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# Contribution of extrathymic γδ T cells to the expression of heat-shock protein and to protective immunity in mice infected with *Toxoplasma gondii*

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## SUMMARY

We demonstrated that γδ T cells contribute to protective immunity against *Toxoplasma gondii* by inducing the expression of a 65 000 MW heat-shock protein (hsp 65) in host macrophages. Here we examined the role of extrathymic and intrathymic γδ T cells in protective immunity and hsp 65 expression in mice infected with *T. gondii*. Intrathymic γδ T cells were obtained from severe combined immunodeficiency (SCID) mice grafted with syngeneic fetal thymus (TG-SCID), in which only T cells derived from the donor thymus developed, whereas extrathymic γδ T cells were obtained from nude mice that lack thymus. Extrathymic γδ T cells from *T. gondii*-infected nude mice differed from intrathymic γδ T cells of infected TG-SCID mice, in terms of Thy1.2 expression and V-region gene usage of T-cell receptor (TCR) γδ. Extrathymic γδ T cells expressed extremely high levels of Thy1.2, and had Vγ7 repertoire but lacked Vγ5,6 and Vδ1,5. On the other hand, intrathymic γδ T cells express intermediate and low levels of Thy1.2. These cells possessed Vγ5,6 and Vδ1,5 but failed to rearrange the Vγ7 gene. Peritoneal macrophages from infected nude mice contained hsp 65, whereas this protein was scarcely expressed in those of infected TG-SCID mice. Transfer of extrathymic, but not of intrathymic γδ T cells to SCID mice enabled their macrophages to express hsp 65. Athymic nude mice were significantly resistant to the infection compared with SCID mice which lack γδ T as well as αβ T cells. The resistance was dependent upon extrathymic γδ T cells, since nude mice depleted of γδ T cells using a corresponding monoclonal antibody became extremely susceptible. These results indicated that extrathymic rather than intrathymic γδ T cells play some crucial roles in protection against *T. gondii* and in hsp 65 expression.

## INTRODUCTION

*Toxoplasma gondii* is one of the most common pathogens of opportunistic infection in patients with acquired immunodeficiency syndrome (AIDS).[1,2] Cellular immunity mediated by T cells is indispensable for eradicative immune responses to this protozoan,[3–5] although some T-cell-independent protective mechanisms reportedly exist.[6–8] T cells are phenotypically divided into two distinct subsets. The majority of T cells in the periphery bears T-cell receptor (TCR)-αβ which recognizes conventional antigens in the context of major histocompatibility complex (MHC) gene products.[9] A minor T-cell population expresses TCR-γδ, which is supposed to recognize MHC-like molecules[10] and mycobacterial antigens, including the 65 000 MW heat-shock protein, hsp 65.[11–13] In contrast to αβ T cells, the antigen recognition mechanism of γδ T cells and their role in protective immunity is not fully understood. This type of T cell reportedly participates in host defence against some pathogens, especially intracellular pathogens.[14–16] In the peripheral blood of patients with acute toxoplasmosis, γδ T as well as αβ T cells increase.[17,18]

Recently, we found that γδ T cells play a crucial role in the expression itself of hsp 65 in host macrophages and its expression is essential for the protective immunity to *T. gondii* at the early phase of infection.[19]

Nude mice lack a thymus which is the central organ for T-cell development. However, a significant level of T cells develops through a thymus-independent (extrathymic) pathway in these mice.[20,21] In contrast, only thymus-derived (intrathymic) T cells develop in severe combined immunodeficiency (SCID) mice by grafting them with a murine fetal thymus.[22] Here, we compare the roles played by extrathymic and intrathymic γδ T cells in protective immunity and hsp 65 expression in mice infected with *T. gondii*. The phenotype, V gene usage of TCR and the ability to induce host hsp 65 were

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Abbreviations: AIDS, acquired immunodeficiency syndrome; hsp 65, 65 000 MW heat-shock protein; mAb, monoclonal antibody; MHC, major histocompatibility complex; RT-PCR, reverse transcription–polymerase chain reaction; SCID, severe combined immunodeficiency; TCR, T-cell receptor; TG-SCID, fetal thymus grafted-SCID; V, variable.

