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Intact Immune Defenses Are Required for Mice To Resist the ts-4 Vaccine Strain of *Toxoplasma gondii*

PETER C. SAYLES* AND LAWRENCE L. JOHNSON

Trudeau Institute, Inc., Saranac Lake, New York 12983

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The ts-4 strain of *Toxoplasma gondii* is a temperature-sensitive mutant that fails to grow at 40°C in vitro. Unlike mildly virulent cyst-forming strains, which can cause fatal chronic infections in certain mouse strains, ts-4 has been widely used to vaccinate mice against virulent *T. gondii* and is a valuable tool with which to investigate mechanisms of acquired resistance to this parasite. In this report, the basis for the avirulence of ts-4 is analyzed. It is shown that ts-4 is able to persist long-term in vivo in mildly immunocompromised mice, which rules out an intrinsic growth defect as a reason for avirulence. ts-4 does not induce body temperatures in mice as high as that needed to kill it in vitro. Moreover, the mild fevers elicited in resistant B6 mice are also seen in susceptible C57BL/6 *scid*/*scid* mice. However, ts-4 elicits strong preimmune defenses, dependent on gamma interferon, which are needed by mice to survive acute infection. Furthermore, $CD4^+$ and $CD8^+$ T-cell-dependent acquired immunity is essential for long-term survival of ts-4-infected mice.

ts-4 is a temperature-sensitive mutant of the RH *Toxoplasma gondii* strain. Unlike the virulent RH parent strain, ts-4 is avirulent for immunocompetent mice. ts-4 does not form tissue cysts and has been reported to be nonpersisting (1, 11, 12, 14). Temperature-sensitive *T. gondii* grows slightly slower in vitro at 37°C than does the wild-type RH parental strain, and its growth becomes progressively slower at 38, 39, and 40°C (11). A significant decrease in viability as defined by growth in vitro does not occur until about 24 h after culture at 40°C (11).

Infection with the temperature-sensitive ts-4 strain of *T. gondii* induces strong protection against rechallenge with virulent toxoplasma in mice. For this reason, it has been widely used to investigate the immunological basis of acquired immunity to normally virulent *T. gondii* strains (5, 6, 10, 13). However, despite its wide use as a vaccine in the laboratory, little is known about the immune response necessary to protect mice from primary ts-4 infection.

ts-4 infection is lethal for T-cell-deficient nude mice (14), but little is known about its virulence or capacity to persist in other immunocompromised mice, its capacity to stimulate preimmune defenses against *T. gondii*, or whether its temperature sensitivity is relevant to its lack of virulence. In particular, it has been inferred in recent published reports that mice resist ts-4 infection because the organisms are unable to survive at 37°C (1, 4).

The foregoing information suggests three possible mechanisms whereby mice might resist primary ts-4 infection. First, the infection may be eventually self-limiting in vivo. Second, infection may result in increased body temperature, thereby limiting growth of this temperature-sensitive parasite. Third, the infection may be controlled by the host immune defenses. In this report, we show that immune defenses are of prime importance in host resistance to primary ts-4 infection and that it is unlikely that the other mechanisms are involved.

MATERIALS AND METHODS

Mice. C57BL/6J (B6) mice, (BALB/cByJ × C57BL/6J)F_1 ($CB6F_1$) mouse neonates, BALB/cByJ mice, and C.B-17 +/+ mice were purchased from the Animal Breeding Facility of the Trudeau Institute. C.B-17 *scid*/*scid* (C.B-17-SCID) mice, C57BL/6 *scid*/*scid* (B6-SCID) mice, gamma interferon (IFN-γ) knockout mice (3), and major histocompatibility complex class II-deficient ($A_\beta^{0/0}$) mice (2) were bred in the Experimental Animal Module of the Trudeau Institute. Immunocompetent mice were maintained under conventional husbandry conditions and were free of common pathogens of mice as evidenced by serological testing performed by the Research Animal Diagnostic and Investigative Laboratory, University of Missouri, Columbia. Mice received commercially prepared chow and acidified water ad libitum. Immunocompromised mice were kept in autoclaved microisolator cages and fed sterile food and water as described previously (8).

