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Recruitment Kinetics and Composition of Antibody-Secreting Cells Within the Central Nervous System Following Viral Encephalomyelitis

Shuen-Ing Tschen,* Cornelia C. Bergmann,†‡ Chandran Ramakrishna,† Shawn Morales,* Roscoe Atkinson,§ and Stephen A. Stohlman2*†

Infection by the neurotropic JHM strain of mouse hepatitis virus produces an acute demyelinating encephalomyelitis. While cellular immunity initially eliminates infectious virus, CNS viral persistence is predominantly controlled by humoral immunity. To better understand the distinct phases of immune control within the CNS, the kinetics of humoral immune responses were determined in infected mice. Early during clearance of the JHM strain of mouse hepatitis virus, only few virus-specific Ab-secreting cells (ASC) were detected in the periphery or CNS, although mature B cells and ASC without viral specificity were recruited into the CNS concomitant with T cells. Serum antiviral Ab and CNS virus-specific ASC became prominent only during final elimination of infectious virus. Virus-specific ASC peaked in lymphoid organs before the CNS, suggesting peripheral B cell priming and maturation. Following elimination of infectious virus, virus-specific ASC continued to increase within the CNS and then remained stable during persistence, in contrast to declining T cell numbers. These data comprise three novel findings. Rapid recruitment of B cells in the absence of specific Ab secretion supports a potential Ab-independent effector function involving lysis of virus-infected cells. Delayed recruitment relative to viral clearance and subsequent maintenance of a stable CNS ASC population demonstrate differential regulation of T and B lymphocytes within the infected CNS. This supports a critical role of humoral immunity in regulating viral CNS persistence. Lastly, altered antiviral ASC specificities following clearance of infectious virus suggest ongoing recruitment of peripheral memory cells and/or local B cell differentiation. The Journal of Immunology, 2002, 168: 2922–2929.

The antiviral activities mediated by humoral and cellular immunity can vary extensively depending on the type of infection (1, 2). Despite the synergy of diverse immune effector mechanisms, which eliminate the majority of infectious virus, sterile immunity is not achieved in many cases. For example, CTL clear lymphocytic choriomeningitis virus during acute infection (3). However, control of persistent infection is dependent upon humoral immunity, cytokines, and CD4+ T cells (4). Similarly, both virus-specific CD8+ and CD4+ T cells mediate clearance of CMV infection in the absence of B cells (5). However, following CMV reactivation induced by immunosuppression, control of virus dissemination is dependent upon antiviral Ab (6). The regulation of latent or persistent viral infections is thus predominantly dependent upon humoral immunity. Distinct antiviral functions by B cells and T cells are also evident during viral infections largely confined to the CNS. While Sindbis virus is eliminated from the CNS predominantly via antiviral Ab (7), the neurotropic JHM strain of mouse hepatitis virus (JHMV)3 is cleared via cell-mediated immune effectors (8, 9) with little apparent participation of humoral immunity (10, 11). Nevertheless, the host is unable to achieve sterile immunity to either infection, resulting in viral persistence associated with the absence of infectious virus (7, 10, 11). Whereas Sindbis virus persistence results in no apparent pathology (7), JHMV persistence is associated with chronic ongoing myelin loss (10, 11). The extent to which the infectious agent, tropism, and the host immune response determine pathogenesis and clinical outcome is complicated by preferential Sindbis virus replication in neurons (7), while JHMV replicates in most other CNS cell types during acute infection (10–12).

Host responses effective in eliminating infectious virus during acute CNS viral infections have been identified in many animal models (3, 7, 10, 11, 13); however, little is known about immune components controlling persistent virus (14, 15). Following JHMV infection all major cell types with potential antiviral function, including NK cells, B cells, macrophages, and CD4+/CD8+ T cells enter the CNS (16–18). However, virus-specific CD8+ T cell infiltration coincides predominantly with reduction of infectious virus (10, 11, 18–20); CD4+ T cells support CD8+ T cell expansion and survival (8, 9). Cell type-dependent antiviral effector functions are demonstrated by control of JHMV replication within macrophage/microglia and astrocytes via a perforin-dependent mechanism (21) and in oligodendroglia by IFN-γ (22).

The inability to detect serum neutralizing Ab until the majority of JHMV has been cleared, in addition to the predominant role of

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3 Abbreviations used in this paper: JHMV, JHM strain of MHV; MHV, mouse hepatitis virus; ASC, Ab-secreting cell; CLN, cervical lymph node; CMC, CNS mononuclear cell; N protein, JHMV nucleocapsid protein; p.i., postinfection; S protein, JHMV spike protein; wt, wild type; sIg, surface Ig.
cell-mediated immunity in viral clearance, indicated little or no role for humoral immunity in protecting the CNS from acute infection (10, 11, 21–23). Nevertheless, a protective role for humoral immunity is supported by a more rapid neutralizing Ab response in resistant JHMV-infected rats compared with susceptible JHMV-infected rats (24, 25) and protection from mouse hepatitis virus (MHV)-induced mortality by passive transfer of antiviral mAb (26–29). In addition, suckling mice weaned on immunized dams are protected from acute JHMV-induced encephalomyelitis (30). However, the role of Ab in this model is not clear. Delayed onset of CNS disease in a variable percentage of maternal Ab-protected mice (30) is associated with productive JHMV replication due to preferential expansion of CTL escape variants (31). The importance of humoral immunity was further demonstrated by incomplete elimination of infectious virus and subsequent virus reactivation in B cell-deficient mice, despite initial clearance from the CNS with kinetics similar to wild-type (wt) mice (19). This contrasts dramatically with complete elimination and only very rare recovery of infectious JHMV from the CNS of wt mice following acute infection (10, 11, 32). The absence of CTL escape virus variants, coupled with the ability of antiviral Ab to prevent JHMV reactivation (19), suggests that separate mechanisms are used by the host to eliminate infectious virus and to control persistent CNS infection.

