γδ T Cell-Deficient Mice Exhibit Reduced Disease Severity and Decreased Inflammatory Response in the Brain in Murine Neurocysticercosis

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In a recently developed mouse model for neurocysticercosis, the immune response was characterized by a massive influx of γδ T cells and a type 1 pathway of cytokine expression. To understand the role of γδ T cells during this infection, the cellular and cytokine response was analyzed in mice that lack γδ T cells (TCRδ−/−). In TCRδ−/− mice, Mesocestoides corti metacestodes preferentially invaded the extraparenchymal areas of the brain. Furthermore, parasites were able to escape from the brain and establish a systemic infection with liver and peritoneal involvement. Immunopathological studies indicated that TCRδ−/− mice develop little inflammatory response and less neurological symptomatology. Significantly reduced numbers of T cells, macrophages, dendritic cells, and mast cells were present in the brain. The cytokine response in the brain of TCRδ−/− mice appears to be a mixed type1/type 2 response with low levels of IL-2, IL-4, IL-10, IL-12, IL-13, IL-15, and IFN-γ. To further investigate the immunological significance of this cell population, γδ T cells were adoptively transferred into intracranially infected TCRδ−/− mice. γδ T cells were specifically recruited into the CNS in response to this parasitic infection, and they were able to target the infected brain within 12 h after transfer. These results suggest that γδ T cells are key players in the immune response elicited during this CNS infection and direct a type 1 response in wild-type mice upon infection. The Journal of Immunology, 2002, 169:3163–3171.

Neurocysticercosis (NCC) is the most common parasitic disease that affects the human CNS and constitutes a public health challenge for most developing countries (1, 2). The disease is caused by the presence of Taenia solium metacestodes in the brain. The pathology of the human disease is influenced by the number of parasites in the brain, their location, and most importantly by the degree of the host immune response (3–6). T. solium taeniosis/cysticercosis affects millions of persons in Latin America, Asia, and Africa, where the disease accounts for the high prevalence rates of epilepsy (1). Other common clinical manifestations of NCC include increased intracranial (i.c.) pressure, obstructive hydroencephalus, stroke, and encephalitis (2, 7).

In the mouse model for NCC, mice are i.c. inoculated with Mesocestoides corti metacestodes (8). In wild-type mice, CNS infection is characterized by a massive influx of γδ T cells, as determined by in situ immunohistochemistry of brain cryosections (8). In addition, the brain immune response is associated with a predominant type 1 pathway of cytokine response. The γδ T cell response is induced very rapidly after infection (2–3 days postinfection [p.i.]) and predominates during the course of the infection. This finding is of interest because γδ T cells represent a minor T cell population residing in lymphoid organs (9, 10). In contrast, γδ T cells are a predominant T cell population within epithelial tissues such as the skin, intestine, and lung (9–12). γδ T cells have been demonstrated to actively participate in host immune response by regulating and resolving inflammatory processes (13–15). Circulating γδ T cells have been found to increase in several infectious states (16) such as tuberculosis, malaria, toxoplasmosis, and HIV. An early protective role of γδ T cells during Listeria monocytogenes infection has been demonstrated (17). γδ T cells have also been implicated in controlling HSV replication and spread, through potent cytotoxic responses (18). The contribution of γδ T cells in the initiation of inflammatory response during influenza virus infection suggests a role for γδ T cells in viral immunity (14, 19). Furthermore, γδ T cells appear to modulate host immune response in several pathologies by producing several type 1 and/or type 2 cytokines (20, 21), including IFN-γ, IL-2, IL-4, and IL-10 (22, 23). However, under pathological conditions, γδ T cells have not been described as a major lymphocyte population in the CNS.

To investigate the role of γδ T cells during this infection, we characterized the CNS immune response to M. corti in TCRδ−/− mice. The results indicate that γδ T cells are critical immunoregulatory players in the infection and further define the immune function of this T cell subset, including their potential role in the human disease of neurocysticercosis.