T. gondii. The RH and ts-4 strains of *T. gondii* were used. Tachyzoites of ts-4 and RH strains of *T. gondii* were maintained by in vitro cultivation in human foreskin fibroblasts (Hs-68, ATCC CRL-1635) at either 33 or 37°C, respectively (11). Mice were infected with ts-4 tachyzoites by intraperitoneal or subcutaneous inoculation of organisms obtained from cultures not more than 10 days old. RH tachyzoites were obtained from the peritoneal cavities of B6 mice infected with cultured tachyzoites 3 to 4 days previously.

Antibodies. The following monoclonal antibodies (MAbs) were used: anti-CD4 (GK1.5, rat immunoglobulin G2b [IgG2b], ATCC TIB 207), anti-CD8 (2.43, rat IgG2b, ATCC TIB 210), anti-Thy-1.2 (30H12, rat IgG2b, ATCC TIB 107), and anti-IFN-γ (R4-6A2, rat IgG1, ATCC HB170). When appropriate, rat IgG2b (LTF-2, an anti-keyhole limpet hemocyanin antibody developed in this laboratory), rat IgG1 anti-horseradish peroxidase, or normal rat IgG (Sigma Chemical Co., St. Louis, Mo.) was used as a control antibody. MAbs were purified in this laboratory by ammonium sulfate precipitation and DEAE ion-exchange chromatography. IgG concentrations were quantified by an enzyme-linked immunosorbent assay (ELISA). IFN-γ was neutralized in vivo by injecting 2×10^4 neutralizing units intraperitoneally (i.p.) on the day before and the day of infection. The neutralization titer (neutralizing units per milliliter) of the R4-6A2 MAb is defined as the reciprocal of the highest dilution of antibody that, when mixed with an equal volume of IFN-γ (final IFN-γ concentration, 10 U/ml), neutralizes greater than 50% of the antiviral activity, judged by the development of vesicular stomatitis virus cytopathic effect (7). $CD4^+$, $CD8^+$, and Thy-1^+ T cells were depleted by i.p. injection of 1 mg of the appropriate cell-depleting MAb per mouse on the day before infection and repeated with i.p. injections of 0.5 mg of the appropriate MAb per mouse on days 1, 3, and 5 following infection.

Flow cytometric analyses. Flow cytometric analyses (FACscan-LYSIS II software; Becton Dickinson, Sunnyvale, Calif.) were performed on peripheral blood cells obtained from the tail. Erythrocytes were hypoosmotically lysed, and the remaining leukocytes were incubated with MAbs to detect $CD4^+$ (GK1.5), $CD8^+$ (2.43), or Thy-1^+ (30H12) T cells. Each of these antibodies was prepared at this institute as fluorescein isothiocyanate-conjugated $F(Ab')_2$ fragments.

IFN-γ assay. An ELISA was used to quantify IFN-γ in lavage fluids of peritoneal cavities as described previously (9). One milligram of IFN-γ contains 2×10^7 U of IFN-γ.

Assay for NK activity. Cells obtained from the peritoneal cavity of mice were incubated with sodium [^{51}Cr]chromate-labeled YAC-1 target cells as described previously (9). The percent specific chromium release was calculated from the formula $100\ (T - S)/(M - S)$, where T is the counts per minute in test wells, S is the counts per minute in wells containing only target cells (spontaneous

* Corresponding author. Mailing address: Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, NY 12983. Phone: (518) 891-3080. Fax: (518) 891-5126. Electronic mail address: sayles@northnet.org.

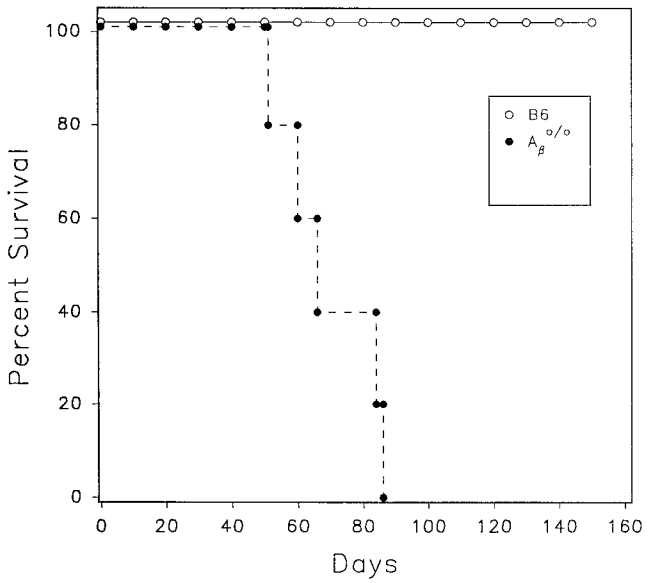

FIG. 1. Survival of groups of five female B6 or $A_\beta^{0/0}$ mice infected by i.p. injection with 2×10^5 ts-4 tachyzoites on day 0.

release), and *M* is the counts per minute in wells containing target cells in 0.5% Triton X-100 (maximum release).

