Control of Parasitemia and Survival During *Trypanosoma brucei brucei* Infection Is Related to Strain-Dependent Ability to Produce IL-4[1]

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We studied non-MHC gene-dependent expression of a number of cytokines in relation to host defense and survival during *Trypanosoma brucei brucei* (Tbb) infection in mice. In particular, the role of IL-4 was explored with use of genomically IL-4-disrupted mice and in vivo Ab blocking. Splenocytes from MHC-identical B10.Q (relatively resistant) mice showed day 5 postinfection higher numbers of IL-4 mRNA expressing cells than C3H.Q (highly susceptible). A trypanosome-derived lymphocyte triggering factor, which is released by Tbb to polyclonally activate $CD8^+$ T cells, stimulated naive splenocytes in vitro to a higher IL-4 response in B10.Q than in C3H.Q mice. The C3H.Q mice developed an extremely high parasitemia, showed a low Ab response against the variant surface glycoprotein (VSG), and had a mean survival time of 42 days. Conversely, B10.Q mice had lower parasitemia, mounted higher anti-VSG response, and had a mean survival time of 56 days. Deletion of the IL-4 gene had no influence on the infection in C3H.Q mice, while in B10.Q mice the deletion was associated with lower anti-VSG Ab levels and higher parasitemia. Paradoxically, B10.Q mice with disrupted IL-4 gene survived longer than the wild type. Anti-IL-4 Ab-blocking experiments in vivo displayed an enhanced parasitemia and prolonged survival in infected B10.Q mice. We conclude that 1) a non-MHC gene-related and $CD8^+$-dependent ability to produce IL-4 partly determines the susceptibility to Tbb infection; and 2) IL-4, although involved in controlling the levels of parasitemia by its effects on immunoglobulin synthesis, also can have toxic effects on the animals. *The Journal of Immunology,* **1996, 157: 3518–3526.**

In experimental infection with the extracellular hemoflagellate *Trypanosoma brucei brucei* (Tbb),[3] $CD8^+$ cells and IFN-γ have been shown to be key elements in the parasite's interplay with its host (1). Tbb releases a molecule, the trypanosome-derived lymphocyte triggering factor (TLTF), which binds to the CD8 molecule on T cells. TLTF-CD8 binding activates the T cells, via tyrosine kinases (2, 3), to cytokine production (4, 5). IFN-γ then promotes growth of the parasites (2, 4, 6). Depletion of $CD8^+$ T cells with Abs in rats and genomic deletion of CD8 in mice (5) reduce levels of parasitemia and prolong survival. This scenario is in contrast to that of intracellular parasites such as *Leishmania* spp. (7) and *Trypanosoma cruzi* (8). In such infections a T cell response with IFN-γ production is critical for host defense. The host defense against extracellular pathogens such as Tbb is in general mostly dependent on B cell responses with production of antimicrobial antibodies (9). Such B cell responses may in turn depend on Th2 cells producing cytokines such as IL-4, IL-5, and IL-6 (10). The elimination of Tbb depends on Ab responses to its variant surface glycoprotein (VSG), where many of these Ab responses are T cell dependent (11). Interestingly, existence of peritoneal VSG-specific $CD4^+$ T cells producing IL-2 and IFN-γ has been demonstrated (12). Tbb can escape this immune response by transcriptional switching among hundreds of VSG genes, resulting in the characteristic waves of VSG-specific parasitemia (13–15). There are well known differences in the control of parasitemia between mouse strains due to their different abilities to mount anti-VSG responses during infection (9). For example, C57B1/6J or B10 mice are good Ab responders and relatively resistant, while C3H/He or C3H mice are poor responders and are highly susceptible (16). These differences are regulated by non-MHC genes, since MHC-identical mouse strains with differing background genomes show dramatic differences in their Ab response (17). Of further interest is that both the susceptible and relatively resistant mouse strains show comparable responses to VSG immunization in the absence of the active infection (18). Thus, active infection, which involves more complex phenomena than VSG immunity alone, is crucial in determining the strain-dependent differences. We here first characterize the cytokine profile and its correlation to parasitemia and death in Tbb-infected MHC-identical mice with background genomes conferring susceptibility or partial resistance. Second, the role of IL-4 is evaluated in the same strains using IL-4 gene-disrupted mice and Ab blocking.

Materials and Methods

Parasites

The pleomorphic strain of Tbb, An Tat 1/1, was obtained from the Laboratory of Serology, Institute of Tropical Medicine, Prins Leopold, Antwerp, Belgium and passaged once in rats before infection.

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[3] Abbreviations used in this paper: Tbb, *Trypanosoma brucei brucei*; TLTF, trypanosome-derived lymphocyte triggering factor; VSG, variant surface glycoprotein.

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Animals and infection procedure

B10.Q mice originated from Professor Jan Klein, Tübingen, Germany and C3H.Q mice from Professor Shreffler, St. Louis, MO. The male IL-4$^{-/-}$ (129/Ola × C57B1/6)F_2 mouse, kindly provided by Dr. Werner Müller, Cologne, Germany (19), was mated with female mice of B10.Q and C3H.Q strains. The offspring were screened for the destroyed IL-4 gene by PCR and repeatedly backcrossed further into each strain. Heterozygous offspring of the third generation, having approximately 90% of the backcross parental genome, were intercrossed. Homozygous offspring (IL-4$^-$) of each intercrossed strain were determined by absence of IgE by ELISA (20). In the experiments, 3- to 4-month-old mice were used, and homozygous mice (B.10.Q/IL-4$^-$ and C3H.Q/IL-4$^-$) were compared with littermates that were homozygous or heterozygous for the wild type of the IL-4 gene showing the presence of IgE (B10.Q/IL-4$^+$ and C3H.Q IL-4$^+$). All mice were kept and bred in a climate-controlled environment with a 12-h light/dark cycle, housed in polystyrene cages containing wood shavings, and fed standard rodent chow and water ad libitum. Each animal was injected i.p. with 0.1 ml of a suspension of trypanosomes in a phosphate saline/glucose buffer, pH 8.0, containing about 10^6 parasites/ml. Every second or third day, the number of parasites in the blood was counted in a Bürker chamber, and the clinical condition was assessed. At day 5 postinfection, mice from each strain were killed to study the number of mRNA-expressing cells among splenocytes (see below) for different cytokines. Spleens from noninfected mice of the different strains were also used for in vitro study of TLTF lymphocyte triggering (for total number of mice used see *Results*).