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examined in γδ T cells from those mice. Some mice were depleted of γδ T cells by giving them a corresponding monoclonal antibody (mAb) prior to infection with *T. gondii*.

## MATERIALS AND METHODS

*Animals*

Euthymic and athymic BALB/c mice were obtained from Japan SLC Inc. (Hamamatsu, Japan), and CB-17 +/+ and *scid/scid* (SCID) mice were from CLEA Japan Inc. (Tokyo, Japan). The CB-17 and BALB/c mice have the same genetic background, except for the immunoglobulin heavy chain gene. Then CB-17 mice were used as normal control in some experiments. These mice were studied at 7–10 weeks old.

*Establishment of fetal thymus-grafted SCID (TG-SCID) mice*

Fetal thymuses were isolated under aseptic conditions from 15-day-old CB-17 +/+ mouse embryos. One lobe of the fetal thymus was grafted under the subcapsular space of the left kidney of SCID mice using a 21-gauge needle as described.[22,23] To prove the reconstitution with T cells, peripheral blood mononuclear cells from TG-SCID mice were analysed with flow cytometry 3 weeks after the operation.

*Micro-organisms*

Bradyzoites of the Beverley strain of *T. gondii* were used as described.[24] In brief, the brains of chronically infected mice were homogenized with RPMI-1640 (Gibco, Grand Island, NY) containing 10% fetal calf serum (FCS; Flow, MacLean, VA), 100 U/ml penicillin, 100 μg/ml streptomycin and 10 mM HEPES buffer. The homogenate was centrifuged at 800 *g* for 20 min at 15° through a 1·057/1·070 gum arabic (resolved with 0·02% ethylenediaminetetraacetic acid (EDTA) saline) (Sigma Chemical Co., St Louis, MO) discontinuous gradient. To obtain the bradyzoites, precipitated cysts were exposed to 0·25% trypsin (Difco Laboratories, Detroit, MI) in phosphate-buffered saline (PBS) for 5 min at 37°, followed by centrifugation at 590 *g* for 10 min at 4° and resuspension in RPMI-1640. Mice were infected with 70–100 bradyzoites of the Beverley strain of *T. gondii* by intraperitoneal injection.

*Monoclonal antibodies*

Anti-TCR-γδ [UC7–13D5 (hamster IgG)] and anti-TCR-αβ [H57–597 (hamster IgG)] were provided by Dr G. Matsuzaki, Department of Immunology, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan. Anti-Thy1.2 [30-H12 (rat IgG)] was purchased from the American Type Culture Collection (Rockville, MA). Phycoerythrin (PE)-anti-CD3 was purchased from Pharmingen (San Diego, CA). Anti-TCR-γδ and anti-TCR-αβ were conjugated with fluorescein isothiocyanate (FITC). Anti-CD4 [GK1.5 (rat IgG)], anti-CD8 [53–6.7 (rat IgG)], and anti-Thy1.2 were biotinylated according to the standard procedure. The murine IgG mAb, termed IA10, specific for amino acids 172–224 of hsp 65 derived from *Mycobacterium bovis* was provided by Dr J. DeBruyn, Institute Pasteur de Brabant, Belgium.

*Flow cytometry analysis and cell sorting*

Non-adherent peritoneal exudate cells (PEC) were stained with various combinations of fluorescence-conjugated mAb and analyzed by two or three-colour flow cytometry (FACScan, Becton Dickinson, Mountain View, CA). Three-colour analysis was achieved using Streptavidin-Cy-Chrome (Pharmingen). Before analysis, the lymphocyte population was gated by light scatter signals to exclude dead and non-lymphoid cells. T-cell subsets were purified with a cell sorter (EPICS ELITE, Coulter Electronics, Hialeah, FL). For use *in vivo*, γδ T cells were negatively sorted.