Temperature measurement. B6 and B6-SCID mice were injected i.p. with phosphate-buffered saline or 2×10^4 ts-4 tachyzoites on day 0. The body temperature of mice, lightly anesthetized with a halothane-O_2 mixture, was measured with a digital thermistor thermometer with a flexible rectal microprobe (Cole-Parmer Instrument Company, Chicago, Ill.) at 9 a.m. on each indicated day of infection.

Irradiation. Mice were irradiated (500 rads) with a ^{137}Cs source (Gammacell 40; Atomic Energy of Canada, Ltd.) that delivers 132 rads/min.

Cell reconstitution. Single-cell suspensions of spleen cells obtained from C.B-17 mice were prepared by gently pushing the organs through sterile wire mesh, freed of erythrocytes by hypoosmotic lysis, and filtered through fine nylon mesh prior to intravenous (i.v.) injection of 1.2×10^8 spleen cells into C.B-17-SCID mice.

Statistical analysis. One-way analysis of variance and the Student-Newman-Keuls test were used to assess differences in numbers of T cells and IFN-γ levels. Statistical significance of survival data were analyzed by Kruskal-Wallis one-way analysis of variance on ranks followed by Dunn's method of multiple comparison to isolate groups that differ.

RESULTS AND DISCUSSION

Persistence of primary ts-4 infection in $A_\beta^{0/0}$ mice. To test the possibility that ts-4 infection may be self-limiting in vivo, $A_\beta^{0/0}$ mice, which lack $CD4^+$ T cells, were injected i.p. with ts-4 tachyzoites. The results presented in Fig. 1 indicate that $A_\beta^{0/0}$ mice survive for up to 86 days after injection prior to dying as a result of primary ts-4 infection. These results indicate that in a mildly immunocompromised mouse strain, ts-4 does not appear to be self-limiting. Similar results have been observed in mice deficient in $CD8^+$ T cells (unpublished observations).

Effect of primary infection with ts-4 tachyzoites on body temperature of mice. ts-4 tachyzoites are known to grow more slowly in vitro at temperatures above 38°C and to be killed at temperatures above 40°C (11). To investigate the effects of infection on body temperature, B6 and B6-SCID mice were injected with ts-4 tachyzoites and rectal temperature measurements were made over the course of acute infection. As shown in Fig. 2A, ts-4-infected mice had increased body temperatures when compared with those of control mice on days 5, 6, and 7 after injection. However, infected B6 mice rarely had temperatures above 38°C, and as they became ill from infection, they had lower body temperatures than those of control mice (Fig. 2A). These results are similar to the findings of Weir (15), who studied body temperatures of mice infected with *Salmonella typhimurium*.

Temperatures of infected B6-SCID mice (Fig. 2B) indicate that these mice, although ultimately succumbing to infection, also had significantly higher temperatures on days 5 and 7 of infection than those of control mice. These results indicate that temperatures high enough to have significant inhibitory effects on parasite growth are not reached in ts-4-infected mice. More importantly, the fevers in B6-SCID mice were as high as those in B6 mice. This finding argues against the hypothesis that immunocompetent B6 mice are resistant because they develop a higher fever in response to ts-4 than do B6-SCID mice.

Effect of primary infection of mice with ts-4 tachyzoites on IFN-γ levels and NK cell activity in peritoneal lavages. We have previously shown that primary infection of immunocompetent and severe combined immunodeficient (SCID) mice with ME49 *T. gondii* cysts results in increased levels of IFN-γ and natural killer (NK) cell activity controlled by Thy^+ $CD4^-$ $CD8^-$ cells at the site of infection (9). We have since determined, by fluorescence-activated cell sorter analysis with antibodies specific for NK1.1 and Thy-1, that most of the induced Thy^+ $CD4^-$ $CD8^-$ cells are $NK1.1^+$ cells (unpublished observations). Since ts-4 infection is resolved quickly in immunocompetent mice and induces resistance to rechallenge with virulent *T. gondii* RH, we sought to determine whether increases in levels of IFN-γ and NK cell activity occurred in the