Materials and Methods

Animals

Female BALB/c mice 3–5 wk old were purchased from the National Cancer Institute Animal Program (Bethesda, MD). Female C57BL/6 and δ-chain TCR−/− mice (TCRδ−/−) were purchased from The Jackson Laboratory (Bar Harbor, ME). Animal experiments were conducted under the...
guidelines of the University of Texas System. The U.S. Department of Agriculture, and the Department of National Health.  

Parasites and inoculations  

*M. corti* metacestodes were maintained by serial i.p. inoculation of 8- to 12-wk-old female BALB/c mice (8). Intracranial inoculations were performed in 100 μl cold PBS, as previously described (8). Control mice were injected with 100 μl sterile HBSS using the same protocol. Before i.c. inoculation, mice were anesthetized i.m. with 20 μl mouse mixture containing 100 mg/ml ketamine and 20 mg/ml rompum (Laboratory Animal Resource, University of Texas Health Science Center, San Antonio, TX). Animals were sacrificed at several time points after inoculation and analyzed for parasite burden and various immune parameters. Before sacrifice, animals were anesthetized with 100 μl mouse mixture and perfused through the left ventricle with 10 ml cold PBS.  

Tissue processing  

The brain was immediately removed from perfused animals, embedded in O.C.T. resin (optimal cutting temperature), and snap frozen. Serial horizontal cryosections 10 μm in thickness were placed on silane prepped slides (Sigma-Aldrich, St. Louis, MO). One in every four slides was fixed in formalin for 12 min at room temperature and stained with H&E. The remainder of the slides was air dried overnight and fixed in fresh acetone for 20 s at room temperature. Acetone-fixed sections were wrapped in aluminum foil and stored at ~80°C or processed immediately for immunohistochemistry or immunofluorescence.  

H&E staining  

After fixation in 4% Formalin for 10 min at room temperature, slides were washed twice in deionized water, dehydration 30 s in 100% ethanol, stained 30 s in hematoxylin, and washed in distilled water for 2 min. Tissue sections were stained with eosin for 15 s, followed by 2-min treatment with 95% and 100% ethanol each. Slides were allowed to air dry, submerged in xylene for 3 min, and mounted using cytoseal mounting medium (Stephens Scientific, Riverdale, NJ). The number and location of parasites were determined by microscopic examination of the stained tissues. Tissues were also analyzed for the presence or absence of mononuclear infiltrates.  

Antibodies  

Abs purchased from BD PharMingen (San Diego, CA) include GL3 (pan anti–γδ), H57-597 (pan anti–αβ), 5E6 (anti–NK), M1/70 (anti–Mac1), Cy3.41 (anti–CD22), XMG12.1 (anti–IFN-γ), SXC-1 (anti–IL-10), BV6D-24G2 (anti–IL-4), JES6-SH4 (anti–IL-2), G277-3960 (anti–IL-15), RB6-8C5 (anti–Gr1), and 9A5 (anti–IL-12 heterodimeric p70). The purified anti-mouse polyclonal Ab against IL-13 was purchased from R&D Systems ( Minneapolis, MN). The purified Ab, NLDC-145 (anti–DEC205 Ab specific for dendritic cells and thymic epithelial cells), was obtained from Serotec (Raleigh, NC). All Abs were titrated in spleen sections from i.p.-infected mice to determine optimal concentrations. Spleen sections were used as positive controls in all experiments. Mast cells were detected using toluidine blue staining.  

Brain mononuclear cell isolation  

Mice were sacrificed 7 days after adoptive transfer. Each perfused brain was gently minced through a fine 150-μm Nitex screen (Sefar America, Depew, NY) using a syringe plunger and collected into 10 ml HBSS (Invitrogen, Carlsbad, CA) containing 0.05% collagenase D (Roche Diagnostics, Indianapolis, IN), 0.1 μg/ml L-1-chloro-3-[4-tosyl-amid]-7-aminoo-2- heptanone-HCI (Sigma–Aldrich), 10 mg/ml DNase (Sigma–Aldrich), and 10 mM HEPES buffer, pH 7.4 (Invitrogen). The mix was gently rocked at room temperature for 1 h and allowed to settle at unit gravity for 30 min to deplete undigested debris. Supernatant was collected and pelleted at 200 x g for 5 min and resuspended in 3 ml 0.4% Trypsin/EDTA-free HBSS (Invitrogen) for each brain. Cells were washed three times in 1 ml 0.1% BSA (Sigma–Aldrich) in HBSS and counted. Cells were diluted to 2 x 10^6 cells/ml and a 0.5-ml aliquot was cytocentrifuged in a Cytospin 2 (Shandon, Pittsburgh, PA) onto silane prepped slides (Sigma–Aldrich).  