In vivo blocking of IL-4

For this purpose, the mouse monoclonal anti-IL-4 Ab (11B11) was used (21). The 11B11 Ab was purified from hybridoma supernatant by NH_4SO_4 precipitation. Each B10.Q mouse (10 mice were used) received 0.5 mg of Ab by i.p. injection at day 28 postinfection. At the same time, 10 other mice received an isotype-matched control Ab (H5), which reacts with trinitrophenyl. The hybridoma producing H5 was obtained from Dr. Birgitta Heyman (Uppsala, Sweden), and Abs were prepared from culture supernatants. Ten untreated normal and 10 IL-4$^-$ B10.Q mice were infected in parallel.

Preparation of splenocytes

Mice were killed (10 infected and 10 uninfected control mice from each C3H.Q and B10.Q mouse strain, i.e., 40 mice in total); the spleens were dissected and crushed through a stainless steel meshwork. The cells were washed once in tissue culture medium. The medium consisted of Iscove's modified Dulbecco's medium (Flow Lab, Irvine, U.K.) supplemented with 5% FCS (Life Technologies, Paisley, U.K.), 1% MEM (Flow Lab), 2 mM glutamine (Flow Lab), 50 μg/ml penicillin, and 60 μl/ml streptomycin. Erythrocytes in the cell pellets were hemolyzed by adding 2 ml of cold sterile water for 30 s, followed by the addition of 1 ml of 2.7% saline. The cells were then washed in medium twice and rediluted to obtain a cell concentration of 5 × 10^6/ml.

Single-cell assay for INF-γ secretion

The method described by Czerkinsky et al. (22) as adapted to rat or mouse IFN-γ (23) was used to detect IFN-γ production by single secretory cells. In principle, 96-well microtiter plates in which the bottom consists of nitrocellulose (Millipore, Bedford, MA) were coated overnight with 100-μl aliquots of the mouse mAb DB1, which is specific for IFN-γ at a concentration of 15 μg/ml (24). After repeated washings with PBS, 2% BSA was applied for 2 to 4 h, the plates were washed in PBS, and mononuclear cell suspensions were applied followed by incubation overnight at 37°C in humidified atmosphere of 7% CO_2. Cells were then removed by flicking the plate, followed by repeated washings in PBS. Polyclonal rabbit anti-rat IFN-γ (25), diluted 1/1000, was applied for 4 h. After washing, biotinylated goat anti-rabbit IgG (Vector Lab, Burlingame, CA) was applied for 4 h followed by avidin-biotin-peroxidase complex (ABC Vectastain Elite Kit, Vector Lab). Peroxidase staining with 3-amino-9-ethylcarbazole and H_2O_2 was performed (26). Spots corresponding to cells that had secreted IFN-γ were counted by use of a dissection microscope.

Preparation of TLTF

Trypanosomes were purified from blood of infected Sprague-Dawley rats 5 days postinfection. A pure population of mobile parasites without any contamination of mononuclear cells was obtained (25). This preparation was pelleted and sonicated, and the material was subjected to Ab affinity chromatography using a mouse monoclonal anti-TLTF Ab (MO1), as described previously (2). Eluted TLTF had retained bioactivity in the form of triggering lymphocytes to IFN-γ production after purification, and migrated as a single band at around 43 kDa after SDS-PAGE and silver staining.

Table I. *Probes used for detection of cytokine mRNA expression by in situ hybridization*

Probe	GenBank Acc. No.	Bases		Reference
IFN-γ	M29315	298–345	(exon 1)	(28)
	M29316	80–125	(exon 2)	
	M29317	303–350	(exon 3)	
		180–227	(exon 4)	
IL-4	X16058	83–130	(exon 1)	(29)
		209–256	(exon 2)	
		270–317	(exon 3)	
		331–378	(exon 4)	
TGF-β	X02812	1363–1410	(exon 1)	(30)
		1457–1504	(exon 2)	
		1766–1813	(exon 3)	
		1953–2000	(exon 4)	
IL-10	M37897	79–126	(exon 1)	(31)
		134–181	(exon 2)	
		184–231	(exon 3)	
		402–449	(exon 4)	
IL-12	M86771	147–194	(exon 1)	(32)
	M86672	595–642	(exon 2)	
		190–238	(exon 3)	
		706–753	(exon 4)	
TNF-α	D00478	913–960	(exon 1)	(33)
		2059–2106	(exon 2)	
		2152–2199	(exon 3)	
		2316–2363	(exon 4)	
TNF-β	Y00137	118–165	(exon 1)	(34)
		202–249	(exon 2)	(35)
		342–389	(exon 3)	
		502–549	(exon 4)	
Cytolysin	M37897	238–285	(exon 1)	(36)
		706–753	(exon 2)	
		769–816	(exon 3)	
		878–925	(exon 4)	