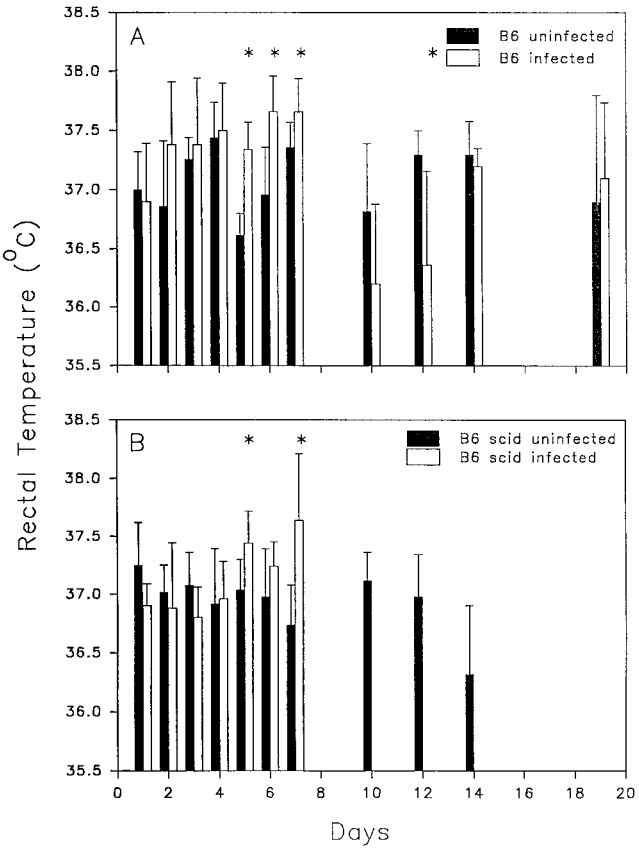

FIG. 2. Body temperatures of uninfected B6 or ts4-infected B6 mice (A) and uninfected B6-SCID or ts4-infected B6-SCID mice (B). *, infected mice differ significantly from uninfected mice. B6-SCID mice started dying by day 10 of infection.

TABLE 1. Amount of IFN-γ produced and NK cell activity in mice with primary ts-4 infection[a]

Day of infection	IFN-γ (U/host)[b]	YAC-1 cell kill (% ^{51}Cr release)[c]
1	BDL[d]	8.7 ± 5.4
4	9.3 ± 1.5	30.2 ± 6.6
7	412.8 ± 2.8	26.5 ± 3.1
11	22.0 ± 1.3	ND[e]

[a] Groups of five B6 female mice were given 2×10^4 ts-4 tachyzoites i.p. on day 0. Peritoneal cavities were flushed with 3 ml of medium on the days indicated.
[b] IFN-γ was assayed in peritoneal lavage fluids (3 ml per host) on the days indicated. Control mice have undetectable levels of IFN-γ and background levels of NK cell activity.
[c] Values are percent specific ^{51}Cr released from peritoneal lavage cells at an effector-to-target cell ratio of 20:1 as described previously (9).
[d] BDL, below detectable level.
[e] ND, not done.

peritoneal cavity of mice with primary ts-4 infection. Table 1 indicates that by day 4 of infection with ts-4, increased levels of IFN-γ and NK cell activity were observed. Levels of IFN-γ and NK cells activity peak on day 7 and are no longer detectable by day 11 of infection. These results indicate that preimmune responses similar to those seen in ME49-infected mice occur in mice infected with ts-4.

Early death due to ts-4 infection in SCID mice treated with IFN-γ-neutralizing MAb or in IFN-γ genetic knockout mice. We have previously shown that SCID mice survive up to 3 weeks after i.p. injection of 20 ME49 cysts and can be made significantly more susceptible by treatment with an IFN-γ-neutralizing MAb (8). SCID mice infected with ts-4 respond much like ME49-infected SCID mice in that increased levels of IFN-γ and NK cell activity are observed (data not shown) and can be made more susceptible to ts-4 infection by neutralizing IFN-γ (Fig. 3A). Mice genetically incapable of producing IFN-γ also succumb early as a result of ts-4 infection when compared with infected control mice (Fig. 3B). These findings further support the hypothesis that early resistance to primary ts-4 infection is IFN-γ dependent and, in the case of SCID mice, that the IFN-γ is produced by cells other than $CD4^+$ and $CD8^+$ T cells.

