the brain with the ensuing associated pathology, the results reported in this study broadly support previous findings: IL-4 allows increased parasite multiplication and thus limits disease control, which results in increasingly severe pathological changes. Nevertheless, toxoplasmic encephalitis and cyst burden are primarily under major histocompatibility complex control (1, 5, 52), and IL-4 probably plays a comparatively minor contributory role. Thus, encephalitis, meningitis, and microglial nodules are found in both IL-4-deficient and wild-type mice. However, numerous free nonencysted parasites are present throughout infection in the immunocompetent animals, while comparatively few free organisms are found in those mice deficient in IL-4. Severe necrotic lesions containing free parasites also develop in the immunocompetent, but not the IL-4-deficient, mice. IL-4 presumably mediates this effect by down regulating both the production of IFN-γ and its activity (20, 41). Current evidence indicates that IFN-γ prevents cyst rupture, and the use of IFN-γ neutralizing antibodies in vivo consequently induces reactivation and exacerbates encephalitis (51). Whether IFN-γ induces encystment remains to be fully determined, though increasing evidence suggests that IFN-γ induces tachyzoites to express bradyzoite antigens (3). Nevertheless, mice deficient in IL-4 had fewer free observable parasites and developed significantly fewer, though often larger, cysts than their immunocompetent counterparts, suggesting that IL-4 either impeded encystment or allowed sustained reactivation. Our previous studies have demonstrated that in immunocompetent mice, intracerebral mRNA transcripts for IL-4 are present between 5 and 20 days postinfection, and thereafter IFN-γ, IL-2, and tumor necrosis factor alpha are the major transcripts detected in the absence of IL-4 (28). This coincides with a later large reduction in cyst burden (6). In vitro studies have demonstrated the ability of these cytokines to activate microglial cells to kill *T. gondii* via nitric oxide production (10, 32). Later postinfection, at day 70, mRNA transcripts for IL-10 are detected (6). Presumably this cytokine, in its ability to limit IFN-γ production, release, and activity, is thereafter necessary to suppress inflammation and limit pathology as also shown for mice recovering from autoimmune encephalomyelitis (33).

Finally, these experiments comparing IL-4-deficient mice with their immunocompetent wild-type counterparts indicate how the interplay between supposedly inflammatory and regulatory cytokines is delicately balanced. Inappropriate or excessive production of either can increase morbidity and/or mortality. Thus, IL-4 during the course of a single *T. gondii* infection in an individual host can play both disease-protective and exacerbative roles.

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