Detection of cytokine mRNA expression by in situ hybridization

Splenocytes from infected mice (5 days postinfection) and from noninfected controls were prepared and directly applied onto glass (Probe On; Fisher Scientific, Pittsburgh, PA) at 10^5 cells. A quadriplicate of this cell number was applied on each slide. The cells were left to dry at 55°C for 30 min. Aliquots (200 μl) of splenocyte suspensions from the different noninfected mouse strains were plated in round-bottom microtiter plates at a number of 5 × 10^6 splenocytes/ml medium for in vitro experiments. Cultures received either no stimulus, 10-μl aliquots of Con A (5 μg/ml; Pharmacia, Uppsala, Sweden), or an optimum dilution of TLTF. Thus, after a 24-h incubation, cells were washed in PBS and 10^5 splenocytes from each culture were dried onto restricted areas of microscope slides (Probe On; Fisher Sientific) at 55°C for 30 min. In situ hybridization was performed essentially as described by Dagerlind et al. (27), with ^{35}S-labeled synthetic oligonucleotide probes (Scandinavian Gene Synthesis AB, Köping, Sweden). The labeling was performed with deoxyadenosine 5′-[α-^{35}S]thiotriphosphate (New England Nuclear, Boston, MA) with terminal deoxynucleotidyl transferase (Amersham, Little Chalfont, U.K.). For each cytokine, a mixture of four different oligonucleotide probes were employed in order to increase the sensitivity of the method. The oligonucleotide sequences were obtained from GenBank through the use of the MacVector system (see Table I for details). A constant ratio of the GC content of approximately 60% was employed. The oligonucleotide probes were approximately 48 bases long and checked for the absence of palindromes or long sequence of homology within the species against available GenBank data.

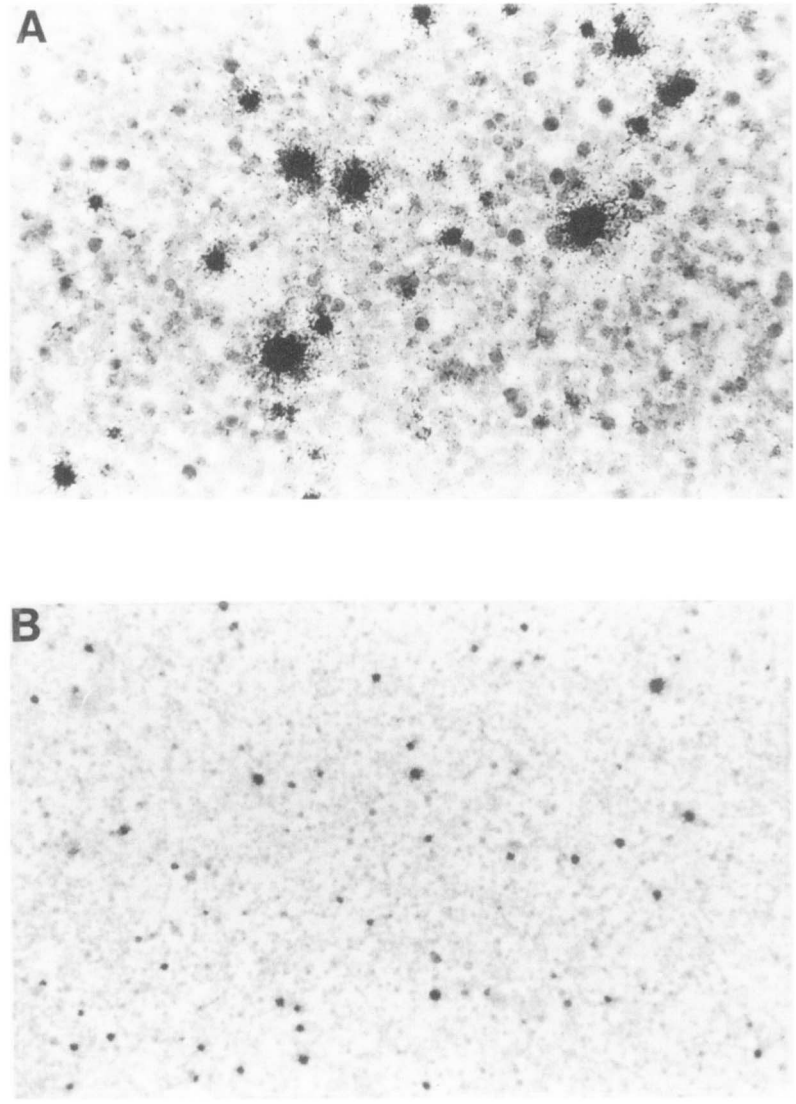

FIGURE 1. Cytokine mRNA expression in splenocytes. In this example, IL-4 mRNA was detected by in situ hybridization, as described in *Materials and Methods*. Splenocytes were prepared from the spleens of B10.Q mice 5 days after infection. *A* and *B* show hybridizing cells in bright field microscopy (×640 and ×160, respectively).

After emulsion autoradiography, slides were coded and cells with more than 10 grains/cell were counted by dark field microscopy. The results were expressed as the number of labeled cells per 10^5 plated cells.

ELISA for anti-VSG Ab responses

Aliquots of 100 μl at different concentrations (0.1, 1, 5, and 10 μg/ml) of VSG from Tbb (An Tat 1.1) were used to coat wells of polystyrene microtiter plates (Nunc, Copenhagen, Denmark) overnight. The plates were washed with PBS, pH 7.4, and the wells were incubated with 0.5% BSA (Sigma) for 24 h at room temperature. After washing with PBS, 100-μl aliquots of different dilutions of sera from infected mice and controls were added for 2 h. After washing with PBS, biotinylated horse anti-mouse IgM (diluted 1/80; Vector) and avidin-biotin-alkaline phosphatase complex (ABC alk P dilution 1/100; Vector) were added sequentially. Enzyme substrate solution was added, and absorbance was measured at 405 nm in a multiscan photometer (Labsystem, Helsinki, Finland).