Resistance to primary ts-4 *T. gondii* infection is dependent on Thy-1$^+$ CD4$^-$ CD8$^-$ T cells followed by later dependence on CD4$^+$ and CD8$^+$ T cells. To determine whether depletion of certain T-cell subsets impairs resistance of mice to ts-4 infection, groups of B6 mice were treated with control rat immunoglobulin or with MAbs to deplete $CD4^+$, $CD8^+$, or Thy^+ cells or to neutralize IFN-γ and then infected with ts-4 *T. gondii*. B6 mice treated with $CD4^+$ and $CD8^+$ T-cell-depleting MAbs survived significantly longer after ts-4 infection than did mice given antibodies that deplete Thy^+ T cells or that neutralize IFN-γ. These results further support the hypothesis that immune defenses are required for mice to resist primary ts-4 infection. Moreover, as is the case with ME49 *T. gondii* infection (9), Thy^+ cells other than those expressing CD4 or CD8 surface markers are responsible for protection very early during acute infection with ts-4.

The mice depleted of both $CD4^+$ and $CD8^+$ T cells, similar to infected SCID mice, eventually died from ts-4 infection, presumably because they failed to generate *T. gondii*-specific immune $CD4^+$ and $CD8^+$ T cells. Mice depleted only of $CD4^+$ or $CD8^+$ T cells survived ts-4 infection for at least 44 days (Fig. 4A). Experiments in progress that utilize mice deficient in $CD4^+$ or $CD8^+$ T cells indicate that each of these T-cell subsets are important in resistance to primary ts-4 infection (unpublished data). Cells of mice treated with normal rat immunoglobulin are not affected (Fig. 4B). The results shown in Fig. 4 indicate that B6 mice possess an IFN-γ-dependent mechanism of early protection against primary ts-4 that is independent of $CD4^+$ or $CD8^+$ T cells.

Increased susceptibility to infection with *T. gondii* ts-4 in mice with immune impairments. To test more extensively the hypothesis that an intact immune system is required to survive infection with *T. gondii* ts-4 (14), we inoculated SCID mice as well as neonatal mice or sublethally irradiated mice with various numbers of ts-4 tachyzoites. The results shown in Table 2 indicate that B6, $CB6F_1$, and C.B-17 adult mice survive inoculation of up to 10^5 ts-4 tachyzoites, while infection of C.B-17-SCID mice or mice with other immune impairments due to irradiation or immature immune systems (neonates) resulted in death. These results, confirmed in several such experiments, demonstrate that mice require an intact or mature immune system to survive infection with *T. gondii* ts-4.

Formal proof that the susceptibility of SCID mice to ts-4 is immunological is shown in Fig. 5. SCID mice resist ts-4 infection if first reconstituted with C.B-17 splenocytes on the day of infection. Furthermore, such mice become immune if inoculated with ts-4 and are no longer susceptible to rechallenge with strain RH tachyzoites. Resistance to RH rechallenge is presumably due to the presence of $CD4^+$ and $CD8^+$ T cells specific for *T. gondii* antigens.