Peritoneal leukocyte isolation  

Intrapерitoneal leukocytes were isolated from C57BL/6 mice that were infected i.p. for 3-5 wk with *M. corti* or from normal uninfected mice. The peritoneal cavity was washed with 10 ml HBSS, and the preparation was filtered through a 150-μm nylon screen (Sefar America) to remove parasites. Cells were pelleted at 300 x g for 10 min and washed three times in 0.1% BSA in PBS and counted. For immunocytochemistry, cells were diluted to 2 x 10^6 cells/ml in 0.1% BSA, and 0.5 ml of this cellular suspension was cytocentrifuged into “super plus” covered slides (VWR Scientific Products, Sugar Land, TX) at 1000 rpm for 7 min. For positive selection of γδ T lymphocytes, the cell suspension was incubated with 10 μg/ml Dynabeads M-280 streptavidin (Dynal, Lake Success, NY) previously coated with 500 μg/ml biotinylated GL3 Ab (BD PharMingen) for 20 min at 4°C. Cells were then selected using a magnetic particle concentrator (Dynal) and washed eight times, according to manufacturer’s instructions.  

CSFE labeling  

CSFE (Molecular Probes, Eugene, OR) was diluted to 5 mM in DMSO (Sigma–Aldrich), aliquoted, and stored at ~20°C, protected from light under nitrogen. Cells were washed with 10 ml cold PBS, and cell concentrations were determined. Cells were resuspended at 5 x 10^6 cells/ml with 10 μM CSFE diluted in CSF medium 1640 (Invitrogen) supplemented with 20 mM HEPES, 0.01 M sodium bicarbonate (Invitrogen), 0.05 μg/ml penicillin G (Invitrogen), 0.1 mg/ml streptomycin (Invitrogen), and 10% FCS (Sigma–Aldrich). This medium will be subsequently referred to as RPMI-10. A total of 10 μl of 5 μM CSFE diluted in RPMI-10 was added to the cells and incubated at 37°C with inversion every 3 min. To stop labeling, several volumes of ice-cold RPMI-10 were added, and cells were washed twice in RPMI-10. Cells were then resuspended to 5 x 10^6 cells/ml when positively selected γδ T cells were labeled or 2 x 10^6 cells/ml for total peritoneal lymphocytes. CSFE-labeled cells were immediately injected into recipient mice.  

Adoptive transfer of γδ T cells into TCRδ−/− mice  

An adoptive transfer experiment was designed with six animals per group, in which three brains were processed for cytosactination and three for isolation of brain leukocytes for analysis by immunocytochemistry. Recipients were TCRδ−/− mice infected with *M. corti* for 4 wk (i.e., TCRδ−/−) or injected i.e. with HBSS (HBSS TCRδ−/−). Two populations of CSFE-labeled donor cells were used: 1) total peritoneal leukocytes from i.p.-infected C57BL/6 mice referred to as infected cells, and 2) total peritoneal leukocytes from normal C57BL/6 mice (normal cells). Experimental groups are described as follows: group I, i.e., TCRδ−/− transfected with infected cells; group II, TCRδ−/− transfected with normal cells infused at 10^7 cells/l; group III, HBSS TCRδ−/− transfected with infected cells. Control animals included i.e. infected TCRδ−/− mice and HBSS TCRδ−/− mice i.e. injected with 200 μl RPMI-10. Tissues were analyzed for the presence and distribution of infiltrating cells. CSFE-labeled lymphocytes (green fluorescence) were detected by direct microscopic examination of brain sections under a fluorescent microscope using the FITC filter. Immunofluorescence performance cross-reacted with the biotinylated with the streptavidin-Alexa Fluor 568 fluor (red fluorescence) allowed the visualization of dual-labeled γδ T cells as yellow fluorescence. The proportion of γδ T cells, αβ T cells, and Mac-1-positive cells was obtained after immunocytochemistry of isolated brain leukocytes 3 and 7 days after adoptive transfer. In a separate experiment, γδ T cells were first isolated from peritoneal leukocytes of i.p.-infected C57BL/6 mice and then labeled with 10 μl CFSE before injection into recipients. Three TCRδ−/− recipient mice i.e. infected with *M. corti* were injected with 1 x 10^6 CFSE-labeled γδ T cells in 200 μl and brain cryosections were analyzed after 12 h for the presence and distribution of γδ T cells, as described above.  

