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*J Immunol* 2002; 168:1204-1211; doi: 10.4049/jimmunol.168.3.1204

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*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852

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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Mechanisms of Central Nervous System Viral Persistence: the Critical Role of Antibody and B Cells

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Contributions of humoral and cellular immunity in controlling neurotropic mouse hepatitis virus persistence within the CNS were determined in B cell-deficient J1D and syngeneic H-2d B cell+ Ab-deficient mice. Virus clearance followed similar kinetics in all mice, confirming initial control of virus replication by cellular immunity. Nevertheless, virus reemerged within the CNS of all Ab-deficient mice. In contrast to diminished T cell responses in H-2d B cell-deficient μMT mice, the absence of B cells or Ab in the H-2d mice did not compromise expansion, recruitment into the CNS, or function of virus-specific CD4+ and CD8+ T cells. The lack of B cells and lymphoid architecture thus appears to manifest itself on T cell responses in a genetically biased manner. Increasing viral load did not enhance frequencies or effector function of virus-specific T cells within the CNS, indicating down-regulation of T cell responses. Although an Ab-independent antiviral function of B cells was not evident during acute infection, the presence of B cells altered CNS cellular tropism during viral recrudescence. Reemerging virus localized almost exclusively to oligodendroglia in B cell+ Ab-deficient mice, whereas it also replicated in astrocytes in B cell-deficient mice. Altered tropism coincided with distinct regulation of CNS virus-specific CD4+ T cells. These data conclusively demonstrate that the Ab component of humoral immunity is critical in preventing virus reactivation within CNS glial cells. B cells themselves may also play a subtle role in modulating pathogenesis by influencing tropism. The Journal of Immunology, 2002, 168: 1204–1211.

The effector mechanisms, which limit viral replication and damage to the host during acute infection, depend upon tissue and cell tropism as well as virus cytopathogenicity (1). Although the individual mechanisms involved are becoming increasingly clear (1, 2), their role(s) in establishing or maintaining persistent viral infections are less well understood (2, 3). For example, lymphocytic choriomeningitis virus (LCMV)3 infection is largely controlled by virus-specific CTL (1, 4–6). However, regulation of persistent LCMV infection is dependent upon humoral immunity, CD4+ T cells, and cytokines (5, 7). Similarly, CMV is cleared from the majority of tissues by CD8+ T cells, while clearance from the salivary gland is mediated by CD4+ T cells (8). Although these T cells function effectively in the absence of B cells, dissemination of reactivating virus is controlled by antiviral Ab (9). T cell-mediated and humoral immunity also play distinct roles in the control of virus-induced pathogenesis of the CNS. Although the CNS differs from most peripheral tissues due to the restricting blood brain barrier, absence of lymphoid drainage, few professional APC, and low levels of MHC expression (10–12), viral CNS infections generally induce effective immune responses (11). However, the mechanisms crucial for viral control may differ depending on the CNS cell types infected. For example, HSV, measles virus, and Alphaviruses infect and persist in neurons (13–15). Although virus-infected neurons are not susceptible to CTL recognition (16), replication can be controlled via an Ab-mediated mechanism distinct from Ab-dependent cell-mediated cytolysis or C-dependent lysis (14). By contrast, virus-infected glial cells appear more dependent on T cell-mediated immunity for effective clearance (17–19).

Pathogenesis of the JHM strain of mouse hepatitis virus (JHMV) provides a rodent model of an acute viral CNS infection, which progresses to a chronic infection associated with ongoing myelin loss (17–19), pathologically similar to the human demyelinating disease, multiple sclerosis. JHMV replicates in a variety of CNS cell types, which require distinct mechanisms of CD8+ T cell-mediated clearance during acute infection (20–24). The absence of detectable antiviral Ab before clearance of infectious virus indicates that Ab plays little or no role during acute infection (18, 24). Consistent with a redundant early role of Ab, JHMV is cleared from the CNS of B cell-deficient C57BL/6-m1Cgrm (μMT) mice with kinetics similar to wild-type (wt) mice (25). The critical role for Ab in controlling persistent infection was revealed by viral recrudescence in the CNS of μMT mice, which was prevented by passive transfer of JHMV-specific Ab (25). However, subsequent analysis demonstrated severely reduced T cell responses in JHMV-infected μMT mice compared with wt mice (26), in striking contrast to other viral infections of μMT mice (5–7, 27, 28). Therefore, it is not clear whether JHMV persistence is solely controlled by antiviral Ab or is also dependent on the magnitude of the virus-specific T cell response. Another antiviral mechanism disrupted in these mice is the potential cytolyis of virus-infected cells by naive B cells, recently suggested as a B cell-mediated innate immune mechanism during JHMV pathogenesis (29). Such direct participation of B cells in JHMV clearance could

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Received for publication August 14, 2001. Accepted for publication December 3, 2001.