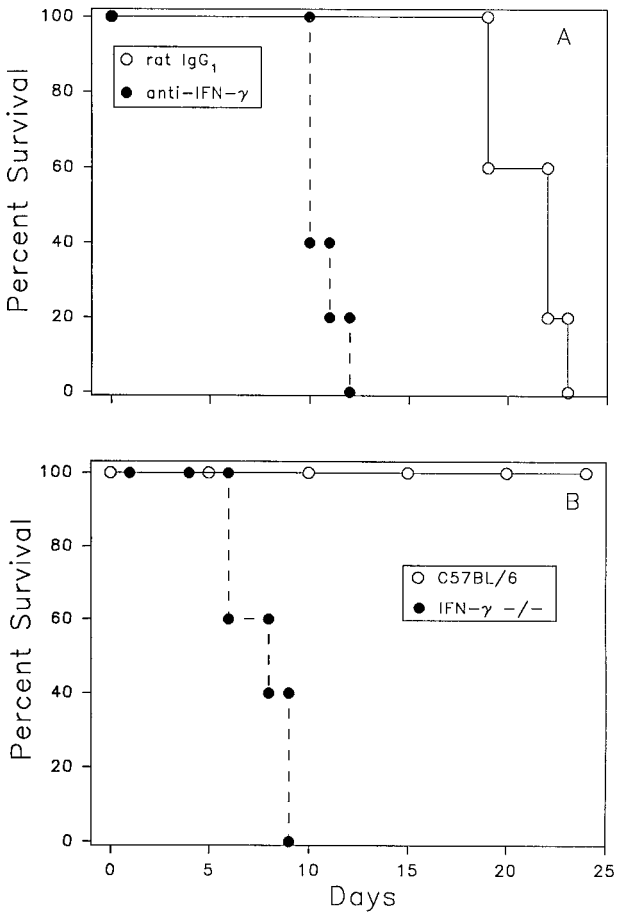

FIG. 3. Survival of groups of five female SCID mice injected on day −1 with control IgG1 MAb or anti-IFN-γ MAb (A) or five female IFN-γ −/− mice infected by i.p. injection of 10^4 ts-4 tachyzoites on day 0 (B).