Immunohistochemistry  

Brain tissue sections or cytocentrifuged brain leukocytes were stained with specific biotinylated Abs, as described previously (8). For detection of IL-12, sections were incubated with purified anti-IL-12 Ab, followed by incubation with biotinylated goat anti- rat IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD). Both Abs were diluted in 3% BSA and incubated for 30 min at 37°C. Tissues were then incubated with streptavidin–alkaline phosphatase for 30 min at room temperature and developed using the alkaline phosphatase substrate kit 1 ( Vector Laboratories, Burlingame, CA). In between incubations, tissues/cells were washed three times for 3 min each. Similarly, the biotinylated Ab rat anti-goat IgG was used for detection of anti-IL-13 and anti-DEC205 Abs. A semiquantitative analysis of the cellular infiltrates was done by counting the number of cells of a given cell type/cytokine per section. All markers were tested on at least three animals. Positive cells were counted, and results were scored as: +, 1–100 positive cells per section; ++, 100–300; ++++, 300–500; and ++++, ≥500. After staining of cryocentrifuged leukocytes, the proportion of positive cells was obtained by counting the number of positive lymphocytes in a total of 1000 cells using ×600 magnification.
Immunofluorescence

All incubations were conducted at room temperature and, between incubations, slides were washed six times for 5 min each in 0.1% BSA in PBS. Tissue sections were incubated with the biotinylated GL3 for 1 h, followed by incubation with streptavidin-fluor 568 conjugate (Molecular Probes) for 30 min. Sections were washed and mounted using fluoresce reagent (Calbiochem, La Jolla, CA) containing 0.3 μM 4',6'-diamidino-2-phenylindole dilactate (Molecular Probes).

Results

Parasites preferentially invade the extraparenchyma areas of the brain in TCRδ−/− mice

Serial horizontal sections of infected mouse brain were stained with H&E to determine the number and location of M. corti metacestodes. The associated immunopathology was followed for 13 wk after infection. In BALB/c and C57BL/6 mice, parasites invaded brain parenchyma in a time-dependent manner. During the first 5 days after infection, most of the organisms remained outside the brain. By 1 wk p.i., the majority of the parasites were located in extraparenchymal regions, which include meninges, subarachnoid spaces, and ventricles. By 3 wk p.i., approximately one-half of the organisms had penetrated brain parenchyma (Table I, Fig. 1a). In contrast, in mice lacking γδ T cells, parasites preferentially invaded extraparenchymal areas of the brain, particularly subarachnoid spaces (Fig. 1b). At each time point analyzed (1, 3, 5, 8, and 13 wk p.i.), fewer parenchymal parasites were consistently found in TCRδ−/− mice compared with wild-type mice (Table I). In the 13-wk period analyzed, nearly 60% of the parasites were located in brain parenchyma in wild-type mice, compared with 26% in TCRδ−/− mice.