*Reverse transcriptase–polymerase chain reaction (RT–PCR)*

Non-adherent PEC from mice infected with *T. gondii* were collected for RNA preparation. Non-adherent PEC were lysed in 0·5 ml of lysis solution D containing 4 M guanidine thiocyanate, 25 mM sodium citrate (pH 7·0), 0·1 M 2-mercaptoethanol, and 0·5% (wt/vol) sarcosyl. To shear high molecular weight DNA, the solution was drawn through a 22-gauge needle fitted onto a 1-ml syringe. Thereafter, 50 μl of 2 M sodium acetate (pH 4·0), 500 μl of phenol and 100 μl of chloroform/isoamylalcohol (49 : 1) were added, and mixed well. The mixture was chilled on ice and centrifuged at 12 000 *g* for 20 min at 4°, and RNA in the supernatant was extracted with 1 ml of ice-cold ethanol at −20° for 60 min. The aliquot was centrifuged as described above, then the precipitate was incubated with 150 ml of solution D and 1 ml of ice-cold ethanol at −20° for 60 min. After centrifugation, the precipitated RNA was washed with 75% ice-cold ethanol and was dissolved with sterile diethyl pyrocarbonate-treated water.

RNA (100 ng) was reverse-transcribed using hexanucleotide random primers (Boehringer Mannheim, Mannheim, Germany) in a reaction mixture (Takara Shuzo, Tokyo, Japan), then the cDNA was amplified with Taq DNA polymerase (Takara Shuzo). The PCR consisted of 34 cycles of relaxation and denaturation of cDNA for 30 seconds at 94°, primer annealing for 30 seconds at 60° and a primer extension for 1 min at 72°, followed by a prolonged extension cycle for 10 min at 72° on a DNA thermal controller (Funakoshi, Tokyo, Japan). The primers were as follows: 3′ primers for Vγ and Vδ usage are CTTATGGAGATTTGTTTCAGA, and CGAATTCCACAATCTTCTTG, respectively. The 5′ primers were as follows: Vγ 1/2, ACACAGCTATACATTGGTAC; Vγ 2, CGGCAAAAAACAAATCAACAG; Vγ 4, TGTCCTTGCAACCCCTACCC; Vγ 5, TGTGCACTGGTACCAACTGA; Vγ 6, GGAATTCAAAAGAAAACATTGTCT; Vγ 7, AAGCTAGAGGGGTCCTCTGC; Vδ 1, ATTCAGAAGGCAACAATGAAAG; Vδ 2, AGTTCCCTGCAGATCCAAGC; Vδ 3, TTCCTGGCTATTGCCTCTGAC; Vδ 4, CCGCTTCTCTGTGAACTTCC; Vδ 5, CAGATCCTTCCAGTTCATCC; Vδ 6, TCAAGTCCATCAGAATTGTC; and Vδ 7 CGCAGAGCTGCAGTGTAACT. TCR nomenclature is according to Reilley *et al.*[25] Ten microliters of PCR products were electrophoresed on a 1·7% agarose gel containing ethidium bromide and visualized under ultraviolet fluorescence. *Hin*cII digested φX174 was used as a molecular weight marker.

*Western blotting*

hsp 65 was detected by Western blotting as described.[24] Briefly, protein extracts of a plastic-adherent fraction of PEC from infected mice were separated by sodium dodecyl sulphate–polyacrylamide gel (SDS–PAGE), then gels were electroblotted onto a polyvinylidene difluoride membrane (Millipore Co., Bedford, MA). The mAb IA10 culture supernatant diluted 1 : 200 was used as the first antibody, and

goat peroxidase-conjugated anti-mouse IgG (Pierce, Rockford, IL) as the second. Binding antibodies were detected using a Konica immunostaining horseradish peroxidase kit (Konica, Tokyo, Japan).