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1 This work was supported by National Institutes of Health Grants NS178146 (to S.A.S.), AI43603 (to M.J.S.), and AI47249 (to C.C.B.).
2 Address correspondence and reprint requests to Dr. Cornelia C. Bergmann, Departments of Neurology and Pathology, Keck School of Medicine, University of Southern California, 1333 San Pablo Street, MCH 142, Los Angeles, CA 90033. E-mail address: cbergman@hsc.usc.edu
3 Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; CLN, cervical lymph node; CMC, CNS-derived mononuclear cell; JHMV, JHM strain of mouse hepatitis virus; p.i., postinfection; S protein, spike protein; wt, wild type.

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potentially alter viral pathogenesis in the absence of Ab specific for the viral spike protein (S protein), which inhibits B cell-mediated cytolyis (29).

To discern the relative contributions of Ab and T cells and the role(s) of both B cells and lymphoid architecture in the control of acute and persistent JHMV infection within the CNS, JHMV pathogenesis was examined in three types of H-2^d mice with distinct defects in the B cell compartment: 1) B cell-deficient JH^D mice; 2) transgenic mice, designated mlgM, with a near normal splenic architecture and B cells that express surface IgM but are unable to secrete antiviral Ab; and 3) transgenic (m+sl) IgM mice, which have surface IgM^+ B cells but a limited secretory repertoire restricted to the IgM isotype (30). T cell activation following microbial infections or immunization of these mice is comparable to wt mice (31–33). Similar to infected mHMT mice (25), JHMV was initially cleared from the CNS but subsequently reactivated in both B cell/Ab-deficient and Ab-only-deficient mice. However, in contrast to infected mMT mice, no defects within the T cell compartment were detected in H-2^d B cell- or Ab-deficient mice. Nevertheless, even an intact virus-specific T cell response did not suffice to control virus reactivation. These results clearly demonstrate that maintenance of JHMV persistence within CNS glial cells is solely dependent upon antiviral Ab, reminiscent of Ab-mediated control of neuronal infection by Alphaviruses (14). No evidence for an Ab-independent antiviral function of B cells in vivo was detected. However, a novel innate role of B cells in subverting viral infection to a cell type less susceptible to T cell-mediated regulation was revealed by the restriction of virus replication to specific CNS resident cell types in B cell^- Ab-deficient mice during reemergence. In summary, the data provide compelling evidence that two separate effectors regulate virus replication during acute infection and maintenance of viral persistence, i.e., cellular and humoral immunity, respectively.

Materials and Methods

Mice

B cell-deficient mice homozygous for a disruption at the JH locus (JH^D), backcrossed six times with BALB/c mice, were bred in an accredited animal facility at the University of Southern California (Los Angeles, CA) under pathogen-free conditions. The absence of B cells and Ig was confirmed by the inability to detect either CD19 or surface IgM cells by flow cytometry or serum Ig by ELISA. A transgene containing a rearranged VDJ for the (4-hydroxy-3-nitrophenyl)acetyl hapten from which the secretory exon and polyadenylation site had been excised was used to produce transgenic mice (mIgM) with normal splenic architecture and B cell compartment. These transgenic mice express surface IgM but no circulating Ab (30) and were maintained by mating with JH^D mice, expresses the IgM transgene as a secretory molecule and therefore contains a normal lymphoid architecture, and a limited secretory IgM Ab repertoire, but no other isotypes (our unpublished observation). Both the mlgM and (m+sl) IgM mice were identified by PCR as previously described (30). They both have approximately normal numbers of splenic B cells as determined by expression of CD19 and surface IgM. No antiviral IgG was detected in JH^D mice or in mlgM and (m+sl) IgM transgenic mice by ELISA following JHMV infection. Antiviral IgG was not detected until day 21 postinfection (p.i.) in (m+sl) IgM-infected mice. Furthermore, the Ab titer in JHMV-infected (m+sl) IgM-infected mice did not exceed 10% of that detected in wt BALB/c mice. No neutralizing activity was detected in the sera of JHMV-infected (m+sl) IgM mice (data not shown). Age-matched wt BALB/c mice were purchased from the National Cancer Institute (Frederick, MD). Mice of both sexes were used between 7 and 8 wk of age.