Infected TCRδ−/− mice develop fewer neurological manifestations

CNS infection with M. corti metacestodes in C57BL/6 and BALB/c mice was associated with the presence of severe neurological symptoms. Usually during the first 1–3 wk p.i., mice exhibited symptoms such as staggering, abnormal vestibular functions, including abnormal landing foot splay, repetitive walking in circles, and weight loss. With an inoculum of 40 parasites, wild-type mice typically did not survive longer than 13 wk after infection. During that period of time, ~60% of M. corti-infected mice (14 of 25 C57BL/6 mice and 19 of 31 BALB/c mice) exhibited intense neurological manifestations. In contrast, in TCRδ−/− mice, symptoms were only detected after 8 wk p.i. and were not as severe as those observed in wild-type mice even at 13 wk. During the course of the infection, ~30% (11 of 38) of the TCRδ−/− mice exhibited an extremely quiet state. This might be explained by the presence of large number of parasites in subarachnoid spaces perhaps causing increased i.c. pressure. However, TCRδ−/− mice with an inoculum of 40 parasites typically survived for 20 wk or more after M. corti infection, a substantially longer period than the 13 wk for normal background mice.

Reduced CNS pathology after M. corti infection in TCRδ−/− mice

H&E-stained sections of infected brains were also evaluated to determine changes in nervous tissue integrity (Fig. 2). Fig. 2a represents brain tissue from control animals that were i.c. inoculated with HBSS. In wild-type mice, areas of active necrosis were identified by the lack of cellularity in parenchymal tissue (Fig. 2b). Areas with spongiosis were initially detected within the first 2 wk p.i. and were present throughout the infection. These areas were determined by the presence of cell drop in areas close to the parasite (Fig. 2c). In TCRδ−/− mice, areas of necrosis in brain parenchyma were not detected. However, some of the parasites located in brain parenchyma were associated with small regions in which spongiosis was present (Fig. 2d), but to a lesser extent than wild-type infected mice.

TCRδ−/− mice exhibit a systemic disseminated infection

One of the most interesting observations during the infection of i.c. inoculated TCRδ−/− mice was the detection of parasites outside the CNS. During the first 7 wk after i.c. infection, ~7% (2 of 14) of the TCRδ−/− mice revealed the presence of parasites in liver parenchyma that were detected macroscopically during the perfusion procedure. By 8 wk p.i., 40% (11 of 24) of i.c. infected mice displayed a disseminated infection with encystment of the liver and a massive peritoneal infection. In normal background mice, the infection was maintained in the CNS. Parasites were not detected in the liver or peritoneal cavity. These observations indicate that γδ T cells are critical in maintaining a localized CNS infection.

Decreased abundance of immune cells in the brain of TCRδ−/− mice

The immune response in the CNS after infection with M. corti was analyzed by in situ immunohistochemistry (IHC). The infection in wild-type mice was associated with a strong inflammatory response in both extraparenchymal (Fig. 3a) and parenchymal (data not shown) areas of the brain. In wild-type mice, γδ T cells and macrophages appear associated with extraparenchymal parasites as early as 2 days p.i., and the numbers increase dramatically after 1 wk p.i. (Fig. 3a). Interestingly, γδ T cells constitute the predominant T cell population during the course of infection (Fig. 3, a and Fig. 3b).
b). γδ T cells (Fig. 3b) and macrophages were initially found in meninges and subarachnoid spaces. As the immune response progresses, a large number of cells accumulates in ventricles. αβ T cells, B cells/plasma cells (Fig. 3a), and dendritic cells (Fig. 3c) were also found in extraparenchymal infiltrates, although in less abundance. In contrast to the prominent cellular response elicited by the infection in wild-type mice, TCRδ−/− mice developed a less intense inflammatory response (Fig. 3d). Macrophages were detected by 2 days p.i. (Fig. 3d) and constituted the major leukocyte population in the brain of γδ T cell-deficient mice (Fig. 3e). Moreover, TCRδ−/− mice exhibited less accumulation of αβ T cells, B cells/plasma cells, and dendritic cells (Fig. 3f) within extraparenchymal infiltrates.

Compared with the immune response that develops in extraparenchymal areas, the immune response induced in brain parenchyma in both wild-type and γδ T cell-deficient mice was delayed and less prominent (data not shown). In wild-type mice, γδ T cells and macrophages were consistently present and associated with parenchymal parasites by 1 wk p.i. In both wild-type and TCRδ−/− mice, αβ T cells and B cells/plasma cells were found associated with parenchymal parasites after 1 and 5 wk, respectively. As described previously, dendritic cells were detected in ventricles and subarachnoid spaces in wild-type (Fig. 3c) and TCRδ−/− mice (Fig. 3f). However, in wild-type mice, dendritic cells rapidly mobilized toward brain parenchyma within 1 wk p.i. In contrast, in TCRδ−/− mice, few dendritic cells were present in parenchymal tissue by 3–5 wk p.i. and were not directly associated with parasites. In both wild-type and γδ T cell-deficient mice, polymorphonuclear cells were found in very low abundance (less than 15 Gr-1-positive cells/tissue section) in both extraparenchymal and parenchymal infiltrates.

