Impaired T Cell Immunity in B Cell-Deficient Mice Following Viral Central Nervous System Infection

Cornelia C. Bergmann, Chandran Ramakrishna, Margaret Kornacki and Stephen A. Stohlman

*J Immunol* 2001; 167:1575-1583; doi: 10.4049/jimmunol.167.3.1575

http://www.jimmunol.org/content/167/3/1575

References

This article cites 54 articles, 34 of which you can access for free at:
http://www.jimmunol.org/content/167/3/1575.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Impaired T Cell Immunity in B Cell-Deficient Mice Following Viral Central Nervous System Infection

Cornelia C. Bergmann, Chandran Ramakrishna, Margaret Kornacki, and Stephen A. Stohlman

CD8+ T cells are required to control acute viral replication in the CNS following infection with neurotropic coronavirus. By contrast, studies in B cell-deficient (μMT) mice revealed Abs as key effectors in suppressing virus recrudescence. The apparent loss of initial T cell-mediated immune control in the absence of B cells was investigated by comparing T cell populations in CNS mononuclear cells from infected μMT and wild-type mice. Following viral recrudescence in μMT mice, total CD8+ T cell numbers were similar to those of wild-type mice that had cleared infectious virus; however, virus-specific T cells were reduced at least 3-fold by class I tetramer and IFN-γ ELISPOT analysis. Although overall T cell recruitment into the CNS of μMT mice was not impaired, discrepancies in frequencies of virus-specific CD8+ T cells were most severe during acute infection. Impaired ex vivo cytolytic activity of μMT CNS mononuclear cells, concomitant with reduced frequencies, implicated IFN-γ as the primary anti-viral factor early in infection. Reduced virus-specific CD8+ T cell responses in the CNS coincided with poor peripheral expansion and diminished CD4+ T cell help. Thus, in addition to the lack of Ab, limited CD8+ and CD4+ T cell responses in μMT mice contribute to the ultimate loss of control of CNS infection. Using a model of virus infection restricted to the CNS, the results provide novel evidence for a role of B cells in regulating T cell expansion and differentiation into effector cells. *The Journal of Immunology*, 2001, 167: 1575–1583.

The CD8+ T cells comprise a dominant component in the resolution of many primary viral infections. However, chronic stimulation, especially under conditions of high Ag load, can lead to down-regulation of effector function, anergy, and ultimately Ag-specific CD8+ T cell exhaustion (1–3). Regulatory interactions involving CD4+ T cells, B cells, and Ig can delay the exhaustion process by providing help and/or by reducing Ag load. Thus, both the CD4+ T cell and humoral immune compartment play an active role in determining the outcomes of CD8+ T cell function ranging from complete viral clearance to uncontrolled viral replication concomitant with functional exhaustion (1–8).

The contribution of B cells and Ig in viral clearance as well as activation and maintenance of Ag-specific CD4+ and CD8+ T cells has been studied in several viral infection models using C57BL/6-Igh-Cμ(−/−) B cell-deficient (μMT) (3) mice (7, 9–17). These mice lack mature B220+ cells and consequently both germinal center formation and the ability to secrete Ig (9). Trapping of Ag-Ab immune complexes on follicular dendritic cells potentially maintaining T cell memory is thereby abrogated. However, no differences in viral replication or clearance were observed comparing infection of μMT and wild-type (wt)3 mice with lymphocytic choriomeningitis virus (LCMV), influenza virus, or murine cytomegalovirus (7, 8, 10, 11, 14). CD8+ T cell function appeared to be independent of B cells during numerous acute infections (7, 8, 10, 13). Furthermore, the CD8+ memory population remained stable in the absence of B cells or Ab following viral clearance (10, 13), and immune μMT mice effectively clear challenge virus (10, 15). Similarly, the frequency of CD4+ T cells was not altered significantly during acute influenza virus infection nor in the memory phase comparing wt and μMT mice (11). Thus, for these primary viral infections, CD8+ and CD4+ T cell activation and memory were independent of B cells.

By contrast, B cells and/or Ab play a critical role in controlling recrudescence and spread of viruses establishing persistent or latent infections (7, 13, 14, 17–20). Uncontrolled LCMV infection in μMT mice is associated with CD8+ T cell exhaustion, similar to high-dose exhaustion in wt mice (8, 13). Furthermore, LCMV immune T cells from μMT mice adoptively transferred into LCMV-infected recipients were less efficient in clearing infectious virus compared with immune splenocytes from wt mice (16). Despite containing similar frequencies of Ag-specific T cells, splenocytes from immune μMT mice had a reduced capacity to secrete both IL-2 and IFN-γ, suggesting impaired T cell effector function (16). Contrasting the majority of data implicating no role of B cells in T cell expansion and differentiation, the latter data suggested that B cells do participate in the generation and maintenance of fully competent CD4+ and CD8+ T cells during systemic LCMV infection.