Virus infection and titer determination

The J2.2v-1 neutralization mAb-derived variant of neurotrophic JHMV (34, 35) was injected intracerebrally in a volume of 30 μl containing 500 PFU. Tissue levels of infectious virus were determined from clarified homogenates prepared from one half brain by plaque assay on monolayers of de-layered brain tumor cells as previously described (20, 21). Plaques were counted following 48 h incubation at 37°C and the data represent the average of duplicate determinations from groups of four or more mice.

Clinical disease

Clinical disease was scored as previously described (34, 35). Briefly, mice were graded as follows: 0, healthy; 1, ruffled hair and hunchbacked appearance; 2, reduced mobility and inability to upright; 3, paralysis and wasting; 4, death. Data represent averages of four mice per time point and are representative of three or more experiments for each group of mice.

Isolation of mononuclear cells

Mononuclear cells were isolated from spleen, cervical lymph nodes (CLN), and the CNS as previously described (20, 36, 37). Splenocytes and CLN cells were washed and resuspended in RPMI 1640 medium supplemented with 25 mM HEPES (pH 7.2) (RPMI-HEPES) before analysis. To isolate CNS-derived mononuclear cells (CMC), brains and spinal cords were removed, homogenized in RPMI-HEPES using Tenbroeck tissue homogenizers, and adjusted to 30% Percoll (Amersham Pharmacia Biotech, Piscataway, NJ). CMC were concentrated by centrifugation at 800 × g for 20 min at 4°C onto a 1-ml cushion of 70% Percoll. Cells were collected from the interface and washed twice in RPMI 1640 before analysis.

CTL assays

CMC were evaluated for ex vivo CTL-mediated cytolytic activity at days 9, 14, and 21 p.i. as previously described (20, 36, 37). Briefly, JH7/4.1 (H-2^d) target cells were labeled with Na^255CrO_4. The L^d-restricted, immuno- nodominant nucleocapsid protein-derived peptide, designated pN (APTA GAFPPP, Ref. 38), was added at 1 μM to labeled target cells before addition of CMC at various E:T ratios. 255Cr release was determined in 100 μl of supernatant following a 4-h incubation at 37°C. Specific lysis is defined as 100 × (experimental release – spontaneous release)/detergent release – spontaneous release). Spontaneous release values were <20% of total release in all experiments.

Flow cytometry

Cells obtained from spleen, CLN, and CNS were analyzed by flow cytometry for expression of cell surface molecules. Cells were preincubated with a mixture (10%) of polyclonal mouse and human sera (Atlanta Biologicals, Norcross, GA) and rat anti-mouse FcγIIIIR mAb (2.4.G2; BD Pharmingen, San Diego, CA) for 20 min on ice to block nonspecific binding. PE-, FITC-, or CyChrom C-labeled mAb specific for CD4 (GK1.5), CD8 (53.67), CD44 (IM4), CD62L (MEL-14), CD43 (S7), CD19 (D13), B220 (RA3-6B2), and IgM (R6-60.2) were all obtained from BD Pharmingen. JHMV-specific CDS' T cells were identified by labeling with the L^d-pN class I tetramer as previously described (36). Cells were analyzed with a FACScan flow cytometer (BD Biosciences, San Jose, CA) using CellQuest software.

ELISPOT analysis

Frequencies of JHMV-specific IFN-γ-secreting CDS' and CDS' T cells were evaluated as previously described (36, 37). Briefly, 96-well plates (Millipore, Bedford, MA) were coated with purified anti-IFN-γ mAb (R4-6A2; BD Pharmingen) at 10 μg/ml overnight at 4°C. CDS' T cells were stimulated on BALB/c splenocytes (5 × 10^5 per well), irradiated for 20 min at 2000 rad in the presence or absence of 1 μM mP 9-mer peptide and 2.5% EL-4 supernatant as a source of IL-2. A UV light-inactivated lysate prepared from JHMV-infected delayed brain tumor cells was used to stimulate CDS' T cells for a period of 36 h at 37°C. Plates were developed using biotinylated anti-IFN-γ mAb (XMG1.2; BD Pharmingen) overnight at 4°C, followed by streptavidin peroxidase and 3,3′-diaminobenzidine (Sigma-Aldrich, St. Louis, MO) as substrate. Spots from two effector cell lines from each mouse (n = 6) were counted using a Leica stereo microscope (Leica, Bannockburn, IL).