Toluidine blue staining of infected brain sections was used to determine the presence and distribution of mast cells (Fig. 4). In wild-type mice, mast cells were detected in extraparenchymal infiltrates (Fig. 4a), both closely associated to the parasites as well as scattered in brain parenchyma (Fig. 4b). In TCRδ−/− mice, fewer mast cells were detected (Fig. 4, c and d). In wild-type mice, mast cells were often found in a degranulated stage.

Reduction of the levels of type 1 cytokines in TCRδ−/− mice after M. corti infection

To further characterize the immune response in the brain, the expression of several cytokines was analyzed by IHC. In wild-type mice, the infection was associated with high expression of IL-2, IL-12, IL-15, and IFN-γ (Fig. 5a). These cytokines were induced during the first week after infection, and colocalized to the areas in which γδ T cells were present. Relatively low expression of the type 2-related cytokines IL-4 and IL-13 was detected (Fig. 5b), and...
These results indicate that whereas in wild-type mice IL-12 promoting an inflammatory response in the brain.

To further examine the role of γδ T cells, we analyzed their migration pattern and distribution in the brain of TCRδ-/- mice. Cells were labeled with the vital fluorescent dye CFSE. It was hypothesized that the presence of γδ T cells in the brain of TCRδ-/- mice would be able to cause an increase in the numbers of cells expressing type 1 cytokines and, more importantly, to up-regulate the type 1 inflammatory response. In initial experiments, isolated γδ T cells obtained from the brains of i.c. infected C57BL/6 mice were used as donor cells. It was found that by 12 h after adoptive transfer, γδ T cells were specifically recruited into the brain of TCRδ-/- mice, as detected by the presence of green fluorescent groups of infiltrating CFSE-labeled γδ T cells in ventricles and subarachnoid spaces (Fig. 6a). Even though γδ T cells were found in high numbers in the brain of wild-type i.c. infected mice, it was difficult to isolate the numbers of positive selected cells required for the different groups for adoptive transfer experiment. Moreover, isolation of large numbers of γδ T cells from normal uninfected mice was not possible.

In additional experiments, it was demonstrated that γδ T cells constituted 10–20% of the total peritoneal population of i.p. infected mice. Moreover, the chemokine receptors expressed by γδ T cells in the peritoneum of i.p. infected mice were identical with brain γδ T cells (A. E. Cardona, W. A. Kuziel, and J. M. Teale, manuscript in preparation). Therefore, in subsequent experiments, total peritoneal cells that were CFSE labeled were used instead of brain γδ T cells. The migration of γδ T cells was analyzed at 12 h, 3 and 7 days after adoptive transfer. In addition to the CFSE labeling of adoptively transferred cells, brain sections from recipients were also stained with red fluorescent anti-γδ TCR so that γδ T cells could be specifically identified. γδ T cells were found in the brain 3 days after transfer (Fig. 6b, arrowhead). A population of CFSE-labeled cells was still present in the brain as analyzed at 3 days after transfer (Fig. 6c).

At 7 days, it was determined whether the transfer of γδ T cells in TCRδ-/- mice affected the cell types that infiltrated the CNS. The proportion of specific cell types was determined by immunocytochemistry on isolated brain leukocytes (Table II). The specific recruitment of cells appeared to require infection of recipients with M. corti. Recipient TCRδ-/- mice inoculated with HBSS did not reveal the presence of γδ T cells when donor peritoneal cells from infected mice were injected (Table II). In contrast, transfer of lymphocytes from infected mice into i.c. infected TCRδ-/- mice

Adoptively transferred γδ T cells into TCRδ-/- mice are found in the inflammatory infiltrates in infected brains

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caused a significant increase in the number of infiltrating cells into the brain (Table II). γδ T cells accounted for ~2% of the brain lymphocytes. Furthermore, four times more αβ T cells and twice as many Mac-1-positive cells were found after adoptive transfer. In contrast, when TCRδ−/− mice were transferred with peritoneal cells from uninfected mice, no statistically significant changes were detected in the abundance of αβ T cells and macrophages (Table II).

