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Cutting Edge: Infection of Mice Lacking the Common Cytokine Receptor γ-Chain (γ_c) Reveals an Unexpected Role for CD4$^+$ T Lymphocytes in Early IFN-γ-Dependent Resistance to *Toxoplasma gondii*

Tanya Scharton-Kersten,* Hiroshi Nakajima,† George Yap,* Alan Sher,[1]* and Warren J. Leonard[1]†

Mice lacking the common cytokine receptor γ-chain (γ_c) gene exhibit defective development of NK cells and CD8$^+$ T cells and greatly diminished production of IFN-γ. Because resistance of SCID mice to *Toxoplasma gondii* requires IL-12-dependent IFN-γ production by NK cells, we expected that γ_c-deficient mice would succumb rapidly to the parasite. Surprisingly, however, most γ_c-deficient mice survived the acute phase of *T. gondii* infection. As in wild-type mice, this resistance required IL-12 and IFN-γ; nevertheless, whereas wild-type mice depleted of CD4$^+$ T cells survived, anti-CD4$^+$ treated γ_c-deficient mice displayed diminished production of IFN-γ and all succumbed to acute infection. These data not only reveal a role for CD4$^+$ T lymphocytes in IFN-γ-dependent host defense but also establish SCID and γ_c-deficient mice as powerful complementary tools for assessing the function of NK vs CD4$^+$ T cells in immunopathophysiologic responses. *The Journal of Immunology,* 1998, 160: 2565–2569.

The common cytokine receptor γ-chain, or γ_c,[2] is a component of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 (1–3). In humans, mutations of γ_c can result in X-linked severe combined immunodeficiency (XSCID) (4), a disease characterized by profoundly diminished numbers of T cells and NK cells (1–4). Without successful bone marrow transplantation, patients with XSCID typically die from opportunistic infections. Like humans with XSCID, mice in which the γ_c gene has been targeted by homologous recombination exhibit a profound immunodeficiency (5–8). The number of thymocytes is approximately 1 to 5% of normal; interestingly, although splenocytes are also diminished in γ_c-deficient mice up to 3 wk of age, there is a subsequent age-dependent accumulation of CD4$^+$ peripheral T cells that exhibit an activated-memory phenotype (9). The accumulation of these cells appears to result from defective peripheral deletion (10). In contrast, CD8$^+$ T lymphocytes are essentially absent at all ages. Interestingly, no NK1.1$^+$ cells were detected, and γ_c-deficient splenocytes failed to produce IFN-γ when stimulated with anti-CD3 plus anti-CD28 (6).

IFN-γ production by NK cells is a major mechanism of innate defense against intracellular pathogens (11). Because γ_c-deficient mice are deficient in NK cells and defective in IFN-γ synthesis (6), they provide an excellent in vivo model system for evaluating the importance of the NK/IFN-γ pathway in host defense. We now have assessed the resistance of γ_c-deficient mice to *Toxoplasma gondii*, an intracellular protozoan that is a major cause of opportunistic infection in immunocompromised individuals (12). In immunocompetent hosts, early growth of *T. gondii* is controlled by IFN-γ produced largely by IL-12-stimulated NK cells (13, 14). In contrast, later in infection, CD8$^+$ T lymphocytes appear to be the major effectors of adaptive resistance, and their protective function is also thought to result from IFN-γ synthesis (15, 16). Since γ_c-deficient mice have been reported to lack NK and CD8$^+$ T cells, we predicted that they would be highly susceptible to *T. gondii*. Unexpectedly, most γ_c-deficient mice survived the acute phase of infection. This NK/CD8$^-$-independent control of pathogen growth is dependent on CD4$^+$ T cells and requires host synthesis of IFN-γ and IL-12.

Materials and Methods

Experimental animals

γ_c-deficient mice were generated as previously reported (6, 9, 10) and back-crossed to C57BL/6 for more than five generations. Five- to twelve-week-old male and female mice were used for experiments.