Histology

Brains bisected in the midcoronal plane and spinal cords were examined for inflammation, distribution of viral Ag, and myelin loss. Tissues were fixed for 3 h in Clark’s solution (75% ethanol, 25% glacial acetic acid) before embedding. Sections were stained with either H&E or luxol fast blue to determine inflammation and demyelination, respectively. Distribution of viral Ag was determined by immunoxenostaining (Vectorstain-ABC kit; Vector Laboratories, Burlingame, CA) using the anti-JHMV mAb J.3.3 specific for the carboxyl terminus of the viral nucleocapsid protein as the primary Ab (39, 40) and horse anti-mouse as
secondary Ab (Vector Laboratories). Sections were scored in a blinded fashion.

Statistical analysis

Results, presented as means ± SEM, were analyzed using a paired Student’s t test and ANOVA. A value of p < 0.05 indicated a statistically significant difference.

Results

Persistent virus replication in the CNS and disease progression are controlled by Ab

Viral recrudescence in the CNS of JHMV-infected B cell-deficient μMT mice (25) may be attributed to severely reduced T cell responses within the CNS compared with wt mice (26), in addition to the absence of a humoral response. To dissect the relative importance of these defects to JHMV reactivation, transgenic mice containing B cells that either only express surface IgM but no circulating Ab (mlgM), or secrete a limited repertoire restricted to the IgM isotype (m(+)s)IgM (30), were infected and compared with syngeneic mice devoid of B cells and wt mice. All groups of mice survived the acute disease induced by JHMV infection (Fig. 1A). However, in contrast to 100% survival of wt mice, >50% of J H D and mlgM mice died between days 13 and 30 p.i. Similar results were obtained for (m(+)s)IgM mice (data not shown). Mild clinical signs initially developed at 8–9 days p.i. and progressed to more severe wasting and hind limb paralysis in all groups (Fig. 1B). Whereas clinical disease in wt mice improved after day 14 p.i., no improvements were noted in any of the Ab-deficient mice (Fig. 1B). Thus the presence of B cells did not affect the morbidity and mortality observed in all Ab-deficient groups. Furthermore, the inability to secrete antiviral Ab, rather than the mere presence of B cells, altered the clinical outcome of JHMV persistence following resolution of acute disease.

To ensure that increased morbidity and mortality resulted from JHMV recrudescence, virus replication in the CNS of the H-2d Ab-deficient and wt mice was compared. Infectious virus peaked and was cleared from the CNS of all groups with similar kinetics (Fig. 1C; days 5–12 p.i.). Although there was little or no detectable virus in the CNS of J H D, mlgM, and (m(+)s)IgM mice at days 12 or 14 p.i., virus recovery from these mice increased at days 21 and 30 p.i. (Fig. 1C), consistent with enhanced clinical disease and mortality. Although virus appeared to reactivate more slowly within the CNS of the mlgM and (m(+)s)IgM mice compared with the B cell-deficient J H D mice (Fig. 1C), the difference was not statistically significant. JHMV replication continued to increase within the CNS of the limited number of survivors in all three groups of Ab-deficient mice until day 45 p.i., the last time point analyzed (data not shown). An Ab-independent innate B cell function affecting acute virus replication (29) was thus not evident in vivo. JHMV reactivation within the CNS of Ab-deficient mice thus appears independent of genetic background, lymphoid architecture, and the presence of B cells. These data support the concept that antiviral Ab is critical for maintaining infectious JHMV at undetectable levels during CNS persistence (25).

The CNS of Ab-deficient and wt mice were compared during acute infection to verify similar inflammatory responses and viral cell tropism. Inflammatory cells were localized to both perivascular and intraparenchymal areas at day 14 p.i.; the pattern of distribution in all Ab-deficient groups was identical to that in JHMV-infected wt mice (Fig. 2, A and B). Similarly, virus Ag was detected in microglia, astrocytes, and oligodendroglia in all three Ab-deficient groups analogous to wt mice (Fig. 2, C and D). The number and distribution of virus-infected cells (Fig. 2, C and D), as well as demyelination (data not shown), were thus similar in all four groups of mice. Thus, acute JHMV pathogenesis was not altered by the absence of B cells, the inability to secrete antiviral Ab, or the ability to secrete only a limited IgM repertoire.