Discussion

Characterization of the immune response to M. corti in TCRδ−/− mice indicates that γδ T cells are instrumental immunoregulatory cells that promote and enhance host immune responses. The cellular inflammatory type 1 response that predominates in wild-type mice appears mediated by cytokines such as IL-12 and IFN-γ that are released early during the infection by γδ T cells. In sharp contrast, TCRδ−/− mice exhibited little inflammation and a direct effect of the host CNS immune response, presumably by their production of crucial cytokines such as IFN-γ and IL-12. In addition, γδ T cells were demonstrated to produce several CC chemokines early after M. corti infection, contributing to the recruitment of inflammatory cells into the brain. Interestingly, the transferred γδ T cells appeared to preferentially target the brain of infected mutant mice, as γδ T cells were found in very low numbers (5–20 cells/section) in the spleen of recipients and were localized to marginal areas of the germinal centers. Moreover, the number of splenic γδ T cells in recipient mice was essentially the same for all experimental groups. Such findings correlate with our previous studies in that when normal mice are i.p. infected with the same organism, the γδ T cell response in spleen and liver is much less predominant than that found in the brain. Thus, it is possible that γδ T cells are important immune cells for the brain similar to their importance in various mucosal tissues.

γδ T cells also affected the infiltration of dendritic cells and mast cells into the infected brain. Thus, lower numbers of both of these cell types were found in TCRδ−/− mice infected with M. corti compared with normal background mice. In addition, the mutant mice exhibited fewer dendritic cells in parenchymal tissue. This is of interest in that dendritic cells are usually found in the meninges, choroids plexus, and cerebrospinal fluid (CSF), but not in the parenchyma, unless there is inflammation (24–27), again confirming

Submitted for publication.

FIGURE 4. Mast cells in the brain of M.corti-infected mice. Toluidine blue staining of brain cryosections was used to determine the presence of mast in infected mice. a, Numbers of mast cells in infected C57BL/6 mice. The scored cell numbers represent 1, 1–40 cells; 2, 40–100 cells; 3, 100 cells. Results represent the average of two mice at each time point. b, Toluidine blue staining of a C57BL/6 mouse 1 wk p.i. showing the presence of mast cells in brain parenchyma (arrowheads). c, Numbers of mast cells in infected TCRδ−/− mice. Cells were counted and scored as explained in a. d, Toluidine blue staining of a TCRδ−/− mouse 1 wk p.i. Magnification ×630 (b and d).
FIGURE 5. Cytokine response in brain after *M. corti* infection. Cytokine-producing cells were detected by IHC of brain cryosections. 

(a) and (b), Numbers of cytokine-specific producing cells in C57BL/6 mice. 

(c) and (d), Cytokine response in TCRδ−/− mice. Scored cell numbers represent 1, 1–100 cells; 2, 100–300 cells; 3, 300–500 cells; 4, 500 positive cells. The results represent the average of two mice at each time point.

FIGURE 6. γδ T cells are recruited to the brain of TCRδ−/− mice after adoptive transfer.

(a), Brain cryosections were analyzed 12 h after i.v. injection of CFSE-labeled γδ T cells into i.c. infected TCRδ−/− mice 4 wk p.i. γδ T cells were found within inflammatory infiltrates in ventricles and subarachnoid spaces as green fluorescent (arrow). 