Statistics

The Mann-Whitney test was used for statistical significance, and the Wilcoxon rank test was used for homogenity over strata for comparison of survival distribution functions.

Results

As a first step, the in vivo expression of cytokine mRNA in splenocytes from C3H.Q and B10.Q mice was assessed 5 days postinfection to study strain-related differences in cytokine production during Tbb infection. Both C3H.Q and B10.Q mouse strains showed similar and strikingly increased numbers of cells expressing mRNA for IFN-γ and TNF-α, while the highly susceptible C3H mice showed higher numbers of TGF-β ($p < 0.001$) and TNF-β ($p < 0.001$) mRNA-expressing cells than the relatively resistant B10.Q mice. In contrast, B10.Q mice showed higher numbers of IL-4 mRNA-expressing cells ($p < 0.005$) than C3H.Q mice. No mRNA-expressing cells for IL-10, IL-12, and cytolysin were detected in any of the mice (Figs. 1 and 2). Uninfected controls from both mouse strains did not show any cytokine expression (data not shown).

The observed in vivo expression of cytokines may result from numerous parasite-induced events including classical Ag-induced triggering of T cells to a cytokine production and polyclonal activation of lymphoid cells by parasite products. It was shown previously that TLTF polyclonally activates lymphoid cells to IFN-γ and TGF-β expression both in vivo and in vitro. This expression is dependent on $CD8^+$ cells (5). It was of interest, therefore, to examine whether there were any strain-dependent differences in TLTF activation of lymphoid cells to IL-4 and IFN-γ expression in vitro. Thus, TLTF was added to splenocyte cultures from naive CH3.Q and B10.Q mice. TLTF induced in both mouse strains similar high numbers of cells expressing mRNA for IFN-γ compared with nonstimulated cells ($p < 0.001$ for both strains) (Fig. 3*A*). However, IL-4 was only induced in the B10.Q mice compared with

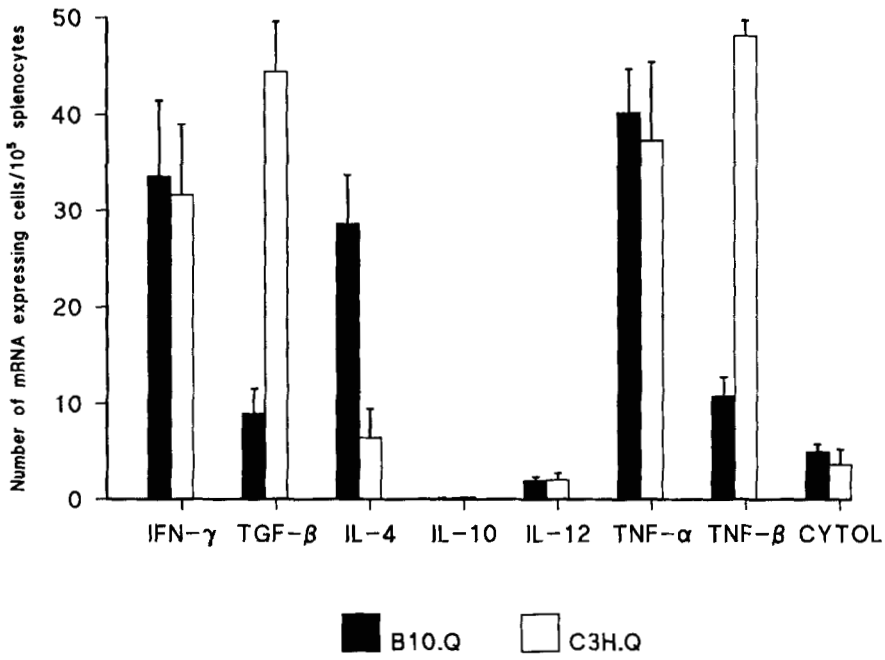

FIGURE 2. Cytokine mRNA expression in vivo. Number of cells expressing mRNA for IFN-γ, TGF-β, IL-4, IL-10, IL-12, TNF-α, TNF-β, and cytolysin was detected by in situ hybridization technique, as described in *Materials and Methods*. Splenocytes were prepared from spleens of B10.Q and C3H.Q mice (10 mice in each group) 5 days after infection. Note the high induction of IL-4 only by the relatively resistant B10.Q mice, while TGF-β and TNF-β are produced in large amounts by the highly susceptible C3H.Q mice. Both mouse strains showed high mRNA expression for IFN-γ and TNF-α. For each mouse, quadruplicate spots were made. Means and SD of 10 mice per group are shown.