T cell activation, recruitment, and function in the CNS is independent of B cells and/or Ab

Frequencies of virus-specific T cells were measured in spleen and CLN cells from infected J H D, mlgM, (m(+)s)IgM, and wt H-2d mice to assess potential T cell defects, which are clearly evident in JHMV-infected μMT mice (26). Irrespective of the overall low frequency of tetramer+ T cells within the spleen following JHMV infection (36, 37), infected J H D mice consistently had a significantly reduced percentage of tetramer+ cells within splenic CD8+ T cells at day 5 p.i. compared with both wt and B cell− Ab-deficient mice (Fig. 3A). However, none of the H-2d Ab-deficient mice had significantly reduced splenic tetramer+ CD8+ T cells after day 5 p.i. (Fig. 3A). Few tetramer+ CD8+ T cells were detected in CLN of any group of JHMV-infected H-2d mice (data not shown), consistent with observations in other mouse strains (26, 33). IFN-γ ELISPOT analysis of spleen and CLN cells confirmed a reduced frequency of virus-specific CD8+ T cells only in infected J H D mice but not in B cell− mice (Fig. 3, B and D). In contrast to CD8+ T cell responses, there were no differences in the frequencies of virus-specific CD4+ T cells in either the spleen or CLN during the acute infection in Ab-deficient compared with wt mice (Fig. 3, C and D). These data indicated that any potential role of B cells or lymphoid architecture in regulating CD8+ T cell activation can only be transiently observed at day 5 p.i. Therefore, in contrast to JHMV-infected μMT mice (26), no peripheral defects in the T cell compartment were detected following day 7 p.i. in either B cell− or Ab-deficient H-2d mice.
FIGURE 2. CNS immunopathology following viral infection. Inflammation within the spinal cords of infected wt (A) and mlgM mice (B) at 14 days p.i. (H&E staining, ×500). Note comparable perivascular (arrows) and intraparenchymal infiltration. Viral Ag in spinal cords of infected wt (C) and mlgM (D) mice at day 14 p.i. (immunoperoxidase with hematoxylin counterstain). Arrowheads indicate infected oligodendroglia; arrows indicate infected astrocytes (×500).

During acute infection CD4⁺ and CD8⁺ T cells were recruited into the CNS of Ab-deficient and wt mice at essentially equivalent frequencies (Fig. 4); by day 14 p.i. CD8⁺ T cells decreased in wt mice but not in the Ab-deficient groups. Tetramer⁺ CD8⁺ T cells within the CNS of all groups were similar (Fig. 4B). The CNS of JH D mice even contained a slight increase in tetramer⁺ CD8⁺ T cells at day 7 p.i. compared with B cell⁺ Ab-deficient mice. A trend toward increased recruitment/survival of tetramer⁺ CD8⁺ T cells in all Ab-deficient groups was evident at day 14 p.i., when CD8⁺ T cell percentages were only reduced in wt mice; however, tetramer⁺ populations remained similar in all groups. Recruitment of virus-specific CD4⁺ and CD8⁺ T cells into the CNS was confirmed by IFN-γ ELISPOT analysis (Fig. 4, C and D). Surprisingly, all Ab-deficient groups revealed higher frequencies of virus-specific IFN-γ-secreting CD8⁺ T cells in the CNS compared with wt mice throughout days 7–14 p.i. Nevertheless, all frequencies progressively declined during viral clearance. The apparent discrepancy between phenotypic (tetramer⁺) and functional (IFN-γ secretion) analysis may indicate down-regulation of CD8⁺ T cell effector function as virus is cleared (20, 36, 37). Frequencies of virus-specific CD4⁺ T cells were also higher in Ab-deficient mice compared with wt mice throughout days 7–14 p.i. (Fig. 4), with a relative decline evident by day 14 p.i., similar to virus-specific CD8⁺ T cells. JHMV-infected H-2D⁺ Ab-deficient mice thus revealed no impairment in T cell activation, expansion, or recruitment into the infected CNS, in contrast to the severely reduced virus-specific T cells in the CNS of infected μMT mice (26). This was also confirmed by phenotypic analysis, which showed equivalent expression of activation/memory surface markers (CD44highCD62LlowCD43⁻) in both wt and Ab-deficient mice (data not shown).