(b) and (c), Immunofluorescence performed in brain cryosections 3 days after i.v. injection of CFSE-labeled peritoneal cells from i.p. infected mice. γδ T cells were detected with the GL3 Ab, followed by streptavidin-fluor 568 conjugate (red fluorescent). Dual-labeled γδ T cells are seen in yellow (arrow), single-positive γδ T cells in red (arrowhead), and CFSE-labeled lymphocytes in green. The 4',6'-diamidino-2-phenylindole was used to visualize cell nuclei in blue. Magnification ×100 (a), and ×630 (b and c).
a reduced inflammatory state in the absence of γδ T cells. In the normal CNS, mast cells have been identified in meninges, thalamus, and periventricular regions, and generally localized adjacent to blood vessels (28, 29). However, the increased numbers of mast cells during neuropathological conditions and studies from experimental allergic encephalomyelitis (EAE) model suggest that mast cells play a role in neurologic inflammation (30, 31). This correlates with the findings shown in this study in that infected TCRδ−/− mice exhibited fewer mast cells and reduced inflammation and pathology. Mast cells are known to release mediators that cause tissue edema and extracellular matrix degradation (32, 33) and are most likely involved in amplifying the inflammatory response down-regulates the type 2 Ab response that may physically force the organisms out of the CNS.

In human NCC, active parenchymal infection is the most common form of the disease (2, 34, 35). However, it is thought that symptoms and associated diagnosis occur late in the disease. Parallel to the human findings, in wild-type mice parasites progressively penetrate brain parenchyma and the disease worsens as the immune response develops. As mice often present large number of parasites in the brain, the magnitude of the disease appears to be a combined effect of both the presence of parasites in ventricles and brain parenchyma and the associated inflammatory response. Although γδ T cell-deficient mice presented the vast majority of parasites in subarachnoid spaces, they exhibited reduced CNS manifestations and survive longer. These observations further suggest that the degree of the host immune response highly correlates with the severity of the disease.

Table II. γδ T cells are recruited to the brain of infected TCRδ−/− mice and cause an increased accumulation of infiltrating leukocytes

<table>
<thead>
<tr>
<th>Recipients</th>
<th>Donor Cells</th>
<th>% Immune Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.c. TCRδ−/−</td>
<td>RPMI only</td>
<td>γδ cells 0.68 ± 0.19 1.89 ± 0.12</td>
</tr>
<tr>
<td>i.c. TCRδ−/−</td>
<td>Infected</td>
<td>αβ cells 2.99 ± 0.23 3.69 ± 0.48</td>
</tr>
<tr>
<td>i.c. TCRδ−/−</td>
<td>Normal</td>
<td>— 2.56 ± 0.60</td>
</tr>
<tr>
<td>HBSS TCRδ−/−</td>
<td>Infected</td>
<td>— 0.07 ± 0.12</td>
</tr>
<tr>
<td>i.c. C57BL/6</td>
<td>RPMI only</td>
<td>— 11.25 ± 0.75</td>
</tr>
</tbody>
</table>

*Immunocytochemistry was performed in brain lymphocytes 7 days after transfer of 4 × 10⁶ peritoneal cells from wild-type mice into TCRδ−/− mice. At least 1000 cells were counted in ×600 magnification and the proportion of positive cells obtained. Data represent the average of three mice. —, No positive cells were found. Value of significance according to ANOVA analysis when the groups were compared to i.c. infected TCRδ−/− mice indicates p values shown below.

a p < 0.01, b p < 0.05.

The differential pattern of parasite distribution in wild-type and γδ T cell-deficient is not completely understood. M. corti meta-ccestodes most likely release proteolytic enzymes as a mechanism to penetrate brain parenchyma. Although hypothetical, it is possible that the immune response plays a critical role in enhancing the invasion process and parasite migration toward brain parenchyma by damaging the peripheral tissue of the brain and further breakdown of the blood brain barrier. In the absence of a strong inflammatory response as exhibited in TCRδ−/− mice, parasites might require increased time to penetrate the parenchyma, resulting in their accumulation in extraparenchymal spaces. γδ T cells also appear to control parasite migration in terms of their restriction to the CNS during infection. Thus, normal mice that are i.c. infected with M. corti exhibit an infection confined to the CNS, whereas TCRδ−/− infected mice often show a disseminated infection with encystment of the liver. The mechanism associated with this remains unclear, although it is possible that the tremendous accumulation of parasites in the extraparenchymal areas of mutant mice may physically force the organisms out of the CNS.

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References