Infection with the neurotropic JHM strain (JHMV) of mouse hepatitis virus provides a model of localized infection in which the absence of B cells results in uncontrolled, persistent CNS infection.
Despite initial viral reduction by T cells (17). In wt mice, a potent regional CD8+ T cell response is sufficient to completely eliminate infectious virus from the CNS (21–24). There is little mortality and the clinical symptoms of disease partially resolve following acute infection (25, 26). Nevertheless, viral Ag and RNA persist exclusively within the CNS (25, 27, 28). Persistence is associated with ongoing demyelination, but little or no clinical abnormalities, thus providing a model for the human demyelinating disease multiple sclerosis (25, 26). Humoral immune responses were thought to play a minor role based on observations that infectious virus is eliminated from the CNS before detection of neutralizing anti-viral Ab (29). Consistent with a primary role of cell-mediated immunity in controlling acute JHMV replication, infectious virus declines with similar kinetics in JHMV-infected μMT and wt mice (17). However, in contrast to wt mice, infectious virus recrudesces in the CNS of μMT mice following initial control of replication. Concomitant with virus reactivation, clinical symptoms continue to worsen and the majority of mice succumb to infection by days 30–45 postinfection (p.i.). Importantly, passive transfer of polyclonal anti-JHMV Ab, but not control Ab from naive mice, following initial clearance prevents virus reactivation (17). These data suggest that although T cell-mediated immunity is sufficient to reduce infectious virus during acute disease, immunological control is not maintained in the absence of humoral immunity.

Recrudescence of infectious virus in μMT mice could not be associated with selection of CTL escape variants nor an apparent limitation of virus replication. Similarly, immunization with 100 μCi of Na35CrO4 (New England Nuclear, Boston, MA) and S510 peptide was added to washed target cells at a final concentration of 1 μM before addition of CTL at the indicated E:T ratios. Supernatants (100 μl) were removed after 4 h of incubation and specific 35Cr release was determined. Specific lysis was defined as 100 × (experimental release − spontaneous release)/detergent release − spontaneous release). Maximum spontaneous release values were <10% of the total release values. The D0-restricted S510 peptide (37, 38) was synthesized by the University of Southern California Norris Cancer Center Microchemistry Laboratory and purity assessed by HPLC and mass spectrometry. The I-Aβ-restricted M133 peptide (39) was purchased from Genemed Synthesis (South San Francisco, CA). Peptides were solubilized at 1 mM in DMSO and diluted in sterile PBS.

**Materials and Methods**

**Mice, viruses, and CD4 depletion**

Male C57BL/6 (H-2b) mice were purchased from the National Cancer Institute (Frederick, MD) at 6 wk of age and certified naive to prior mouse hepatitis virus exposure. μMT mice, obtained as breeding pairs from The Jackson Laboratory (C57BL/6-Igh-6tm1Cgn; Bar Harbor, ME), were bred at the University of Southern California Keck School of Medicine under pathogen-free conditions. Mice were housed in microisolator cages in an accredited animal facility at the University of Southern California and infected at 6–7 wk of age. CNS infections were induced by intracranial injection of 30 μl containing 200–500 PFU of the 2.2v-1 mAb-derived variant of JHMV as previously described (30). This variant produces paralysis associated with demyelination and replicates predominantly in oligodendrocytes. Virus was propagated in the presence of neutralizing mAb J.2.2 and quantified by plaque assay using monolayers of the murine delayed brain tumor astrocytoma cell line (17). Intraperitoneal injections were conducted with 4 × 106 PFU of the parental JHMV isolate designated DM variant (31).

Mice were depleted of CD4+ T cells by i.p. injection of 250 μg of anti-CD4 mAb GK1.5 at days −2, 0, and +2 relative to virus infection as described elsewhere (22, 32); control mice received PBS injections at the same time points. This treatment resulted in >98% depletion of CD4+ T cells at day 8 p.i. as analyzed by flow cytometry.

**Tissue sampling and isolation of lymphocytes**

CMC were obtained from pooled brains and spinal cords of 6–10 mice/group at various time points p.i. as described previously (33, 34). Briefly, tissues were minced and homogenized in Tenbroek homogenizers. Cell suspensions were adjusted to 107 cells/ml and concentrated onto a 70% Percoll cushion by centrifugation at 800 × g at 4°C for 20 min, washed, and resuspended in RPMI 1640 medium. Yields varied from 0.8 to 2.0 × 106 cells/mouse, with maximum yields between days 8 and 12 p.i. Single-cell suspensions were prepared from the spleens and cervical lymph nodes (CLN) from individual groups of mice as previously described (35).

**CTL assays and synthetic peptides**

CTL assays were performed as described elsewhere (24, 36). Briefly, EL-4 (H-2b) target cells were labeled with 100 μCi of Na35CrO4 (New England Nuclear, Boston, MA) and S510 peptide was added to washed target cells at a final concentration of 1 μM before addition of CTL at the indicated E:T ratios. Supernatants (100 μl) were removed after 4 h of incubation and specific 35Cr release was determined. Specific lysis was defined as 100 × (experimental release − spontaneous release)/detergent release − spontaneous release). Maximum spontaneous release values were <10% of the total release values. The D0-restricted S510 peptide (37, 38) was synthesized by the University of Southern California Norris Cancer Center Microchemistry Laboratory and purity assessed by HPLC and mass spectrometry. The I-Aβ-restricted M133 peptide (39) was purchased from Genemed Synthesis (South San Francisco, CA). Peptides were solubilized at 1 mM in DMSO and diluted in sterile PBS.