JHMV is cleared from the CNS of infected mice predominantly by CD8⁺ T cell-mediated cytolytic activity (17, 18), an activity impaired in the CNS of JHMV-infected μMT mice (26). By contrast, CMC from infected JH D and mlgM mice expressed equivalent, or slightly increased, JHMV-specific ex vivo cytolytic activity compared with wt mice at day 9 p.i. (Fig. 5). Similar to the loss of this function concomitant with viral clearance in wt mice (20, 26, 36, 37), ex vivo CTL activity declined in all mice by day 14 p.i. (Fig. 5). Thus, both phenotypic and functional analysis of CD8⁺ T cells within the CNS of Ab-deficient mice suggest no defect in the ability of cytolytic activity to contribute to viral clearance during acute infection.

T cells are unable to prevent CNS virus reactivation

The absence of an apparent T cell defect during acute infection of JH D, mlgM, and (m+s)lgM mice provided the opportunity to analyze the effect of JHMV reactivation on the T cell compartment. At days 21 and 30 p.i. CMC yields were equal or slightly reduced in Ab-deficient mice compared with wt mice. CD8⁺ T cell populations within the CNS remained relatively constant during virus
reactivation between days 14 and 30 p.i.; a modest, albeit insignificant, decline was only evident within the CNS of wt mice (Fig. 6A). There were also no significant differences in frequencies of tetramer+ CD8+ T cells when comparing the three Ab-deficient groups to wt mice at day 14 p.i. However, between days 21 and 30, frequencies in wt mice had dropped significantly compared with JhD and mlgM mice (Fig. 6A). Thus, while tetramer+ CD8+ T cells gradually declined after viral clearance in wt mice, they remained elevated but did not increase in mice harboring recrudescent virus. To address the possibility that tetramer+ cells were underestimated due to Ag-induced TCR down-regulation, the frequencies of IFN-γ-secreting CD8+ T cells were assessed by ELISPOT analysis. Overall, frequencies of IFN-γ-responsive CD8+ T cells within each group gradually decreased between days 14 and 30 p.i., opposing the tetramer analysis (Fig. 6B). The ~2-fold drop from day 14 to day 30 p.i. was significant not only in wt mice but in all Ab-deficient mice. Furthermore, when comparing the groups to each other at each time point, IFN-γ+CD8+ T cell frequencies were significantly lower in wt mice only at days 21 and 30 p.i. While the ratios of tetramer+ to IFN-γ+CD8+ T cells were the same for all groups at day 14 p.i. (5:1), they were higher in all Ab-deficient groups by day 30 p.i., suggesting an increased state of nonresponsiveness (Fig. 6B). Down-regulation of effector function was supported by the inability to recover ex vivo cytolytic activity from the CNS during virus reactivation (data not shown). Thus, increasing Ag load within the CNS during virus reactivation was not associated with enhanced recruitment of functional virus-specific CD8+ T cells but rather resulted in down-regulation of cytolytic activity and IFN-γ secretion.

During acute JHMV infection CD4+ T cells enhance virus-specific CD8+ T cell expansion and are required to maintain the CD8+ T cell population within the CNS (23, 26). A potential defect in the CD4+ T cell compartment to explain the lack of increased virus-specific CD8+ T cells during recrudescence was thus assessed. Total numbers of CD4+ T cells within CMC remained constant in all groups (Fig. 6C), irrespective of virus reactivation. ELISPOT analysis revealed a statistically significant decline in frequencies of virus-specific CD4+ T cells in wt, mlgM, and (m+s)lgM mice between days 14 and 30 p.i., despite increased Ag load in the CNS of the mlgM and (m+s)lgM mice. In stark contrast, they were maintained within the CNS of JhD mice (Fig. 6D). Thus, IFN-γ+CD4+ T cells were significantly higher in JhD mice compared with all B cell+ mice at both days 21 and 30 p.i. This surprising result suggests that the absence, rather than presence, of B cells enhances virus-specific CD4+ T cell function. Nevertheless, the overall absence of increased recruitment, concomitant with an apparent decrease in IFN-γ secretion by both CD4+ and CD8+ T cells during virus reactivation in the CNS, suggests that T cells alone cannot control persistent infection.