*T-cell depletion*

Mice were depleted of γδ T cells using a mAb (UC7–13D5) specific for TCR-γδ. Five hundred micrograms of mAb was inoculated intraperitoneally 1 and 3 days prior to infection and 200 mg of mAb was further administered every 3 days from day 5 after infection. The administration of a control antibody (immunoglobulin fraction of hamster serum) did not alter the course of infection with *T. gondii*.[19]

## RESULTS

### γδ T-cell expansion in the peritoneal cavity of nude mice infected with *T. gondii*

Nude mice reportedly possess T cells developed via the extrathymic pathway.[20,21] To investigate whether nude mice have γδ T cells responding to infection with *T. gondii*, T-cell subsets in the peritoneal cavity of nude mice were analysed by flow cytometry 10 days after infection (Fig. 1a). The number of γδ T cells ($CD3^+$, TCR-$\gamma\delta^+$ fraction) increased in the peritoneal cavity of nude as well as in normal mice. Although the number of αβ T cells ($CD3^+$, TCR-$\gamma\delta^-$ fraction) also increased, this increase was not comparable to that of γδ T cells. This finding indicated that extrathymic γδ T cells expanded in response to *T. gondii* infection.

### Characteristics of extrathymic γδ T cells responding to *T. gondii* infection

Extrathymic γδ T cells were found in the peritoneal cavity of nude mice infected with *T. gondii* as described above. Therefore, we characterized these T cells in comparison with thymus-derived γδ T cells (intrathymic γδ T cells) obtained from SCID mice grafted with a syngeneic fetal thymus (TG-SCID). TG-SCID mice possess only T cells derived from grafted thymus.[22] Fluorescence-activated cell sorter (FACS) analysis revealed an increase in the number of intrathymic γδ T cells in these mice after challenge with *T. gondii* (Fig. 1b). There was no difference in TCR expression between extra- and intrathymic γδ T cells, since the levels of TCR and CD3 in γδ T cells from both nude and TG-SCID mice were comparable to those in γδ T cells from control mice. Further phenotypic analysis revealed that neither extra- nor intrathymic γδ T cells expressed CD4 or CD8 (data not shown). The expression of Thy1.2 antigen on γδ T cells was markedly different between euthymic, nude and TG-SCID mice (Fig. 2). The γδ T cells from euthymic normal mice were composed of three subsets expressing Thy1.2 antigen with high, intermediate or low intensity, and those from TG-SCID mice expressed this antigen with intermediate or low intensity, whereas most of the γδ T cells from nude mice were composed of a single population expressing high levels of Thy1.2. These data indicated that intrathymically developed γδ T cells are composed of subsets with low and intermediate levels of Thy1.2, while most γδ T cells developed extrathymically express Thy1.2 antigen at high level.

The cDNA constructed from non-adherent PEC of mice infected with *T. gondii* was analysed for usage of the V region of TCR-γδ, by means of PCR (Fig. 3). The γδ T cells from euthymic BALB/c mice infected with *T. gondii* used all of the Vγ gene segment and Vδ1,4,5 and 6. Intrathymic γδ T cells from infected TG-SCID mice showed rearrangement similar to those of BALB/c mice but lacked Vγ7, whereas extrathymic γδ T cells from nude mice lacked Vγ5,6 and Vδ5 repertoires but possessed Vγ7. Uninfected mice also possessed the similar repertoires of the γδ T cells in terms of Vγ gene usage in each group of mice, but Vδ1,4,5 and Vδ4,5,6 were not transcribed in BALB/c and TG-SCID mice, respectively. These findings indicated that extrathymic and intrathymic γδ T cells differ in the V gene repertoire as well as Thy1.2 expression, and that intrathymic T