**ELISPOT assays**

ELISPOT assays to measure the frequency of Ag-specific IFN-γ-secreting cells were conducted as described previously (24). Serial 2.5-fold dilutions of cells were plated in triplicate into 96-well plates supporting cellulosic ester membranes (Multiscreen HA; Millipore, Bedford, MA) and precoted with R4-6A2 mAb (BD PharMingen, San Diego, CA) and stimulated with R4-6A2 mAb (5–105 cells/ml) in 96-well microplates with R4-6A2 mAb (5–105 cells/ml) or left untreated. EL-4 supernatant was added as a source of IL-2 to a final concentration of 2.5%. Cultures were incubated for 36 h at 37°C. Bound IFN-γ was detected by overnight incubation at 4°C with biotinylated anti-IFN-γ mAb (0.5 μg/ml, XMGI-1.2; BD PharMingen), followed by subsequent incubations with streptavidin-peroxidase (Sigma, St. Louis, MO) and 3,3’-diaminobenzidine as substrate (Sigma).

**Flow cytometry**

Single-cell suspensions were blocked with purified anti-mouse CD16/ CD32 (G-I3; BD Pharmingen) and stained with FITC-, PE-, or Cy-Chrome-conjugated mAb specific for CD8 (53–6.7), CD4 (GK1.5), CD62L (MEL-14), CD43 (1B11), CD44 (IMT), CD69 (H1.2F3), CD25 (PC61), CD11a (2D7), CD49d (R1-2; BD PharMingen), and PE-conjugated D0-S510 tetramer (0.1–0.2 μg/0.5–1.0 × 106 cells) in various combinations. Cells were typically stained at 4°C with mAb for 15–20 min before incubation with tetramer for 30 min in PBS containing 0.1% BSA. The generation of D0-S510 tetramers, composed of D0 class I H chains, β2-microglobulin, and the S510 peptide, was previously described (24). Samples were analyzed by flow cytometry on a FACStar (BD Biosciences, Mountain View, CA). Forward and side scatter signals obtained in linear mode were used to establish a gate that contained intact lymphocytes, while excluding remaining tissue debris. A minimum of 4 × 104 viable cells were subsequently acquired and analyzed.

Synthesis of intracellular IFN-γ in response to Ag stimulation was determined by incubating 4–5 × 106 CMC or 1 × 106 splenocytes in 100 μl of RPMI 1640 complete supplemented with 10% FCS, 1 μM peptide, and ...
1 μg/ml monensin (BD Pharmingen) for 5 h at 37°C (3). Peptide was omitted in negative control samples. Cells were then stored at 4°C overnight and stained 14–16 h later using the Cytofix/Cytoperm kit (BD Pharmingen). Following surface staining, cells were fixed, permeabilized, and stained with mAb specific for IFN-γ (XMG1.2) as recommended by the supplier (BD Pharmingen).

**Results**

*Reduced Ag-specific CD8+ T cell responses within the CNS of μMT mice*

Previous studies of acute JHMV infection in μMT mice suggested that T cell responses within the CNS were comparable to those in wt mice (17). This interpretation was based on 1) similar kinetics of initial virus clearance, 2) no apparent differences in the number of CNS infiltrating CD8+ or CD4+ T cells determined by immunohistochemistry, and 3) similar CTL responses of in vitro-restimulated splenocytes harvested at day 7 p.i. However, the inability of μMT mice to control the infection beyond day 10 p.i. implied that T cells were rendered unresponsive and/or underwent activation-induced cell death due to increasing viral load. To assess the possibility of CD8+ T cell exhaustion, the frequencies of CD8+ T cells specific for the immunodominant S510 epitope were determined at times when virus has been cleared from the CNS of wt mice, but re-emerged in μMT mice (17). CMC from wt and μMT-infected mice were initially analyzed at days 14 and 21 p.i. by flow cytometry and IFN-γ ELISPOT assay (Fig. 1). The percentage of total CD8+ T cells in CMC was similar or elevated in μMT mice (Fig. 1A), consistent with immunohistochemical analysis (17). Nevertheless, as determined by tetramer staining, the population of virus-specific cells within the CD8+ T cell compartment never exceeded 12% in μMT-derived CMC, whereas it ranged between 30 and 40% in wt-derived CD8+ CMC (Fig. 1A). Consistent with a relative decrease in tetramer staining cells, the frequencies of CD8+ T cells secreting IFN-γ at day 14 p.i. were at least 3-fold lower in μMT- compared with wt-derived CMC (Fig. 1B). In wt mice, frequencies of IFN-γ-secreting cells further declined by day 21 p.i., consistent with the decline in viral Ag (17). Decreased frequencies of IFN-γ-secreting CD8+ T cells were also evident in CMC from μMT mice, despite viral recrudescence. This confirmed an inability to respond to increasing virus load and suggested either limited resources of peripheral Ag-specific T cells to replenish the CNS, inhibited infiltration, down-regulation of type 1 CD8+ T cell effector function, or a combination of these factors.