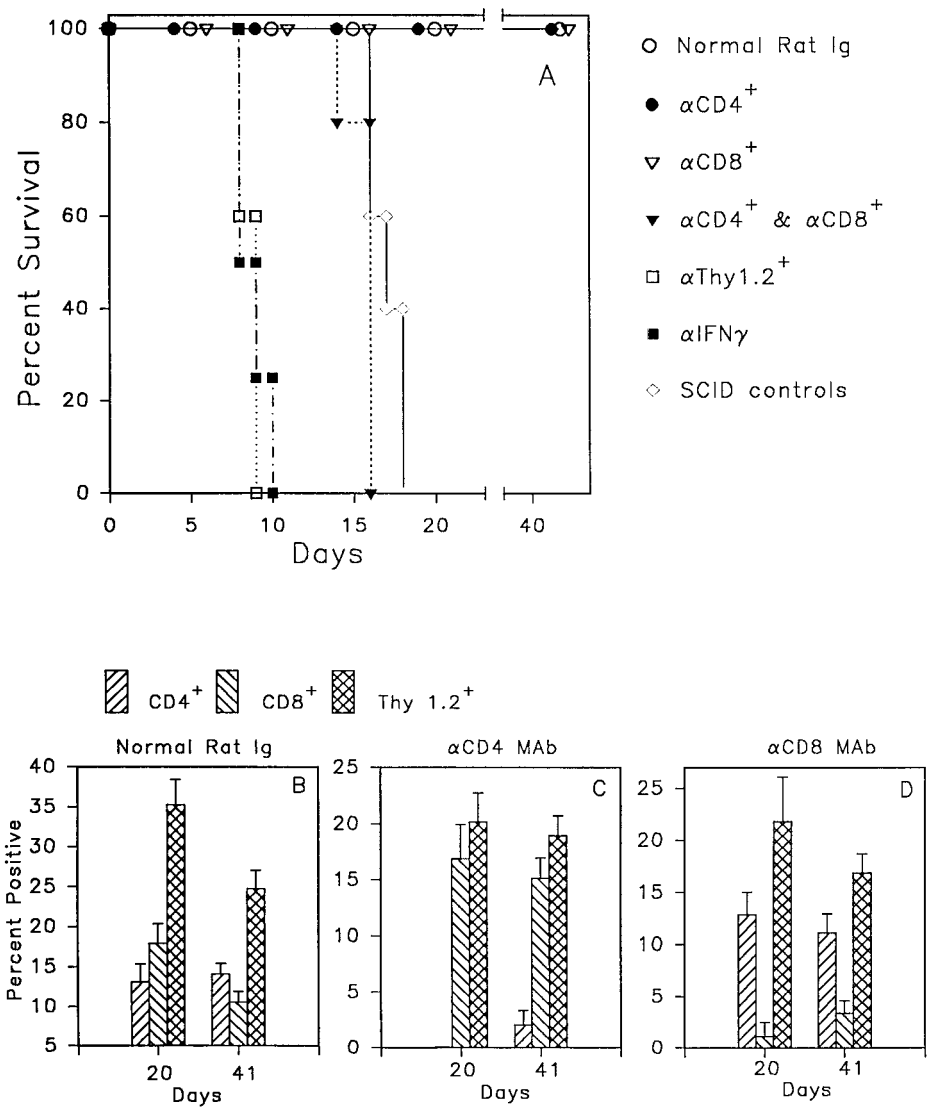

FIG. 4. (A) Survival of groups of five (four in anti-IFN-γ MAb-treated group) female B6 mice inoculated i.p. with 10^5 ts-4 tachyzoites and injected with normal rat immunoglobulin or anti-CD4, anti-CD8, anti-CD4 and anti-CD8, anti-Thy-1, anti-IFN-γ, or untreated, infected SCID mice. MAbs are as described in Materials and Methods. Peripheral blood mononuclear cells obtained from the tail vein of surviving mice (from treatment described for panel A) injected with normal rat immunoglobulin (B), anti-CD4 (C), or anti-CD8 cell-depleting (D) MAbs were stained and analyzed by flow cytometry as described in Materials and Methods. The percentages of positively staining $CD4^+$, $CD8^+$, or Thy-1^+ cells are shown. Symbols or bars represent means ± standard deviations of five mice per group.

The widespread use of the ts-4 strain to study immunological defenses against *T. gondii* led us to study the immune response of mice to ts-4 infection. A specific goal was to demonstrate definitively that the resolution of ts-4 infection in a mammalian host is strictly dependent on host immunological resistance rather than on failure of the mutant to thrive at host body temperatures. The results described above establish the following. (i) A functional immune system is required for resistance to ts-4 infection. (ii) The avirulence of ts-4 in immunocompetent mice is not due to induction of high fever in that the induced fevers are not as high as that needed to kill ts-4 in vitro, nor are the fevers of resistant or susceptible mice markedly different. (iii) Resistance to primary ts-4 infection requires early production of IFN-γ followed by later requirement of $CD4^+$ and $CD8^+$ T cells.

Vaccination with ts-4 tachyzoites is frequently performed in an experimental setting to protect mice against virulent *T.*

TABLE 2. Susceptibility of mice with immune deficiencies to infection with *T. gondii* ts-4

Mouse strain	Immune deficiency	ts-4 dose i.p.	Survival (days) of individual mice after infection
C57BL/6[a]	None	1×10^5	>42, >42, >42, >42, >42
C57BL/6	Irradiated[b]	1×10^5	7, 7, 7, 14, 18
$CB6F_1$	None (adult)	1×10^5	>42, >42, >42, >42, >42
$CB6F_1$	Neonate[c]	1×10^3	6, 6, 10, 11, 17, 25
$CB6F_1$	Neonate	1×10^5	3, 5, 5, 5, 6, 6[d]
C.B-17	None	2×10^4	>42, >42, >42, >42, >42
C.B-17	SCID[e]	2×10^4	17, 17, 18, 19, 19

[a] Immune intact mice of all strains tested survive infection with at least 10^5 ts-4 tachyzoites injected i.p., subcutaneously, or intravenously.
[b] Mice received 500 rads of gamma radiation 1 day prior to infection.
[c] Neonate, infected i.p. 24 to 48 h after birth.
[d] More than 97% of $CB6F_1$ uninfected neonates typically survive to adulthood.
[e] Infection with as few as 2×10^1 ts-4 tachyzoites injected i.p. results in death.

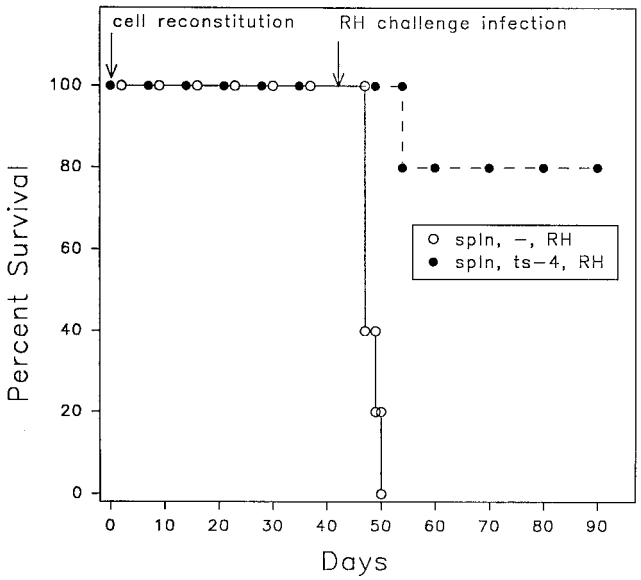

FIG. 5. Effect of reconstitution (day 0) with normal spleen cells on survival of SCID mice injected i.p. with 10^4 ts-4 tachyzoites on day 0. Unreconstituted, ts-4-infected control SCID mice died by day 22 of infection (data not shown). Normal-spleen-cell-reconstituted SCID mice (spln) with or without ts-4 immunization were challenged by i.p. injection with 2×10^3 RH tachyzoites on day 45 after cell reconstitution and ts-4 infection, and survival was monitored. Unreconstituted, RH-infected, control SCID mice died by day 7 of infection (data not shown). N = five mice per group.