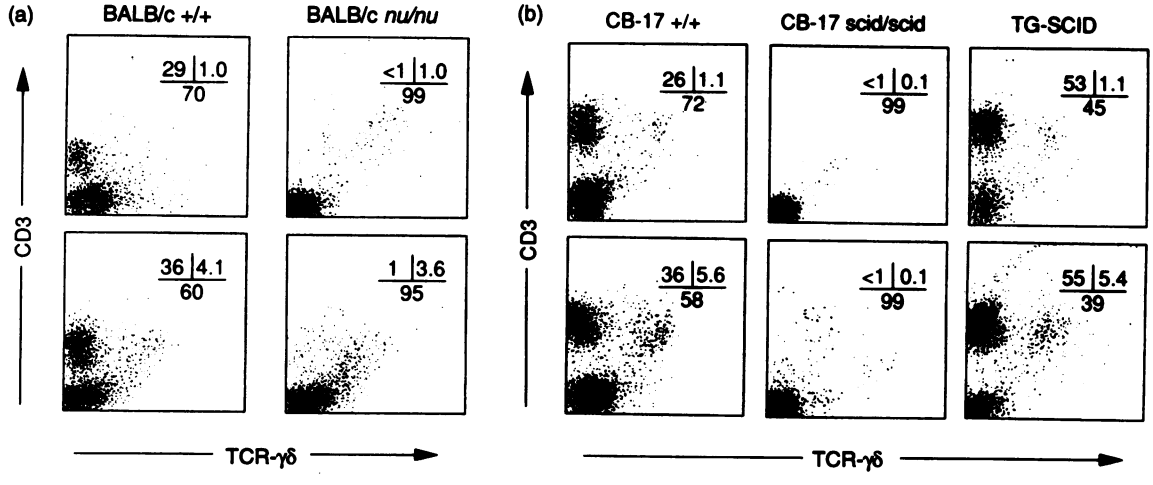


**Figure 1.** γδ T cells in the peritoneal cavity of nude and TG-SCID mice infected with *T. gondii*. Flow cytometry analysis of non-adherent PEC from uninfected (upper panels) and *T. gondii*-infected (lower panels) nude mice (a) and TG-SCID mice (b). Cells were collected 10 (nude) and 12 days (TG-SCID) after infection with 100 bradyzoites and stained with a combination of FITC–anti-TCR-γδ and PE–anti-CD3. These panels show typical two-colour profiles. The numbers represent the percentages of each subpopulation. Similar results were obtained when cells were stained with FITC–anti-TCR-αβ and PE–anti-CD3 (data not shown).

 

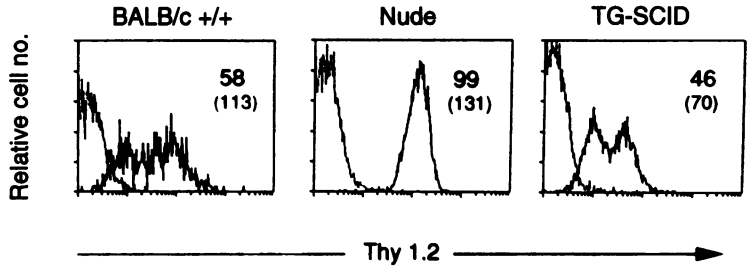


**Figure 2.** Thy1.2 expression by intrathymic and extrathymic γδ T cells. FACS three-colour analysis using the above combination plus biotin–anti-Thy1.2 (bold line) or control antibody (line). Cells from the indicated mice 12 days after infection with 100 bradyzoites were stained. $CD3^+$, TCR-$\gamma\delta^+$ cells were gated and analysed for Thy1.2 expression. Numbers represents the percentage of $Thy1.2^+$ cells and those in parentheses indicate the mean fluorescence intensity of Thy1.2 high and intermediate γδ T cells. Thy1.2 expression by γδ T cells from uninfected mice was comparable to that from infected mice, although the absolute number was much less than that of infected mice (data not shown).

cells bearing Vδ4,5 expanded or were recruited in the peritoneal cavity.