Previous analysis indicated no difference in numbers and distribution of apoptotic cells in the CNS of wt vs μMT mouse (17). Significantly reduced frequencies of S510-specific CD8+ T cells in μMT mice following initial control of viral replication could thus not be attributed to enhanced apoptosis. To uncover potential defects in induction or recruitment of virus-specific CD8+ T cells in μMT mice, CMC were analyzed at early times p.i. (Fig. 2). At day 5 p.i., percentages of total CD8+ T cells were higher in CMC from μMT mice, suggesting that T cell recruitment into the CNS was not impaired in μMT mice; tetramer+ cells within the CMC population of either group were below detection limits at this time (Fig. 2A). Surprisingly however, at day 8 p.i., when viral clearance was similar in both groups (17), CD8+ T cells within CMC derived from μMT mice only contained 4–9% tetramer+ cells, whereas CD8+ T cells from wt mice typically contained 33% tetramer+ cells (Fig. 2A). Despite similar overall CD8+ T cell infiltration, tetramer+ cells constituted a significantly reduced percentage within the CNS of μMT mice compared with wt mice. Overall yields of CMC from either infected group ranged from 1.5 to 2.0×10^6/mouse during acute infection, and differences never exceeded 25% between the groups. These data implied that few of the CD8+ T cells recruited into the CNS of JHMV-infected μMT mice are

**FIGURE 1.** Reduced frequencies of S510-specific CD8+ T cells during viral recrudescence in the CNS of μMT mice. CMC were prepared from JHMV-infected mice at 14 and 21 days p.i. (n = 7–8 per time point). A. CMC were stained for expression of CD8 (FITC-labeled anti-CD8) and S510 epitope-specific TCR using PE-labeled D^b-S510 tetramers. Percentages of CD8+ T cells within CMC are depicted by boxes (left y-axis) and percentages of tetramer+ cells within the CD8 population by circles (right y-axis); ■, wt CMC; □, μMT CMC. Data from two representative experiments of four, designated a and b, are shown. B. The frequency of cells responding to S510-coated feeder splenocytes was measured by IFN-γ ELISPOT. Numbers of cells secreting IFN-γ in response to peptide stimulation were determined within a linear range and standardized to 1×10^6 unfractionated cells. Cells secreting IFN-γ in the absence of peptide were subtracted. Error bars, SD of three to six wells.

**FIGURE 2.** Reduced frequencies of S510-specific CD8+ T cells during CNS infection of μMT mice. CMC of infected mice were prepared at 5 and 8 days p.i. (n = 7–8 per time point) and analyzed as described in the legend to Fig. 1. A. CMC were stained with anti-CD8 mAb and D^b-S510 tetramers. Data from two experiments, a and b, representative of four to five experiments are shown. B. The frequency of cells responding to S510-coated feeder splenocytes was measured by IFN-γ ELISPOT. Ratios of tetramer+ IFN-γ-secreting cells are indicated above each bar at day 8 p.i.
impaired S510 specific or, alternatively, TCR down-regulation due to high stimulation. Frequencies of S510-specific IFN-γ-secreting cells were also at 3-fold lower in μMT-derived CMC (Fig. 2B), confirming reduced numbers of S510-specific T cells at the functional level. Nevertheless, similar or lower ratios of tetramer+ to IFN-γ-secreting CD8+ T cells in CMC from μMT mice compared with wt mice suggests that μMT-derived CD8+ T cells are not inherently impaired in cytokine-mediated effector function.

A reduction of S510-specific CD8+ T cells at day 8 p.i. was not anticipated due to effective virus clearance up to day 10 p.i., suggesting that few virus-specific CD8+ T cells suffice to mediate strong antiviral effector function. However, direct examination of this notion revealed very poor ex vivo cytolytic activity of μMT-derived CMC at day 8 p.i. compared with wt CMC (Fig. 3). Even when E:T ratios were adjusted to reflect only tetramer+ CD8+ T cells, cytolyis on a per cell basis was severely reduced. Furthermore, re-emerging viral replication did not trigger increased cytolytic activity (Fig. 3). Thus, similar to wt mice (24), CNS-resident CD8+ T cells in μMT mice were rendered noncytolytic after initial viral reduction. These data support IFN-γ secretion as a major antiviral mechanism (23), as this function did not appear impaired on a per cell basis (Fig. 2) in contrast to cytolytic function (Fig. 3).