gondii rechallenge. Thus, it is important that we understand the underlying principles of resistance to primary ts-4 infection. A crucial question that remains to be answered is why B6 mice, for example, develop chronic disease and die from ME49 infection, yet survive ts-4 infection and develop strong resistance to rechallenge, given the seemingly similar immune responses mice make to these *T. gondii* strains.

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REFERENCES

1. **Araujo, F. G.** 1994. Immunization against *Toxoplasma gondii*. Parasitol. Today **10:**358–360.
2. **Cosgrove, D., D. Gray, A. Dierich, J. Kaufman, M. Lemeur, C. Benoist, and D. Mathis.** 1991. Mice lacking MHC class II molecules. Cell **66:**1051–1066.
3. **Dalton, D. K., S. Pitts-Meek, S. Keshav, I. S. Figari, A. Bradley, and T. A. Stewart.** 1993. Multiple defects of immune cell function in mice with disrupted interferon-γ genes. Science **259:**1739–1742.
4. **Denkers, E. Y., P. Casper, and A. Sher.** 1994. *Toxoplasma gondii* possesses a superantigen activity that selectively expands murine T cell receptor $V_\beta 5$-bearing $CD4^+$ lymphocytes. J. Exp. Med. **180:**985–994.
5. **Elwell, M., and J. K. Frenkel.** 1984. Immunity to toxoplasmosis in hamsters. Am. J. Vet. Res. **45:**2668–2674.
6. **Gazzinelli, R. T., F. T. Hakim, S. Hieny, G. M. Shearer, and A. Sher.** 1991. Synergistic role of $CD4^+$ and $CD8^+$ T lymphocytes in IFN-γ production and protective immunity induced by an attenuated *Toxoplasma gondii* vaccine. J. Immunol. **146:**286–292.
7. **Havell, E. A.** 1986. Purification and further characterization of an anti-murine interferon-γ monoclonal neutralizing antibody. J. Interferon Res. **6:**489–497.
8. **Johnson, L. L.** 1992. SCID mouse models of acute and relapsing chronic *Toxoplasma gondii* infections. Infect. Immun. **60:**3719–3724.
9. **Johnson, L. L., F. P. VanderVegt, and E. A. Havell.** 1993. Gamma interferon-dependent temporary resistance to acute *Toxoplasma gondii* infection independent of $CD4^+$ or $CD8^+$ lymphocytes. Infect. Immun. **61:**5174–5180.
10. **McLeod, R., J. K. Frenkel, R. G. Estes, D. G. Mack, P. B. Eisenhauer, and G. Gibori.** 1988. Subcutaneous and intestinal vaccination with tachyzoites of *Toxoplasma gondii* and acquisition of immunity to peroral and congenital toxoplasma challenge. J. Immunol. **5:**1632–1637.
11. **Pfefferkorn, E. R., and L. C. Pfefferkorn.** 1976. Toxoplasma gondii: isolation and preliminary characterization of temperature sensitive mutants. Exp. Parasitol. **39:**365–376.
12. **Subauste, C. S., and J. S. Remington.** 1993. Immunity to *Toxoplasma gondii*. Curr. Opin. Immunol. **5:**532–537.
13. **Waldeland, H., and J. K. Frenkel.** 1983. Live and killed vaccines against toxoplasmosis in mice. J. Parasitol. **69:**60–65.
14. **Waldeland, H., E. R. Pfefferkorn, and J. K. Frenkel.** 1983. Temperature-sensitive mutants of *Toxoplasma gondii*: pathogenicity and persistence in mice. J. Parasitol. **69:**171–175.
15. **Weir, J. A.** 1947. The temperature of the mouse in health and disease. Proc. Iowa Acad. Sci. **54:**383–388.