Parasites and experimental infection

Cysts of the avirulent ME49 strain (initially provided by Dr. J. Remington, Palo Alto Research Foundation, Palo Alto, CA) were harvested from the brains of C57BL/6 mice 1 mo following i.p. inoculation with approximately 20 cysts. For experimental infections, mice received 20 ME49 cysts or PBS i.p. Control inoculations with normal brain suspensions failed to

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[2] Abbreviations used in this paper: γ_c, γ-chain; XSCID, X-linked SCID.

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elicit detectable inflammatory responses, NK cytotoxicity, or significant increases in cytokine levels (data not shown). Soluble tachyzoite Ag was prepared as described (17).

In vivo assessment of acute infection

Acute tachyzoite growth was assessed using cytocentrifuge smears of peritoneal cells as previously described (18). Differential analyses, including assessment of intracellular *T. gondii* infection, were performed on 400 to 500 cells per animal.

Cell cultures and serum preparation

Single-cell suspensions were prepared from spleen and peritoneal cells. Peritoneal cells were cultured at 4×10^5 cells and spleen cells at 8×10^5 per well in 200 μl in RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 10% FBS, penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), HEPES (10 mM), and 2-ME (5×10^{-5} M) in the presence or absence of soluble tachyzoite Ag (5 μg/ml). Supernatants were harvested 72 h later for determination of levels of IFN-γ and IL-12.

Blood was collected from mice at the time of sacrifice, allowed to clot at room temperature for 2 h, centrifuged for 5 min at 5000 rpm, and serum was assayed for cytokine levels.

IFN-γ and IL-12 measurements

IFN-γ and IL-12 levels were assayed by two-site ELISAs, as previously described (18), and quantitated by comparison with standard curves generated with rIFN-γ or rIL-12 (provided by Genentech, San Francisco, CA, and Genetics Institute, Cambridge, MA, respectively).

In vivo cell and lymphokine depletion experiments

For cytokine depletion, mice were injected i.p. 1 day before infection with 1 mg anti-IFN-γ mAb XMG6 (rat IgG1) or with 1 mg anti-IL-12p40 mAb C17.8 (rat IgG2a) (cell lines provided respectively by Drs. R. Coffman, DNAX, Palo Alto, CA, and G. Trinchieri, Wistar Institute, Philadelphia, PA). To deplete $CD4^+$ and/or $CD8^+$ T cells, mice were treated i.p. with 1 mg of GK1.5 mAb (19) and/or 1 mg 2.43 mAb (20), respectively, 2 days before and on the day of parasite challenge. Ascites for the various mAbs were produced by Harlan Bioproducts for Science (Indianapolis, IN); the Abs were partially purified by ammonium sulfate precipitation. NK cells were depleted by treating mice with rabbit anti-asialo-G_{M1} (Wako Bioproducts, Richmond, VA) 4 days before injection of parasites (1.5 mg i.p.) and on the day of infection (0.5 mg i.p.). Depletion (as assessed by FACS) was $\geq$90% in each group.

Flow cytometric analysis

Splenocytes were stained and analyzed on a FACSCalibur (Becton Dickinson, San Jose, CA) using CellQuest software (9). Conjugated anti-CD4 Cy-Chrome (H129.12), anti-CD8 FITC (53–6.7), and anti-NK1.1 PE (PK136) mAb were from PharMingen (San Diego, CA). Before staining, Fc receptors were blocked with anti-CD16/32 (clone 2.4G2, PharMingen).

Statistical analyses

Statistical analyses were performed using an unpaired, two-tailed Student's *t* test.

Measurement of NK cell functional activity

To evaluate in vitro NK cell activation, splenocytes were cultured with or without 10 ng/ml (5.6×10^6 U/mg) of rIL-12 (Genetics Institute) and/or 60 IU/ml rIL-2 (Cetus Corp., Emeryville, CA) at 8×10^5 cells/well in triplicate wells in round-bottom 96-well plates. After 24 h, ^{51}Cr-labeled YAC-1 cells (8×10^3/well) were added, and cytotoxicity was assessed as previously described (18).