Phenotypic comparisons of CD8+ CMC derived from acutely infected μMT and wt mice revealed similar expression patterns of activation/memory markers (Table I). The majority of CNS-infiltrating CD8+ T cells at day 8 p.i. were CD44low/int and CD62high, CD11a low, and CD49d+ phenotype, characteristic of an activated/memory phenotype (40). However, μMT-derived CMC consistently contained slightly increased populations of naive CD8+ T cells characterized by the CD44high/int and CD62low, CD11ahigh and CD49d+, specific CD8+ T cells were more evident on the CD8+ populations of μMT-derived CMC (Table I); no differences in CD69 or CD25 expression was evident at days 5 and 8 p.i. Tetramer+ CD8+ T cells were readily detectable in the spleen and CLN of wt mice, but were severely reduced in μMT mice, despite higher CD8+ T cell percentages arising from the lack of B cells (Fig. 4A). Analysis of CMC from the same groups of mice confirmed accumulation of Ag-specific cells specifically at the site of infection. These data suggested that reduced S510-specific CD8+ T cells in the CNS of μMT mice resulted from impaired peripheral expansion following CNS infection. Consistent with these data, the frequency of IFN-γ-secreting S510-specific CD8+ T cells in both spleens and CLN was 2- to 3-fold lower in μMT mice (Fig. 4B). However, each group of mice revealed discrepancies between tetramer staining and ELISPOT analysis. Although splenocytes of wt mice contained higher frequencies of tetramer+ T cells than CLN, the frequency of IFN-γ-secreting cells was higher in the CLN. This inability to detect tetramer+ cells may reside in down-regulation of TCR due to more potent Ag-specific stimulation in the CLN. The same observation was made in μMT mice, despite overall lower frequencies of IFN-γ-secreting cells at both sites. Enhanced frequencies of IFN-γ-secreting cells in the CLN compared with spleen were already pronounced at day 5 p.i., when tetramer+ cells were below detection. These data thus support CLN as the primary site of T cell priming in both wt and μMT mice during CNS infection, as suggested for CNS infection induced by LCMV (42). The lack of higher frequencies of virus-specific CD8+ T cells in the spleen of wt mice compared with μMT mice at day 5 p.i. further indicates that sequestration of virus-natural Ab immune complexes to the spleen (43) plays a minor role in T cell priming in this model of infection.

Impaired CD8+ T cell responses are associated with reduced CD4+ T cell activation

For some viral infections, CD8+ T cell expansion, function, and survival is tightly linked to the CD4+ T cell compartment (22, 44–47). During acute JHMV infection, survival and function of CD8+ T cells in the CNS, but not entry, is dependent on CD4+ T cells (22). Furthermore, CD4+ T cells appear to enhance peripheral CD8+ T cell expansion, as indicated by decreased cytolytic activity of splenocytes from infected mice depleted of CD4+ T cells (22). ELISPOT analysis was therefore used to analyze expansion of CD4+ T cells specific for the dominant M133 epitope located within the viral matrix protein (39). At day 8 p.i., both splenocytes and CMC from μMT mice contained severely reduced frequencies of IFN-γ-secreting CD4+ T cells (Fig. 5). Reduced frequencies of M133-specific CD4+ T cells in the CNS, despite similar total CD4+ populations, again implied a defect in virus-specific CD4+ T cell expansion rather than recruitment, similar to CD8+ T cell expansion. This was indeed supported by the low frequencies of splenic virus-specific CD4+ T cells. Reduced CD8+ T cell responses in μMT mice following JHMV infection may thus be a direct consequence of impaired CD4+ T cell expansion, rather than a CD8+ T cell-specific defect.

To confirm a role of CD4+ T cell function for JHMV-specific CD8+ T cell expansion, wt mice were depleted of CD4+ T cells before infection. CMC from CD4-depleted and mock-depleted infected wt mice were compared with infected μMT mice for production of IFN-γ by peptide-stimulated CD8+ T cells (Fig. 6).
Intracellular IFN-γ staining revealed that CD4⁺ T cell depletion reduced the frequency of S510-responsive CD8⁺ T cells to 60% of that in untreated wt mice. However, only 4% of CD8⁺ T cells from μMT mice produced IFN-γ following stimulation; this represents only 19% of the wt value. Nevertheless, the mean fluorescence intensities of IFN-γ⁺ cells from μMT and CD4-depleted mice were only slightly reduced, indicating no significant impairment in IFN-γ production. These data suggest that although CD4⁺ T cells enhance CD8⁺ T cell responses during JHMV infection, deprivation of CD4⁺ T cell help alone does suffice to account for the impaired CD8⁺ T cell response in μMT mice. Transfer of 2.5 × 10⁷ splenic B cells from naive wt mice into μMT mice a day before infection did not affect priming/expansion of either CD4⁺ or CD8⁺ T cells (data not shown). There were no differences in the frequencies of S510 CD8⁺ T cells or M133 CD4⁺ T cells at 5 or 8 days p.i. in either the spleen, CLN, or CNS. However, CD19⁺ B cells only comprised 1–2% in splenic or CLN populations at the time of T cell analysis. The inability to rescue T cell expansion presumably resides in the previously reported rapid disappearance of B cells from μMT recipients (48). Thus, unlike other peripheral infections studied in μMT mice (10–15), T cell priming and expansion following infection of the CNS appears to require the physical presence of B cells and/or a B cell-dependent component absent in μMT mice.

To evaluate whether decreased T cell priming in μMT mice is correlated to CNS infection, a more virulent, parental JHMV was administered i.p. This route of administration results in an asymptomatic infection associated with limited replication, but effective

**FIGURE 4.** Expansion of virus-specific CD8⁺ T cells in peripheral lymphoid organs. A. Spleen (SPL) and CLN cells, as well as CMC, obtained from μMT and wt mice at day 8 p.i. were stained for expression of CD8 (FITC-labeled anti CD8; x-axis) and S510 epitope-specific TCR (PE-labeled D⁹-S510 tetramer as indicated; y-axis). Density plots of cells from wt mice are shown in the left column, cells from μMT mice in the right column. Numbers in each quadrant represent percentages of the total population. Plots are representative of three separate determinations. B. Splenocytes and CLN cells obtained from mice at 5 and 8 days p.i. stained with anti-CD8 mAb and D⁹-S510 tetramer. Percentages of total CD8⁺ T cells are depicted by bars (left y-axis). Percentages of Db-S510 tetramer⁺ cells within the CD8⁺ population are depicted by circles (right y-axis). C. The frequency of cells responding to peptide-coated feeder splenocytes was measured by IFN-γ ELISPOT as described in the legend to Fig. 1; cells were derived from the same groups of mice analyzed in B. Data are representative of three separate experiments.