An Ab-independent B cell mechanism limits persisting virus to oligodendrocytes

The observation that CD8+ T cells are similarly down-regulated in all groups of mice, independent of viral recrudescence or the presence of B cells, suggested that differential regulation of CD4+ T cells in JhD mice may reside in altered cytokine/chemokine release or altered class II Ag presentation. Therefore, the CNS of Ab-deficient mice were examined for the distribution of mononuclear cell infiltrates, CNS demyelination, and virus cellular tropism during JHMV reactivation. Increased inflammation was present in the CNS of all Ab-deficient mice compared with wt mice (Fig. 7, A–C). However, in contrast to acute infection (Fig. 2), the majority of mononuclear cells were present within the CNS parenchyma. Demyelination was also increased in the Ab-deficient mice com-
pared with wt mice (Fig. 7, D–F), consistent with demyelination induced by actively replicating virus during acute infection (17–19). Ab-deficient mice also exhibited increased numbers of virus-infected CNS cells, most prominently in the spinal cord, compared with wt mice (Fig. 7, G–I). Neurons were not infected in any group. Despite the greater number of Ag-positive cells in the CNS of JH D compared with wt mice, the cell types infected, i.e., astrocytes and oligodendroglia, were similar (Fig. 7, G and I). In striking contrast to tropism of recrudescing virus in JH D mice, oligodendroglia were the predominant CNS cell type infected in both mIgM mice (Fig. 7H) and (m+s)IgM mice (data not shown). Importantly, the decrease in Ag-positive astrocytes was not counterbalanced by increased numbers of infected oligodendrocytes. Similar numbers of Ag-positive oligodendrocytes in B cell+ and B cell-deficient recrudescing mice were consistent with similar severity of demyelination. This restricted tropism for oligodendroglia in B cell Ab-deficient mice suggests that the presence of B cells contributes to virus clearance from astrocytes and microglia. This Ab-independent clearance mechanism suggests a novel role for B cells within the CNS, which manifests itself by limiting infectious virus to oligodendroglia during reactivation.

**FIGURE 7.** Inflammation in spinal cords during viral recrudescence. Increased inflammation in the CNS parenchyma of JH D (A) and mIgM (B) mice compared with wt mice (C) at day 30 p.i. (H&E staining, ×300). Shown is increased demyelination (D and E) and viral Ag (G and H) in JH D (D and G) and mIgM (E and H) mice compared with wt mice (F and I). *, Gray matter/white matter junction. Luxol fast blue staining was used in D–F; immunoperoxidase staining was used in G–I; ×300.
Discussion
The goal of this study was to distinguish between impaired T cell responses and the absence of either B cells or Ab in contributing to viral recrudescence within the CNS. Control of JHMV CNS infection was thus examined in H-2b B cell-deficient JpD mice and compared with syngeneic mice, which contain B cells but are either unable to secrete antiviral Ab or secrete only a limited IgM repertoire. Increased mortality and morbidity rates in JpD mice, both on the H-2b background (31). B cell deficiency also manifests itself differently during distinct infections. In contrast to JHMV infection, MT mice mount normal CD8⁺ CTL responses following LCMV, influenza, and HSV infections (5, 6, 27, 28). Furthermore, neither acute nor memory CD4⁺ T cells are compromised following influenza virus infection of MT mice (27), and murine mammary tumor virus superantigen responses in MT mice are also comparable to wt mice (43). By contrast, CD4⁺ T cell responses to HSV and keyhole limpet hemocyanin are reduced in MT mice (44, 45). These findings raise the possibility that differences affecting the CD4⁺ T cell compartment may lead to the distinct regulation of JHMV-specific T cells in infected MT vs JpD mice (26). An indirect effect on CD4⁺ T cells, to explain discrepant T cell function in the absence of B cells, is supported by the fact that CD4⁺ T cells enhance JHMV-specific CD8⁺ T cell expansion and function (23, 26).

A role for B cell-mediated cytolysis (29) was examined in consideration of the delay in antiviral Ab responses in wt mice until after virus is essentially cleared from the CNS (18, 24). The ability of B cells from naive donors to lyse virus-infected cells through an interaction between the viral S protein and the viral receptor is unique to mouse hepatitis virus (29, 46). Although B cells express the mouse hepatitis virus receptor in vivo (29, 47), they appear resistant to infection in vivo based on the inability to detect viral Ag in lymphocytes (22–24). As cytolysis is inhibited by anti-S protein Ab (29, 47, 48), a B cell cytolytic mechanism in vivo can only be active before anti-S protein Ab secretion. However, similar kinetics of virus replication and clearance in mLgM, (m+s)lgM, and JpD mice argue against an innate immune function of B cells during acute infection, consistent with the limited recruitment of B cells into the CNS during acute infection (49).