Results

γ_c-deficient mice are partially resistant to infection with T. gondii

Wild-type or γ_c-deficient mice were infected with *T. gondii*, and cumulative mortalities were monitored. As expected, wild-type animals were resistant and exhibited long term survival (Fig. 1, *A* and *B*, solid lines). Because of the absence of NK cells and IFN-γ production in γ_c-deficient mice (6), we hypothesized that these animals, which typically live longer than 1 yr, would rapidly succumb, analogous to IFN-γ-deficient mice, which routinely die within 2 wk after infection with *T. gondii* (18). Surprisingly, although almost all γ_c-deficient mice died by 140 days following infection (Fig. 1*A*, broken line), >50% of them survived for at least 28 days (Fig. 1*B*, broken line). Moreover, when the presence of intracellular parasites was assayed at the site of infection (peritoneal cavity), few (<1%) infected cells were observed at 7 days following infection in both wild-type and γ_c-deficient mice, in contrast to the high percentage of infected cells (>25%) typically observed in IFN-γ-deficient mice (18). Thus, γ_c-deficient mice display relatively normal control of early *T. gondii* infection. Nevertheless, their increasing mortality at later time points indicated impaired resistance to chronic infection. This conclusion is supported by the observation that 30 days after infection, the surviving γ_c-deficient mice had ~10-fold more cysts in the brain than did wild-type mice (data not shown). We hypothesize that this defective resistance to chronic infection relates to the virtual absence of $CD8^+$ T cells, which are required for adaptive immunity to *T. gondii* (15).

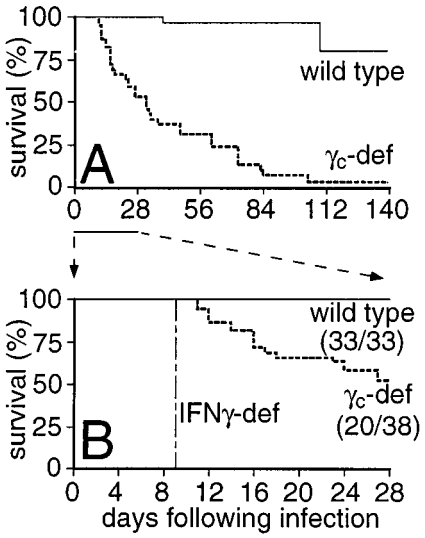

FIGURE 1. Percent survival of wild-type (solid lines) and γ_c-deficient (broken lines) mice following infection with *T. gondii*. *A*, Survival from 0 to 140 days. *B*, Survival from 0 to 28 days. *A* and *B* display the same results on different scales. Data are cumulative results from six independent experiments. In *B*, the survival of previously analyzed IFN-γ knockout mice (18) is also shown (staggered broken line). Essentially, no mortality was observed in uninfected control mice maintained for the same period.

Resistance of γ_c-deficient mice to acute T. gondii *infection is dependent on IL-12 and IFN-γ*

To clarify the mechanism by which γ_c-deficient mice control *T. gondii* infection, we evaluated the synthesis of the host-protective cytokines, IL-12 and IFN-γ. When examined at day 5 postinfection, levels of IL-12 in γ_c-deficient and wild-type mice were similar in serum and in the supernatants of splenocytes and peritoneal exudate cells (Fig. 2*A*). Consistent with a previous report by Cao et al. (6), minimal IFN-γ was produced by splenocytes in most infected γ_c-deficient mice (Fig. 2*B*). Surprisingly, however, substantial quantities of IFN-γ were detected in these mice in serum and at the site of infection (peritoneal cavity).