**FIGURE 5.** Expansion of virus-specific CD4⁺ T cells in μMT mice. CMC and splenocytes obtained from infected wt and μMT mice (n = 6–8 per group) at day 8 p.i. were analyzed for the frequency of CD4⁺ T cells responding to the dominant viral matrix protein-derived M133 peptide. Splenocytes from wt mice coated with 1 μM peptide were used as feeder cells. Frequencies of IFN-γ-secreting cells were calculated as described in the legend to Fig. 1. Total percentages of CD4⁺ T cells (right y-axis) are shown by circles for reference. Data are representative of three independent assays.

**Table 1.** Phenotypic analysis of CMC-derived CD8⁺ T cells

<table>
<thead>
<tr>
<th></th>
<th>S510-TCR</th>
<th>CD62L⁻</th>
<th>CD44high</th>
<th>CD11chigh</th>
<th>CD49d⁺</th>
<th>CD25⁺</th>
<th>CD69⁺</th>
<th>CD43high</th>
<th>CD25⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>32.1</td>
<td>94.8</td>
<td>91.6</td>
<td>96.7</td>
<td>90.0</td>
<td>2.3</td>
<td>30.7</td>
<td>76.1</td>
<td>76.1</td>
</tr>
<tr>
<td>μMT</td>
<td>8.3</td>
<td>87.2</td>
<td>87.5</td>
<td>92.6</td>
<td>75.0</td>
<td>4.2</td>
<td>43.8</td>
<td>43.2</td>
<td>43.2</td>
</tr>
</tbody>
</table>

* CMC obtained from wt and μMT mice at day 8 p.i. (n = 3–4 per group) were stained for expression of CD8, S510 epitope-specific TCR, and the indicated activation markers. Numbers represent percentages within the CD8⁺ population. Data are representative of three different experiments.
T cell priming in wt mice. At day 8 after peripheral immunization, splenocytes of μMT mice revealed severely decreased expansion of SS10-specific CD8\(^+\) T cells compared with wt mice (Fig. 7). Percentages of both tetramer\(^+\) and IFN-γ-producing cells in the CD8\(^+\) population were reduced at least 5-fold in μMT mice. These data confirm a role of B cells in priming CD8\(^+\) T cells and their CD8\(^+\) T cell response compared with mock-treated wt and μMT mice at day 8 p.i. CMC from all three groups were stimulated with 1 μM SS10 peptide for 5 h using endogenous APC as feeders. Production of intracellular IFN-γ was measured in the CD8\(^+\) population by flow cytometric analysis. Numbers in each quadrant represent percentages of the total population. Numbers in parentheses, percentages of IFN-γ\(^+\) cells in the CD8\(^+\) T cell population. Mean fluorescent intensities (MFI) of IFN-γ\(^+\) cells are indicated. Dot plots are representative of three separate determinations.

**Reduced absolute T cell numbers compromise control of infection in μMT mice**

The effects of impaired Ag-specific CD8\(^+\) T cell expansion in μMT mice are compounded by reduced cellularity of both CLN and spleen (11). Although relative percentages of CD4\(^+\) and CD8\(^+\) T cells are increased, cell yields of CLN and spleen per mouse in JHMV-infected μMT mice at day 8 p.i. were typically 2- to 3- and 4- to 6-fold reduced compared with wt mice, respectively. Because tetramer\(^+\) cells were below detection limits in spleen and CLN after d 8 p.i. in both wt and μMT mice, frequencies of SS10-specific IFN-γ-secreting T cells were used to calculate SS10-responding T cells per spleen to adjust for the reduced cellularity in μMT mice throughout the course of infection (Fig. 8). A reduction of splenic SS10-specific CD8\(^+\) T cells in μMT mice was most dramatic on day 8 p.i., but was evident throughout the infection in μMT mice; at day 21 p.i., the frequency of SS10-specific IFN-γ spots was below 1 in 10\(^6\) splenocytes (Fig. 8). This supports the notion that few available circulating memory cells may lead to CD8\(^+\) T cell exhaustion, even when chronic Ag stimulation is confined to the CNS. These data suggest that in addition to the lack of Ab, inefficient CD8\(^+\) T cell expansion and concomitant exhaustion may contribute to viral recrudescence within the CNS of μMT mice.