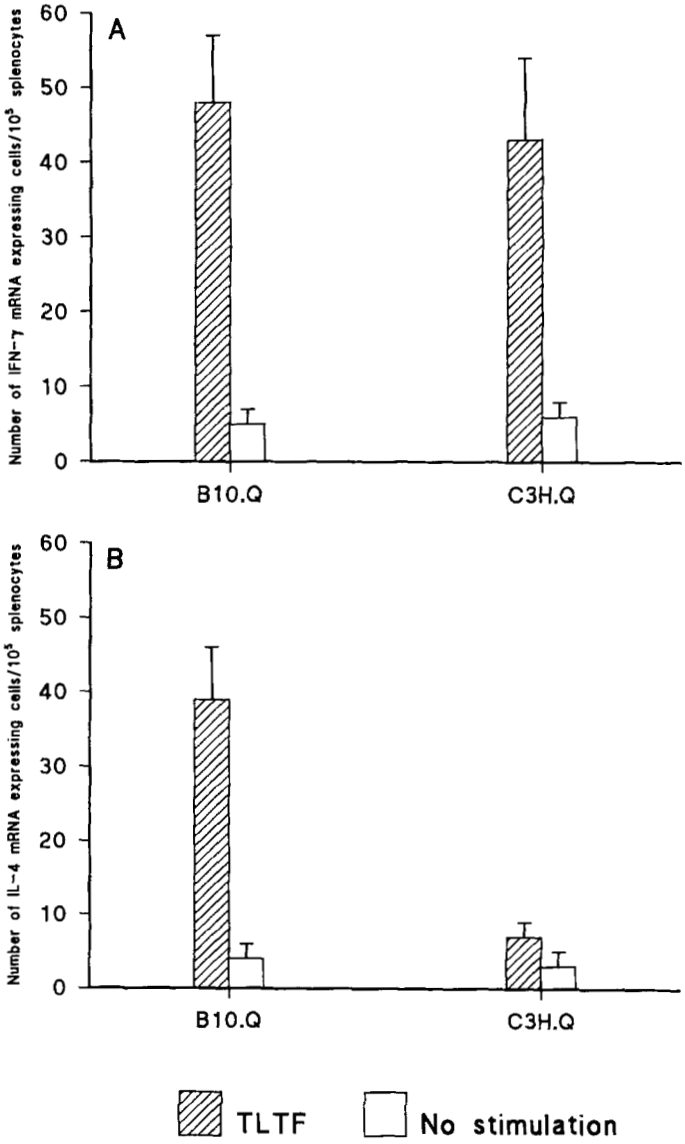

FIGURE 3. Expression of IFN-γ and IL-4 mRNA in vitro. Number of cells expressing mRNA for IFN-γ (*A*) and IL-4 (*B*) was determined after 24 h in the culture of splenocytes from B10.Q and C3H.Q mice. The cultures received either no stimulation or optimally diluted TLTF. Each bar denotes data from quadruplicate cultures of 10 mice. TLTF induced high mRNA expression for IFN-γ in both mouse strains. Note the IL-4 mRNA expression in response to TLTF only by the relatively resistant B10.Q mice. Con A induced high numbers of cells expressing mRNA for IFN-γ and IL-4 in both strains of mice (data not shown). Means and SD of 10 mice per group are shown.