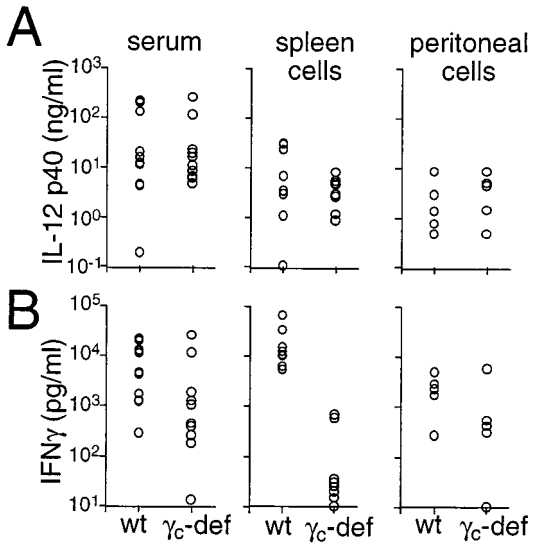

FIGURE 2. Levels of IL-12 and IFN-γ in wild-type and γ_c-deficient mice at day 5 following infection with *T. gondii*. *A*, IL-12 p40 levels in serum and in the supernatants of splenocytes or peritoneal cells incubated for 72 h in vitro. *B*, IFN-γ levels in the same serum and supernatant samples. The results shown are combined from three or more independent experiments and are values measured in individual mice (serum and spleen cell samples) or in supernatants of pooled cell suspensions (peritoneal cells).

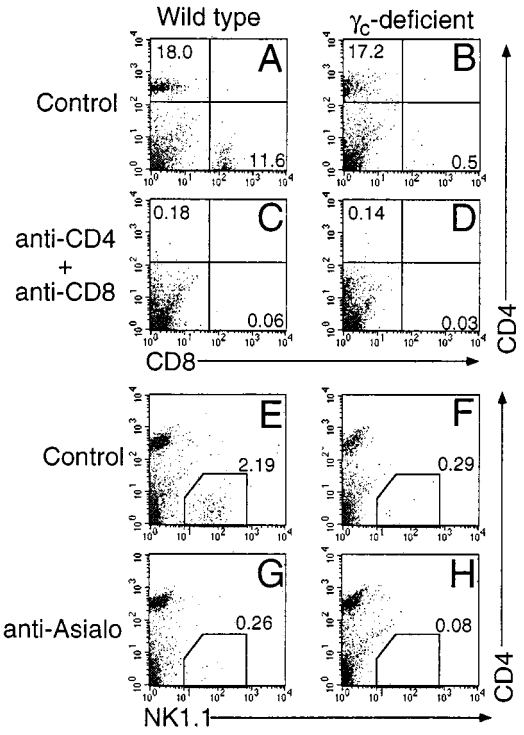

FIGURE 4. Effective depletion of CD4, CD8, and NK cells 5 days following treatment with Abs to CD4 and CD8 or asialo-G_{M1}. *A*, *C*, *E*, and *G*, Splenocytes from control wild-type mice (*A*, *E*) or from animals that were treated in vivo with Abs to CD4 and CD8 (*C*) or asialo-G_{M1} (*G*) were stained for CD4/CD8 expression (*A*, *C*) or CD4/NK1.1 expression (*E*, *G*). *B*, *D*, *F*, and *H*, Splenocytes from untreated γ_c-deficient mice (*B*, *F*) or from animals treated with Ab to CD4 + CD8 (*D*) or asialo-G_{M1} (*H*) were stained for CD4/CD8 expression (*B*, *D*) or CD4/NK1.1 expression (*F*, *H*). Directly conjugated Abs were used for all staining. Data are from one representative experiment of two performed.

Consistent with the role of IL-12 and IFN-γ in the control of acute *T. gondii* infection in wild-type mice (Fig. 3*A*), administration of Abs to IL-12 or IFN-γ resulted in 100% mortality of γ_c-deficient animals by day 13 (Fig. 3*B*), demonstrating that the IL-12/IFN-γ pathway is functional and able to limit parasite growth in these mice.

$CD4^+$ T cells control acute infection in γ_c-deficient mice

During acute *T. gondii* infection, NK cells are believed to play a major role in IFN-γ-mediated resistance. For example, SCID mice (which have NK cells but not T or B cells) survive for at least 20 days following infection with *T. gondii* (21, 22). Because γ_c-deficient mice lack $NK1.1.^+$ cells and NK cytolytic activity (6), we hypothesized that another cell type must be important for IFN-γ-dependent resistance in these animals. To identify the relevant cell population, wild-type and γ_c-deficient mice were injected 2 days before and on the day of infection with Abs to CD4, CD8, CD4 + CD8, or asialo-G_{M1} (which recognizes NK cells). These treatments substantially depleted each targeted population (Fig. 4, *C* vs *A*, *D* vs *B*, *G* vs *E*, and *H* vs *F*) but did not significantly reduce the survival of wild-type mice (Fig. 3*C*), suggesting that multiple cell