### hsp 65 expression in/on macrophages of nude and TG-SCID mice

We examined the biological functions of intrathymic and extrathymic γδ T cells. We showed that host protective immunity to *T. gondii* closely correlates with the intensity of hsp 65 expression in/on host macrophages, and that γδ T cells play a central role in its expression.[19,26,27] We thus analysed whether hsp 65 is expressed in/on macrophages in nude and TG-SCID mice. This protein was expressed in macrophages of normal mice 9 days after infection with 70 bradyzoites of the Beverley strain of *T. gondii*, but not in SCID mice that completely lack γδ T cells. Peritoneal macrophages of nude mice expressed hsp 65, while those of TG-SCID mice showed only marginal expression (Fig. 4a). These results indicated that extrathymic γδ T cells have more potential than intrathymic γδ T cells to induce hsp 65. To confirm this notion, macrophages from SCID mice given $10^6$ sorted extrathymic γδ T cells from nude mice or intrathymic γδ T cells from fetal thymus were examined for hsp 65 expression after infection with *T. gondii*. As shown in Fig. 4(b), the extrathymic γδ T cells transferred to SCID mice enabled their peritoneal macrophages to express hsp 65, whereas those given intrathymic γδ T cells did not. This finding indicated that extrathymic γδ T cells are responsible for hsp 65 expression in macrophages, and supports our previous finding[19] that depletion of $Thy1.2^+$ T cells including extrathymic γδ T cells markedly reduced hsp 65 expression, regardless of the presence of $Thy1.2^{low}$ γδ T cells developed via the intrathymic pathway.

### Contribution of extrathymic γδ T cells to the protection against *T. gondii* infection

Nude mice are susceptible to *T. gondii* infection because of a T-cell defect.[28,29] Here, all of the infected nude mice died of acute infection after being given a dose sublethal for euthymic BALB/c mice (Fig. 5). Thus, nude mice were susceptible to *T. gondii* infection compared with their euthymic counterparts, but were significantly more resistant than SCID mice which lack γδ T as well as αβ T cells, with respect to duration of survival ($21{\cdot}0 \pm 1{\cdot}83$ versus $18{\cdot}2 \pm 2{\cdot}11$ days, $P < 0{\cdot}05$ significant according to Student's *t*-test). This resistance in nude mice should be dependent on extrathymically derived T cells, especially γδ T cells. To examine this possibility, nude mice were depleted of γδ T cells using a mAb specific for TCR-γδ (UC7–13D5). The efficacy of this *in vivo* treatment in depleting the corresponding T-cell subset has been described.[19,27] Nude mice treated with anti-TCR-γδ mAb

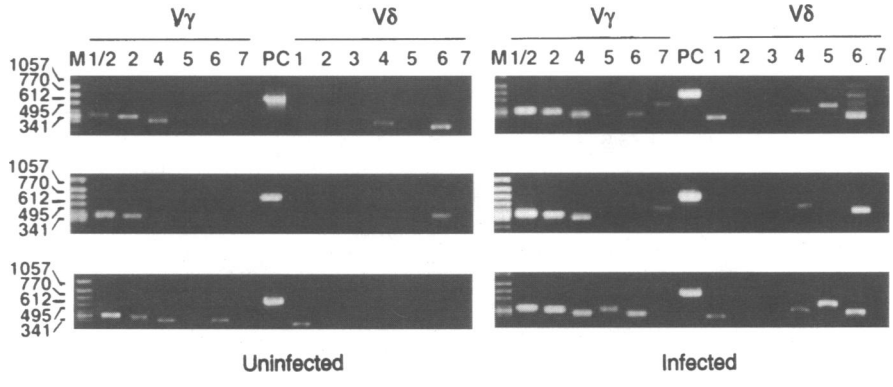


**Figure 3.** V gene usage by intrathymic and extrathymic γδ T cells. The cDNA was constructed from non-adherent PEC of BALB/c (upper panels), nude (middle panels) and TG-SCID mice (bottom panels) before and 12 days after infection with 70 bradyzoites of *T. gondii*. Primers for β-actin were used as the internal control indicated as PC. M indicates molecular markers shown in the left in base pairs.