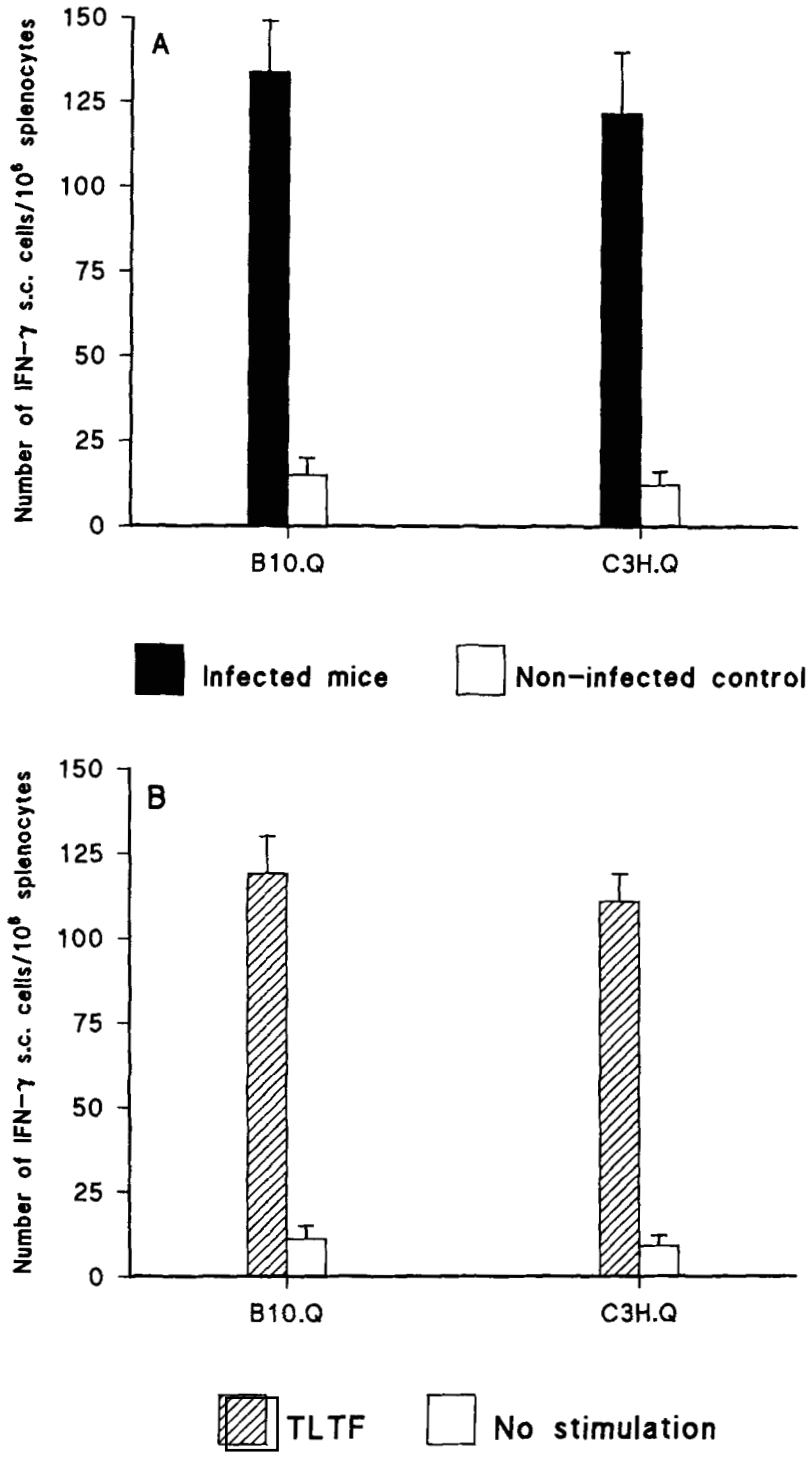

FIGURE 4. Number of IFN-γ secreting cells as determined by the immunospot assay. *A*, Infected mouse splenocytes were prepared 5 days after infection. Splenocytes from B10.Q and C3H.Q (10 mice from each strain) were plated at a number of 10^6 cells per well and cultured for 24 h. Splenocytes from normal noninfected controls were cultured at the same time. Both strains of mice showed increased IFN-γ production compared with noninfected controls. *B*, Normal splenocyte suspensions (from B10.Q and C3H.Q) were plated at a number of 10^6 cells per well and cultured for 24 h. Triplicate cultures were exposed to optimal dilution of TLTF. Control cultures received Con A as a positive control (not shown) or no stimulation. Splenocyte cell suspensions from the spleens of both mouse strains similarly showed high induction of IFN-γ in response to TLTF stimulation. Ten mice were used in each group. Means ± SD of 10 mice per group are shown.

nonstimulated controls or to C3H.Q mice ($p < 0.001$ and $p < 0.002$, respectively) (Fig. 3*B*). Because CD8 is the target for the TLTF, we examined the IL-4 mRNA expression in splenocytes from B10/CD8 knock-out mice. The deletion of CD8 has abrogated the IL-4 mRNA expression in response to TLTF (data not shown).

Because mRNA levels are not necessarily reflected in finally secreted protein, we also measured IFN-γ production by an immunospot assay during infection in vivo and and after TLTF exposure in vitro. Both mouse strains showed a marked increase in the number of splenocyte-secreting IFN-γ at day 5 postinfection ($p < 0.001$ for both strains compared with noninfected controls) (Fig. 4*A*). Splenocytes from noninfected mice of both strains exposed to TLTF for 24 h in vitro also displayed large numbers of cells secreting IFN-γ ($p < 0.001$ for both strains compared to nonstimulated controls) (Fig. 4*B*).

To understand the role of the strain-dependent IL-4 production, we studied the parasitemia and survival time of Tbb-infected normal and genomically IL-4-deleted C3H.Q and B10.Q mice (20 in each group; i.e., 80 total mice). The infected normal C3H.Q mice rapidly developed high levels of parasitemia and had a short survival time after infection with Tbb as compared with infected normal B10.Q mice ($p < 0.03$ and $p < 0.001$, respectively). The