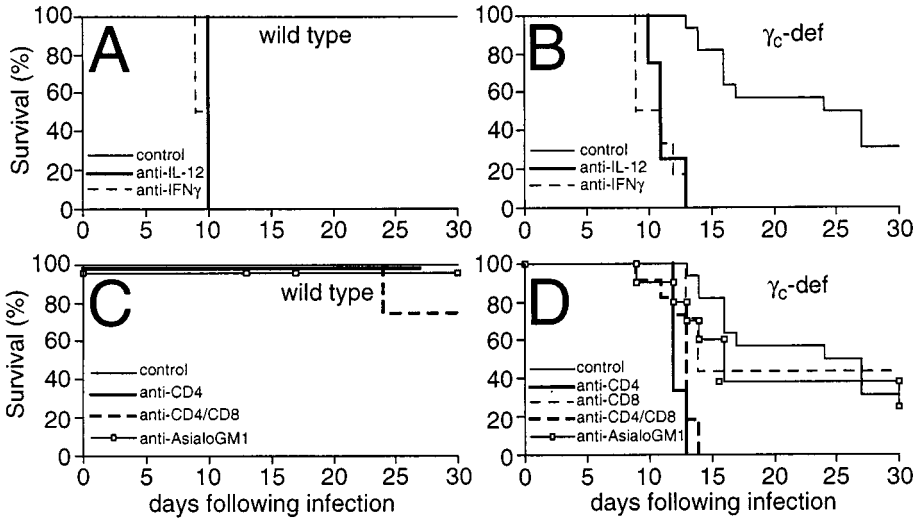

FIGURE 3. Importance of IL-12, IFN-γ, and $CD4^+$ T cells in γ_c-deficient mice for host defense to *T. gondii*. *A* and *B*, Survival following infection with *T. gondii* in wild-type (*A*) and γ_c-deficient (*B*) mice either not treated (control) or treated with neutralizing Abs to IL-12 or IFN-γ. *C* and *D*, Survival following infection with *T. gondii* in wild-type (*C*) and γ_c-deficient (*D*) mice not treated (control) or treated with Abs to CD4, CD8, CD4 + CD8, or asialo-G_{M1}. The results are combined from three individual experiments. Wild-type: untreated (n = 18), anti-IFN-γ (n = 6), anti-IL-12 (n = 4), anti-CD4 (n = 3) anti-CD4/CD8 (n = 8), anti-asialo-G_{M1} (n = 6). γ_c-Deficient: untreated (n = 17), anti-IFN-γ (n = 6), anti-IL-12 (n = 4), anti-CD4 (n = 3), anti-CD8 (n = 7), anti-CD4/CD8 (n = 11), anti-asialo-G_{M1} (n = 10).

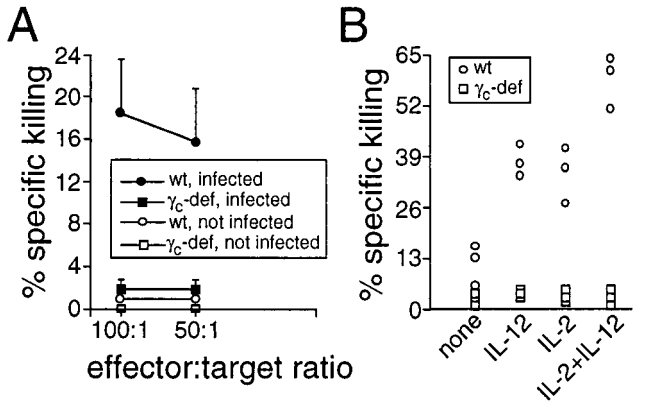

FIGURE 5. *T. gondii* induces NK activity in wild-type but not γ_c-deficient mice. *A*, Significant cytolytic activity against YAC-1 target cells was observed in wild-type (circles) but not γ_c-deficient splenocytes (squares) harvested from infected animals 5 days after i.p. inoculation of *T. gondii* (compare solid with open symbols). *B*, Treatment of splenoctyes from naive mice with IL-2, IL-12, or both cytokines for 24 h induced cytolytic activity in cultures of cells from wild-type (circles) but not γ_c-deficient animals (squares). Data points are values measured in individual mice.