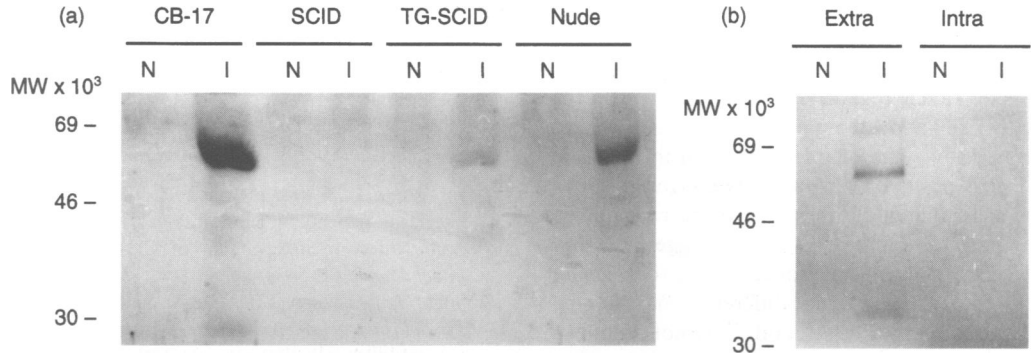


**Figure 4.** Extrathymic γδ T cells play a crucial role in the induction of hsp 65. Cell lysates prepared from peritoneal macrophages of mice 10 days after infection with 100 bradyzoites of the Beverley strain of *T. gondii* were Western blotted. (a) Nude and TG-SCID mice, (b) SCID mice transferred with sorted extrathymic (extra) or intrathymic (intra) γδ T cells were infected. N and I refer to naive and infected mice, respectively. Protein extracts of the parasites and macrophages from uninfected mice did not contain demonstrable levels of hsp 65 as previously described.[19] Eight micrograms of protein was loaded on lanes. Normal mouse IgG did not react with this protein. Standard molecular weight markers are shown on the left in kilodaltons.

became susceptible to *T. gondii* infection compared with naive nude mice (18·3 ± 1·86 versus 21·3 ± 2·05 days, $P < 0·05$ significant with Students's *t*-test). On the other hand, this treatment never altered the course of infection in TG-SCID mice that possessed intrathymic, but not extrathymic γδ T cells. In addition, less hsp 65 was expressed in γδ T cell-depleted, than in untreated nude mice (data not shown). These results indicate that extrathymic, rather than intrathymic γδ T cells play an essential role in the protection and induction of host hsp 65 in/on macrophages.

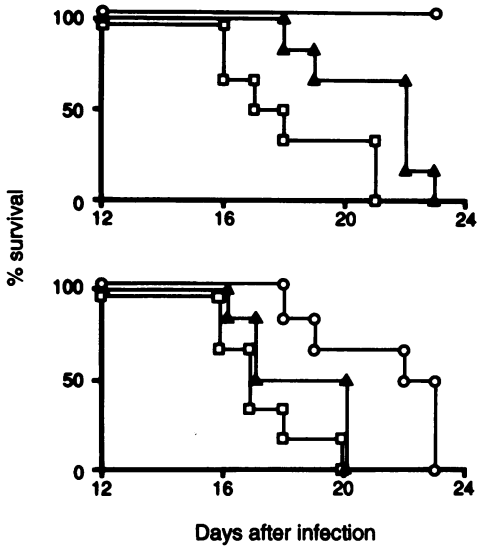


**Figure 5.** Contribution of extrathymic γδ T cells to protective immunity against *T. gondii*. Upper panel, nude mice (closed triangles) exhibited significant resistance to *T. gondii* compared with SCID mice (open squares), but were much more susceptible to a sublethal dose in euthymic mice (open circle). Six mice from each group were infected with 70 bradyzoites of the Beverley strain of *T. gondii*. Lower panel, nude mice treated with mAb to TCR- γδ (closed triangles) or without (open circles) and SCID mice (open squares) were infected with 100 bradyzoites. Each group consisted of six animals. Two repeated experiments yielded similar results.