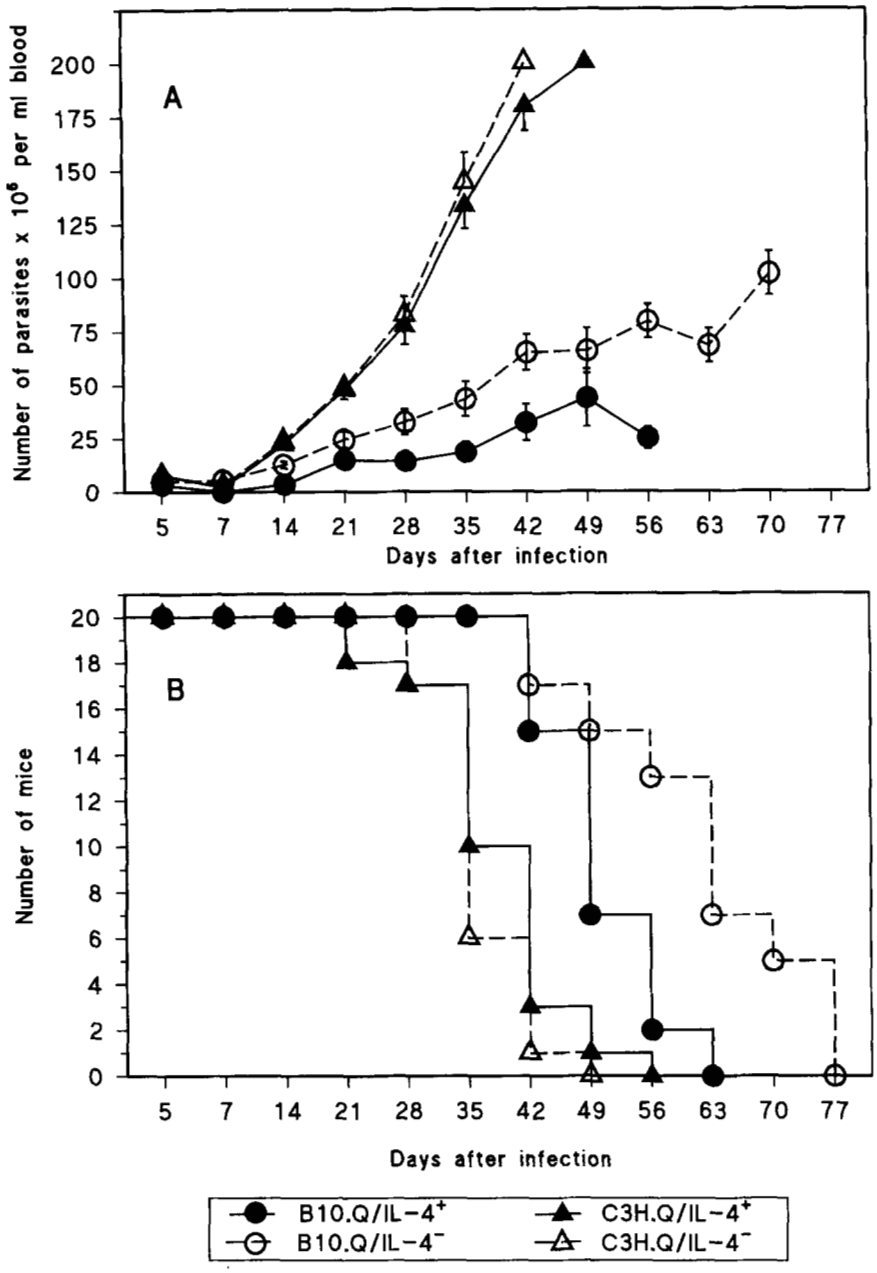

FIGURE 5. *A*, Time course of Tbb parasitemia in normal B10.Q and C3H.Q mice and in IL-4$^-$ mutant mice of both strains. Twenty mice were used in each group. The experiment was repeated twice with similar results. Means ± SD are shown. *B*, Number of mice surviving the Tbb infection. Normal B10.Q and C3H.Q mice and IL-4$^-$ mutant mice from both strains are shown.

deletion of the IL-4 gene had no further effect on the course of infection in C3H.Q mice, while in the B10.Q mice it caused higher parasitemia levels and a paradoxically prolonged survival ($p < 0.01$ and $p < 0.001$, respectively) (Fig. 5, *A* and *B*).

Because the recorded effects of IL-4 gene disruption may be due to ontogenetic adaptation to the defect, we studied the effect of in vivo IL-4 Ab blocking during Tbb infection in B10.Q mice. The anti-IL-4 was given to the B10.Q mice quite late during infection (day 28 postinfection) to allow for observable effects on survival. As in genomically IL-4-deleted mice, there was significantly increased parasitemia in the treated mice compared with untreated wild type-infected controls ($p < 0.01$). Similar to the IL-4 gene-disrupted mice, the anti IL-4-treated mice survived longer ($p < 0.02$) than their IL-4-competent counterparts (Fig. 6*A*). Control experiments with injection of irrelevant isotype-matched mouse mAb did not change the natural course of the Tbb infection (Fig. 6*B*).

Anti-VSG Ab levels in normal and genomically IL-4-disrupted C3H.Q and B10.Q-infected mice were examined. Sera from C3H.Q mice showed low Ab response against the VSG on day 5 postinfection, while sera from B10.Q mice showed a relatively higher anti-VSG Ab response ($p < 0.02$). Deletion of the IL-4 gene in B10.Q IL-4 mice resulted in lower anti-VSG Ab levels as compared with wild-type mice ($p < 0.03$), while the levels did not differ significantly between C3H.Q IL-4$^-$ and IL-4$^+$ mice (Fig. 7).

Discussion

The present study shows both in vivo and in vitro differences in cytokine patterns that are involved in the regulation of Ab production, which mainly determines levels of parasitemia (9, 16). For instance, the more susceptible C3H mice produced high levels of TGF-β and TNF-β during infection. TGF-β exerts suppressive effects on the immune system as demonstrated using genomically TGF-β-deleted mice (37). It also inhibits production of cytokines (38, 39), especially IL-4, IL-5, and IL-6, which regulate B cell proliferation and differentiation (10). TNF-β is a proinflammatory