However, preferential cellular tropism of recrudescing virus for oligodendroglia in B cell⁻ Ab-deficient mice, compared with astrocytes and oligodendroglia in JpD mice, did uncover an apparent Ab-independent role for B cells within the CNS during persistence. Oligodendroglial tropism in both mLgM and (m+s)lgM mice suggests that the negligible antiviral IgM response detected in the (m+s)lgM mice is insufficient to mediate this effect. Furthermore, B cells only facilitate clearance of reemerging virus from astrocytes and microglia/macrophages as the T cell response wanes. Whether this mechanism involves direct B cell-mediated cytolysis or an indirect effect of B cells on other cell types is unclear. B cell cytolysis is solely dependent on viral S protein expressed on target cells (29, 47, 48) but independent of Fas-Fasl interactions, perforin, and TNF-α in vitro (29, 50). If a cytolytic mechanism is indeed functional in vivo, it implies that oligodendroglia are resistant to B cell-mediated lysis, reminiscent to their resistance to perforin-mediated cytolysis by CD8⁺ T cells (24). Alternatively, a potential indirect mechanism may involve enhanced CD4⁺ T cell responses in the presence of B cells. However, IFN-γ-secreting virus-specific CD4⁺ T cells decreased rather than increased in B cell⁻ mice compared with B cell-deficient JpD mice, negating such a causal relationship. Although limited B cell lysis of infected cells in B cell⁻ Ab-deficient mice during recrudescence cannot be excluded, such B cell effector function is unlikely to be active in immune-competent wt mice due to detection of inhibiting antiviral Ab by approximately day 10 p.i. (18, 24).

The circuits linking altered tropism with antiviral B cell function and/or CD4⁺ T cell responsiveness remain elusive. As the CNS chemokine/cytokine profile is not only shaped by infiltrating cells but also by infected resident CNS cells (51), alterations in either compartment will potentially affect the other. B cells may thus potentially alter tropism directly via cytokine expression and/or via an indirect effect on CD4⁺ T cell function (52). Alternatively, enhanced CD4⁺ T cell survival/recruitment in JpD mice may result from 1) class II-mediated TCR stimulation by specific infected cell types, 2) MHC-independent recruitment factors produced by infected astrocytes and microglia/macrophages but not oligodendrocytes, and 3) the absence of potentially negative signals by B cells. The fact that only virus-specific CD4⁺ T cells, but not total CD4⁺ T cells, are enhanced favors a direct interaction with class II resident APC (10–12). These data further indicate that in contrast to infected astrocytes and microglia/macrophages, productively infected oligodendrocytes do not, or only inefficiently, present viral Ag to CD4⁺ T cells.

The most crucial finding relative to persistent viral infection of the CNS is that reactivation does not result in increased inflammation, increased virus-specific T cells, or re-expression of cytolytic effector function. Both virus-specific CD4⁺ and CD8⁺ T cells within the CNS retain the ability to secrete in response to viral Ag. Although IFN-γ is crucial for viral clearance during acute infection (24), this effector function is evidently insufficient to control virus reactivation. The inability to enhance T cell effector function may be due to a decreased ability of peripheral T cells to access the CNS, the inability of Ag to leave the CNS and reactivate peripheral T cells, Ag-induced anergy, or T cell apoptosis. Irrespective of increased tetramer CD8⁺ T cells in all recrudescing mice compared with wt mice, the overall decrease of virus-specific IFN-γ-secreting cells favors Ag-induced down-regulation of T cell effector function. Initial clearance of infectious virus thus appears to predispose the CNS to preempt further T cell-mediated viral clearance. Such an altered CNS environment emphasizes the need for Ab-mediated protection, particularly after immune- or virus-induced CNS pathology. In summary, although neither B cells nor antiviral Ab influence clearance during acute infection, Ab inhibits viral recrudescence (25) and may, in addition, be beneficial to completely eliminate infectious virus during the waning T cell response, especially as CD8⁺ T cells lose effector function (20, 36, 37).

Acknowledgments
We thank Wen Wei for assistance with the histopathology, Maria R. Ramirez for breeding the mice, and Dr. David Hinton for helpful discussions.
References