## DISCUSSION

We investigated the involvement of extra- and intrathymic γδ T cells in protective immunity and hsp 65 expression in *Toxoplasma* infection using nude and TG-SCID mice. Phenotypically, the levels of Thy1.2 antigen expression is extremely different from each other, although that of TCR is similar. This discrepancy between extra- and intrathymic γδ T cells may reflect the state of cell activation,[30] suggesting that extrathymic γδ T cells are highly activated from an early phase of infection. RT-PCR analyses revealed that these two subsets also differ in the V gene usage. The relationship between Vγ repertoire usage, tissue distribution and the origin of γδ T cells is well defined.[31] That is, the Vγ5 is used preferentially by γδ T cells located within epidermis, while γδ T cells with Vγ6 locate under the epithelium of the tongue and in the female reproductive tract, and those with Vγ7 are found under that of the intestine. In contrast to Vγ5 and Vγ6, both of which are supposed to be derived from fetal thymus, Vγ7 develops via the extrathymic pathway. This concept is consistent with our observations that nude mice lack Vγ5, 6 and TG-SCID mice lack Vγ7. It is not known whether and how epidermic or intestinal γδ T cells migrate into the peritoneal cavity. However, the relationship between the homing ability of γδ T cells to epithelia and their Vγ repertoire usage is not stringent.[32,33] We suppose that these cells originally locate in the peritoneal cavity.

Recently, a part of the γδ T cells participating in immune surveillance recognizes ubiquitous compounds in pathogens and mammalian cells in an MHC-independent fashion.[34,35] Thus, our data suggest that extrathymic γδ T cells, which have not undergone thymic education, may recognize protozoan antigens and be activated, which induces hsp 65 expression in/on macrophages. Intrathymic γδ T cells, especially $V\delta4^+$ and $V\delta5^+$ T cells in BALB/c and TG-SCID mice, also expanded after infection, although they seemed not to function in host-defence and hsp 65 expression. As is generally known, hsp 65 is one of the ligands for γδ T cells.[11,12] Thus, these γδ T cells may recognize host-derived hsp 65 on macrophages induced by extrathymic γδ T cells. In fact, our speculation is supported by some reports as follows. That is, $V\delta5^+$ γδ T cells recognize

murine HSP60 in the context of MHC in self mixed lymphocyte reaction,[36] and the early and late expansion of $\gamma\delta$ T cells consisting of $V\delta6^+$ and $V\delta4^+$ cells, respectively, are observed in schistosomiasis.[37] Taken together, extrathymic $\gamma\delta$ T cells bearing $V\delta6$ may be activated and induce hsp 65 in macrophages at an early stage of infection, then intrathymic $V\delta4$ and $5^+$ $\gamma\delta$ T cells reactive to hsp 65 may expand.

We described here that nude mice are more resistant to infection with *T. gondii* than SCID mice, suggesting that extrathymic $\gamma\delta$ T cells are responsible for resistance to *T. gondii* infection. One of the causes of this difference may be the existence of antibodies, but specific antibodies are not produced in nude mice because of a defect in helper T-cell function exerted by $CD4^+$ T cells.[29] Thus, this possibility is negligible and extrathymic $\gamma\delta$ T cells should be important. However, this resistance preferentially operated in the early stages after infection and could not completely control the infection after all. Alternatively, extrathymic $\gamma\delta$ T cells contribute to protection but sequential defence mechanisms from extrathymic $\gamma\delta$ T cells to intrathymic $\alpha\beta$ T cells are required for the complete resolution of *T. gondii* infection. We found that $\gamma\delta$ T cell-depleted euthymic BALB/c mice become extremely susceptible to infection with this organism, despite the fact that $\alpha\beta$ T cells increase even in absolute number,[19] indicating again that $\gamma\delta$ T cells are indispensable to the early stages of protection and to the accomplishment of sequential defence mechanisms. It is reported that $\alpha\beta$ and $\gamma\delta$ T cells play different roles in protective immunity against intracellular pathogens.[38] Thus, it is of importance to elucidate the relationship between $\alpha\beta$ and $\gamma\delta$ T cells in protective immunity. Several studies have demonstrated the existence of host hsp 65-reactive $\alpha\beta$ T cells,[39–41] indicating that hsp 65 derived from host macrophages and/or parasites provides a link between these two types of T cell. The biological role and expression mechanism of hsp 65 in host macrophages is still under investigation. This protein is expressed by cytokines secreted from $\gamma\delta$ T cells, and preserves macrophage functions in terms of evading apoptosis induced by intracellular noxious agents such as nitric oxide (manuscript in preparation).

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