**Discussion**

Acute viral infections generally elicit both cellular and humoral immune responses. Selective depletion of individual effector subsets, commonly used to identify correlates of protective immunity, has uncovered redundancies, but also cooperative mechanisms, between different arms of the adaptive immune response (19, 20, 44, 46, 47). During virus infections typically establishing persistence, the interactions between distinct immune components may be critical in the survival of the host. Thus, acute JHMV infection of the CNS is primarily controlled by CD8\(^+\) T cells (21). However, CD4\(^+\) T cell help enhances CD8\(^+\) T cell expansion, effector function, and survival (22). Neutralizing Ab emerges following clearance of infectious virus (29), but T cells are also maintained within the CNS (24, 34). The long-term inability of T cells to control infection in μMT mice, despite initial clearance of infectious virus,
revealed the protective role of humoral immunity during persistence (17). The fact that infusion of polyclonal JHMV Ab at the time of initial clearance effectively controlled recrudescence (17) suggested that T cells may be redundant after reducing infectious virus to undetectable levels. Precedence for suppression of persisting virus in the CNS by prolonged intrathecal Ab synthesis by B cells within the CNS is provided by control of alphavirus encephalomyelitis (20).

The reactivity of virus-specific T cells was examined to determine the fate of initially protective CD8+ T cells during viral recrudescence in μMT mice. The CNS of μMT mice contained comparable numbers of CD8+ T cells relative to wt mice, but surprisingly lower frequencies of virus-specific CD8+ T cells throughout the entire course of infection. In addition, severely compromised cytolytic function by μMT-derived CMC during the acute response raises the question as to the mechanism which initially controls virus replication. Although perforin-mediated cytolysis clears virus from microglia and astrocytes (29), IFN-γ acts as the dominant antiviral cytokine in oligodendrocytes (23). However, studies of pathogenesis in infected perforin- and IFN-γ-deficient mice indicate that IFN-γ plays a more prominent role in protection (23, 29). By contrast, Fas-Fas ligand interactions do not contribute to viral clearance or pathogenesis (51). Initial viral clearance in μMT mice may thus be predominantly controlled by antiviral IFN-γ rather than perforin-mediated cytolysis. An ineffective cytolytic response in vivo is supported by the similar distribution of viral Ag during viral recrudescence compared with acute infection, i.e., in microglia, astrocytes, and oligodendrocytes (17). Nevertheless, impaired cytoxicity is not intrinsic to μMT-derived CD8+ T cells, as cytolytic function is readily restored upon restimulation with infected wt splenocytes in vitro (17). Furthermore, a pronounced defect in IFN-γ secretion by μMT-derived CD8+ T cells was inapparent taking the overall lower frequencies of virus-specific CD8+ T cells into account. This is supported by high levels of IFN-γ mRNA in the CNS of μMT mice (17), similar IFN-γ+ :tetramer+ T cell ratios, and only moderate reduction in IFN-γ production as measured by intracellular staining (see Figs. 2 and 6). Since NK cells constitute similar percentages of CMC from both groups of mice early during infection (4–5% at day 8 p.i.) and drop below detection by day 14 p.i. (data not shown), they are not believed to contribute significantly to altered pathogenesis in μMT mice. Initial viral clearance in μMT mice, despite reduced T cell effector function, suggests that the response in wt mice by far surpasses a minimal threshold required for antiviral control. Nevertheless, this apparently excessive response may be critical in prolonging viral control and limiting virus spread within the CNS until the emergence of protective Ab.

Reduced primary virus-specific T cell responses in the absence of B cells were not evident following a number of other virus infections (7, 8, 10, 11, 14). Effector function of virus-specific CD8+ T cells, as well as CD8+ memory populations (10, 13), and frequencies of virus-specific memory CD4+ T cells (11) were not altered significantly in μMT mice compared with wt mice. However, impaired antiviral T cell function in μMT mice was suggested by the inability of adoptively transferred T cells primed in these mice to control LCMV infection (16). A reduced capacity of splenocytes from LCMV-immune μMT mice to secrete both IL-2 and IFN-γ, but not reduced frequencies of Ag-specific T cells, contrasts sharply with reduced virus-specific CD4+ and CD8+ T cells but no significant reduction in IFN-γ production observed during JHMV infection. The reasons underlying impaired T cell function in JHMV-infected μMT mice compared with other viral infections is unclear. Ineffective recruitment of T cells into the CNS can be ruled out because total percentages of CD4+ and CD8+ T cells are similar in the CNS of wt and μMT mice. Reduced frequencies of virus-specific CD8+ T cells in both spleen and CLN during the acute response rather implicates inefficient priming/expansion. The fact that CD4+ T cell expansion is also severely limited in infected μMT mice suggests that reduced CD4+ T cell help diminishes CD8+ T cell expansion and differentiation. CD4+ T cell responsiveness was also decreased in B cell-deficient mice early during ocular HSV infection (52), and expansion, but not function, was also impaired in CD4+ T cells from μMT mice immunized with keyhole limpet hemocyanin (53, 54). The defects in keyhole limpet hemocyanin-responding T cells were attributed to ineffective Ag presentation due to the lack of immune complex formation (54). Nevertheless, B cells appear to provide a more critical component than CD4+ Th cells in regulating CD8+ T cell expansion during JHMV infection, as virus-specific CD8+ T cells in peripheral lymphoid organs as well as in CMC were more severely reduced in the absence of B cells than in CD4+ T cell-depleted mice. Efforts to enhance T cell responsiveness via transfer of B cells from naive mice to μMT recipients failed. No differences in virus-specific CD4+ or CD8+ T cell frequencies were evident 5 or 8 days p.i. in these recipients compared with untreated μMT mice (data not shown). B cell transfers also had no effect on virus titers in the CNS at days 5 or 8 p.i. (data not shown). The degree to which the absence of B cells or Ab alone, the lack of CD4+ T cell-B cell interactions, or inhibited follicle formation and disrupted lymphoid architecture in μMT mice contribute to CD8+ T cell expansion therefore remains unclear.