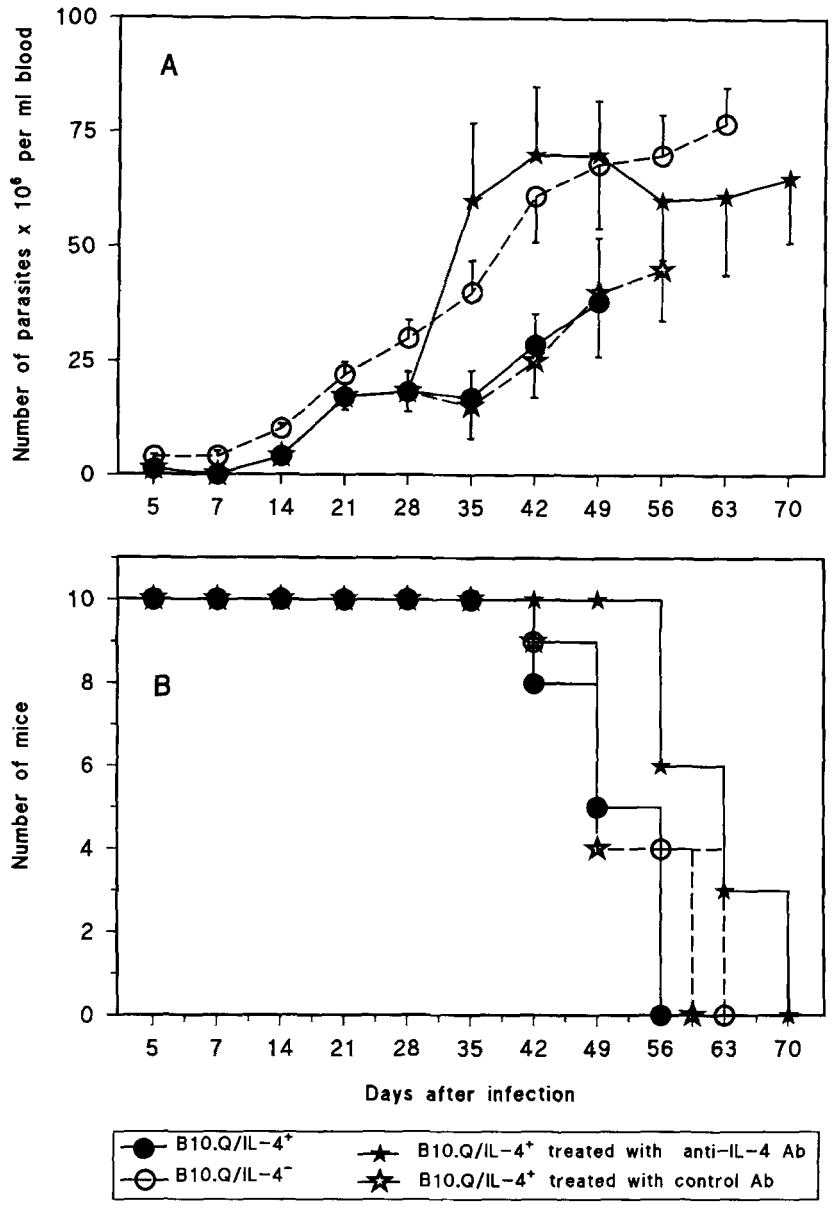

FIGURE 6. Effects of anti-IL-4 Ab treatment in vivo on blood parasitemia (*A*) and on survival (*B*) in B10.Q mice. Note that anti-IL-4 Ab given 28 days postinfection resulted in higher parasitemia and prolonged survival. Controls that received treatment using irrelevant isotype-matched mouse mAbs did not show any changes in the course of the Tbb infection. The experiment was repeated twice with similar results.

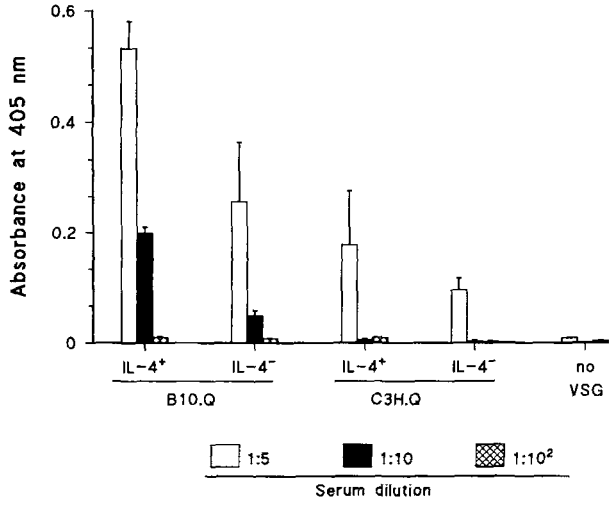

FIGURE 7. *Anti-VSG Ab response during infection as determined by an ELISA technique.*

cytokine that has the ability to induce generation of nitric oxide during parasitic infection (40). Recently, activated macrophages from Tbb-infected mice were shown to release high levels of nitric oxide, which interferes with lymphocyte proliferation and leads to impaired lymphocyte responses and immunosuppression (41). Thus, the observed in vivo TGF-β and TNF-β induction in the highly susceptible C3H strain may add to the immunosuppression that evolved during trypanosomiasis, leading to higher susceptibility in this particular strain. On the contrary, cells expressing IL-4 mRNA, a cytokine needed for Ig synthesis and isotype class switch (42), were induced in higher numbers both in vivo and in vitro upon stimulation with TLTF by the relatively resistant B10.Q strain, but not by the highly susceptible C3H.Q strain. Interestingly, an important difference between the B10 and C3H strains that was confirmed in this study is that only the former is able to mount a VSG-specific Ab response 5 days after infection. Since the main defense mechanism against this extracellular parasite is mediated by Abs (43, 44), it is possible that the ability to produce IL-4 in B10 mice may be casually related to the high levels of

anti-VSG Abs and better host defense in this host. Although B10.Q/IL-4$^{-/-}$ mice have significantly higher numbers of parasitemia compared with B10.Q wild-type mice, they do not show a more marked increase in susceptibility when compared with C3H.Q. This may be related to the still effective-specific anti-VSG Ab that was not totally abrogated by deletion of the IL-4 gene, but was significantly reduced. Also, other genetic factors, which need further investigation, may be involved.

Mutant C3H.Q and B10.Q mice lacking IL-4 (IL-4^{-}) were infected to test directly the role of IL-4 in the host defense. In addition, anti-IL-4 Ab treatment of infected B10.Q mice was performed to exclude the possibility that the observed differences between wild-type and IL-4-deleted mice were due to ontogenetic adaptation events. While the IL-4 deletion had no influence on the infection in C3H.Q mice, the infected IL-4^{-}- and the infected anti-IL-4 Ab-treated B10.Q mice showed a lower anti-VSG Ab level and higher parasitemia than the wild-type mice. This strongly suggests that the non-MHC-related ability of B10.Q mice to produce IL-4 is of relevance in vivo. It will be important to map exactly the putative polymorphic gene for this immunoregulatory influence. Interestingly, in vitro experiments showed that TLTF, which polyclonally stimulates CD8^{+} T cells (4, 5), also induced IL-4 selectively in B10 mice. Our data suggest that anti-VSG Ab production can be promoted by this polyclonal non-antigen-specific activation of CD8^{+} T cells to IL-4 production. This event may be in addition to the demonstrated VSG-specific Th cell activity (9).

Despite higher parasites in the IL-4^{-} mice, the animals survived longer. This phenomenon may be consistent with toxicity of excessive IL-4 secretion. Such toxicity was previously demonstrated in immunodeficient mice challenged with recombinant vaccinia viruses expressing IL-4. It appeared that IL-4 contributed to the deaths of the mice, as they succumbed more rapidly than the immunodeficient mice inoculated with a control virus. At the same time, IL-4-mediated toxicity was confirmed in normal immunocompetent mice (45). In humans, IL-4 toxicity was detected during IL-4 therapy in which patients developed IL-4-associated myocarditis (46) and life-threatening gastrointestinal hemorrhage (47). In conclusion, our data suggest that differences in susceptibility and resistance to Tbb infection in certain mouse strains may depend on their ability to produce IL-4 and that an enhanced production of IL-4 may contribute to the death of the animals.

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