types normally can mediate IFN-γ-dependent host defense. Nevertheless, depletion of either $CD4^+$ or $CD4^+$ + $CD8^+$ lymphocytes diminished resistance of γ_c-deficient mice (Fig. 3*D*) to a similar extent to that observed when the mice were treated with anti-IFN-γ or anti-IL-12 (Fig. 3*B*). In contrast, survival of γ_c-deficient mice was not substantially reduced following depletion of the $CD8^+$ or NK subpopulations (Fig. 3*D*), cells that are present at very low levels in γ_c-deficient mice even before treatment with Abs to CD8 or asialo-G_{M1} (6) (Fig. 4, *B* and *F*). The diminished resistance of mice depleted of $CD4^+$ T cells correlated with a loss of IFN-γ production. This was demonstrated by showing that, at day 9, anti-CD4 treatment of γ_c-deficient mice resulted in a >10-fold decrease in IFN-γ (mean = 2.33 ng/ml in untreated vs 0.15 ng/ml in anti-CD4-treated mice, $p < 0.02$). In contrast, wild-type mice exhibited no decrease in IFN-γ production following $CD4^+$ T cell depletion (mean = 7.42 ng/ml in untreated vs 9.33 ng/ml in anti-CD4-treated mice).

Although no NK cytolytic activity has been observed in splenocytes from naive γ_c-deficient mice (6), it was conceivable that *T. gondii* might induce the activation of NK cells. We therefore measured cytolytic activity in splenocytes of naive and infected wild-type and γ_c-deficient mice. Activity was detected in infected wild-type but not γ_c-deficient mice (Fig. 5*A*). Moreover, the addition of IL-2, IL-12, or both of these cytokines did not enhance cytolytic activity by γ_c-deficient splenocytes (Fig. 5*B*).

Discussion

Humans with XSCID have recurrent opportunistic infections. As a result, we hypothesized that γ_c-deficient mice would also be highly susceptible to opportunistic pathogens and have now investigated host defense in these animals. The resistance of γ_c-deficient mice to acute *T. gondii* infection was unexpected and demonstrated their capacity to display protective immunity despite the unresponsiveness of their T and NK cells to IL-2, IL-4, IL-7, IL-9, and IL-15. $CD4^+$ T lymphocytes are known to accumulate with age in γ_c-deficient mice and to exhibit an activated-memory phenotype (9). We now demonstrate that these cells can mediate IFN-γ-dependent effector activity in response to host infection, further arguing that they represent a functional lymphocyte population. The identification of the signals responsible for the growth and activation of γ_c-deficient $CD4^+$ T cells could lead to the development of strategies for inducing cell-mediated immunity in certain disease settings, including immunodeficiency states, in which conventional IL-2-dependent activation is defective.

Previous studies employing SCID mice indicated that NK cells, through production of IFN-γ, can mediate defense against acute intracellular infection when T and B lymphocytes are lacking (11). We now demonstrate that, in γ_c-deficient mice, which lack NK and $CD8^+$ T cells, $CD4^+$ T cells emerge as important mediators for IFN-γ-dependent resistance. Because SCID and γ_c-deficient mice have abnormal immune systems, it is conceivable that their mechanisms of control of *T. gondii* infection differ from those operating in wild-type animals. Nevertheless, it was striking to observe that selective depletion of either NK cells or of $CD4^+$ + $CD8^+$ T cells in wild-type mice did not significantly diminish resistance. This suggests that immunity in wild-type mice infected with *T. gondii* can be redundantly subserved by more than one cell lineage and that, early in infection, both $CD4^+$ T and NK cell populations may serve as dual effectors of host defense.

Taken together with previous studies of host resistance to acute *T. gondii* infection, the present report demonstrates how γ_c-deficient and SCID mice can provide complementary tools for genetically isolating and identifying the immunologic activities of NK and $CD4^+$ T cells. We suggest that the combined use of both of these immunodeficient mouse strains may provide a powerful general strategy for assessing the respective contributions of these cell lineages in immune responses.

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