Exclusive neurotropism of JHMV may compound inefficient Ag-specific CD4+ and CD8+ T cell expansion in JHMV-infected μMT mice. Although the CNS is efficient in supporting secondary activation of primed T cells, it is poor in priming naive T cells (49, 50, 55). T cell priming during a parenchymal CNS infection is believed to take place in peripheral lymphoid organs where B cells may contribute to activation. The absence of B cell follicle formation is associated with reduced follicular dendritic cell networks in μMT mice and diminished spleen and CLN cellularity (11). Thus, the restricted nature of JHMV replication in the CNS, coincident with limited peripheral Ag presentation, may render T cell activation more dependent on a component provided by B cells compared with other viral infections previously studied. However, perturbed T cell expansion even following peripheral JHMV immunization suggested a mechanism involving Ag presentation rather than CNS infection as responsible for diminished T cell responsiveness. There is no evidence that B cells themselves act as APC during JHMV infection. However, an indirect regulatory role for B cells during priming cannot be ruled out. Although a role for Ab and complement in enhancing Ag presentation in wt mice via sequestration of immune complexes to the spleen cannot be ruled out (43, 54), there was no evidence for enhanced splenic vs CLN priming in wt mice compared with μMT mice (Fig. 4). The observation that JHMV replication in μMT mice follows similar kinetics and never exceeds titers in wt mice also negates a role of immune complexes in delaying CNS infection. Interestingly, a significant contribution of B cells to effector CD8+ T cells was recently uncovered in the clearance of, and recovery from, influenza virus infection (56). However, in contrast to JHMV infection, the as yet unidentified B cell activity was Th cell independent and did not appear to enhance CD8+ T cell function. Furthermore, the lack of Th cell-B cell interaction may indirectly dysregulate APC function. B cells have indeed been shown to affect dendritic cell function by regulating cytokine production. Upon Ag stimulation, dendritic cells from μMT mice produce higher levels of IL-12p70 and fail to induce IL-4 secretion, resulting in potential CD4+ Th1 cell
deviation (57). Although there is no evidence for this pathway during JHMV infection, alternative default interactions of CD4+ T cells with APC rather than B cells may conceivably result in diminished T cell activation.

In summary, the data indicate that not only the lack of Ab, but severely reduced frequencies of virus-specific T cells contribute to viral recrudescence in the CNS of μMT mice. Impaired CD4+ and CD8+ T cell expansion appears to be unique to JHMV infection of the CNS parenchyma and results in reduced T cell effector function within the CNS. Although the limited T cell response is sufficient to reduce viral replication, virus is not cleared below detection and recrudescences. Premature loss of effector function below a minimal threshold may thus provide a window for virus reactivation. An inability to replenish effector cells concomitant with increasing Ag load in the CNS appears to ultimately lead to exhaustion. Reduced numbers of splenic T cells in μMT mice (10, 11) and inadequate CD4+ T cell help may facilitate this process, analogous to high-dose LCMV-induced loss of specific CD8+ T cells (1–3, 7). Thus, a vigorous early T cell response may tip the balance between viral replication and immunity to viral persistence, which is ultimately controlled by Ab.

References


5. Rehermann, B., M. K. Chang, J. McHutchinson, R. Kokka, M. Houghton, J. A. Misplon, and J. R. Bennink. 1998. Mechanism of impaired T cell activation in μMT mice (10, 11) and inadequate CD4+ T cell help may facilitate this process, analogous to high-dose LCMV-induced loss of specific CD8+ T cells (1–3, 7). Thus, a vigorous early T cell response may tip the balance between viral replication and immunity to viral persistence, which is ultimately controlled by Ab.

References


5. Rehermann, B., M. K. Chang, J. McHutchinson, R. Kokka, M. Houghton, J. A. Misplon, and J. R. Bennink. 1998. Mechanism of impaired T cell activation in μMT mice (10, 11) and inadequate CD4+ T cell help may facilitate this process, analogous to high-dose LCMV-induced loss of specific CD8+ T cells (1–3, 7). Thus, a vigorous early T cell response may tip the balance between viral replication and immunity to viral persistence, which is ultimately controlled by Ab.

References


5. Rehermann, B., M. K. Chang, J. McHutchinson, R. Kokka, M. Houghton, J. A. Misplon, and J. R. Bennink. 1998. Mechanism of impaired T cell activation in μMT mice (10, 11) and inadequate CD4+ T cell help may facilitate this process, analogous to high-dose LCMV-induced loss of specific CD8+ T cells (1–3, 7). Thus, a vigorous early T cell response may tip the balance between viral replication and immunity to viral persistence, which is ultimately controlled by Ab.

References