To better characterize the role of B cells and antiviral Ab during the decline of infectious virus and control of persistence, the kinetics of B cell recruitment into the JHMV-infected CNS were examined. Peripheral activation of anti-JHMV Ab-secreting cells (ASC) was delayed until the majority of virus had been cleared from the CNS, consistent with the kinetics of serum antiviral Ab. Similarly, virus-specific ASC were most prominent in the CNS following clearance of infectious virus, although mature B cells, ASC with nonviral specificity, and a few virus-specific ASC were present during acute viral infection. An Ab-independent role of B cells, via lysis of infected cells expressing the viral spike (S) protein (33–36), appears to play a minor, if any, role in virus suppression. The increase of virus-specific ASC in the CNS was most dramatic between days 14 and 21 postinfection (p.i.), spanning the critical time point of virus reactivation within the CNS of B cell-deficient mice (19). Furthermore, ASC remained at relatively high and stable levels during viral persistence, in contrast to the decline of T cells. These results indicate that the emergence of JHMV-specific ASC within the CNS and serum antiviral Ab coincides with regulation of viral persistence. Surprisingly, despite constant frequencies of total virus-specific ASC, plasticity in the response was evidenced by an altered pattern of viral protein specificities. Retention and differentiation of ASC in the CNS thus indicates that viral persistence may be maintained by mechanisms other than neutralizing Ab specific for the viral JHMV S protein. These data are consistent with B cell recruitment into the CNS during viral-induced encephalomyelitis (7, 13, 16, 17, 37–39) and the presence of antiviral Ab in the cerebral spinal fluid (7, 14, 24, 40).

Materials and Methods

Mice

Male C57BL/6 mice were purchased from the National Cancer Institute (Frederick, MD) at 6 wk of age and infected within 7 days of arrival. All mice were caged in groups of four or five in microisolator cages and maintained on standard laboratory food and water ad libitum. No evidence of anti-MHV Ab was detected before use.

Virus

The neutralizing mAb-derived 2.2v-1 variant of JHMV was used for intracerebral infection (41). Virus was propagated and plaque was assayed on monolayers of the murine glioblastoma cell line, designated DBT cells, as previously described (41, 42). Mice were injected in the left hemisphere with 250 PFU of JHMV diluted in Dulbecco’s PBS in a volume of 30 μl or with an equal volume of sterile PBS. CNS virus titers were determined by plaque assay on monolayers of DBT cells as previously described (41, 42). Briefly, one-half of the brains were homogenized in RPMI 1640 medium containing 25 nM HEPS, pH 7.2 (RPMI-HEPES), using Tenbroek tissue homogenizers. Following clarification by centrifugation at 500 × g for 7 min, homogenates were either assayed directly or stored at −70°C.

Clinical scores

Clinical disease was graded as previously described (43): 0, healthy; 1, hunched back; 2, partial hind limb paralysis; 3, complete hind limb paralysis; 4, moribund or dead.

Virus-specific Ab

Serum anti-JHMV Ab were determined by ELISA as previously described using sera pooled from three to five individuals per time point (22, 23). Briefly, 96-well plates were coated overnight at 4°C with a clarified serum-free supernatant derived from JHMV-infected DBT cells (∼6 × 10^7 PFU/well) diluted in 0.1 M sodium phosphate buffer (pH 9). After blocking with PBS containing 10% FCS for 1 h at room temperature, 2-fold serum dilutions were incubated overnight at 4°C. Ab was detected by addition of biotinylated goat anti-mouse IgG, anti-mouse IgM (Jackson Immunoresearch Laboratories, West Grove, PA), or anti-mouse IgA (BD Pharmingen, San Diego, CA). Following a 2-h incubation at room temperature, plates were washed four times with PBS containing 0.05% Tween 20 (PBS-Tween) before the addition of avidin peroxidase. Following an additional 1 h at room temperature the plates were washed four times with PBS-Tween before the addition of 1 mg/ml ABTS (Roche Diagnostics, Indianapolis, IN) in PBS containing H_2O_2. Color intensity was determined at 405 nm using a Microplate Autoreader (Bio-Tek Instruments, Winooski, VT).

Neutralization and fusion inhibition assays were performed as previously described using sera pooled from three to five individuals per time point (22, 23). Briefly, for virus neutralization serial dilutions of heat-inactivated (56°C for 30 min) serum were incubated with 200 PFU of JHMV in 96-well plates for 90 min at 37°C. Following addition of DBT cells (9 × 10^5 cells/well), cultures were incubated for 48 h at 37°C. Neutralization titers represent the highest serum dilution that prevented virus-induced cytopathic effect. For fusion inhibition assays complete layering of DBT cells in 96-well plates were infected with 200 PFU and incubated for 4 h at 37°C before serum addition. Titers represent the highest serum dilution that prevented viral-induced cytopathic effects.

Mononuclear cells

Groups of six to nine JHMV-infected mice were perfused with PBS at various times p.i. Single cell suspensions from spleen and cervical lymph nodes (CLN) were prepared by disassociation in RPMI-HEPES (18). Mononuclear cells (MNC) were prepared by disassociation in RPMI-HEPES (18). Mononuclear cells were recovered from the 30/70% interface, diluted in RPMI-HEPES, collected by centrifugation, washed three times, and resuspended in RPMI-HEPES for analysis.

Flow cytometry

For analysis of surface markers, 5 × 10^5 cells were preincubated with normal mouse serum, human serum, and rat anti-mouse FcRIII/IIR mAb (2.4G2) (BD Pharmingen) for 1 h to inhibit nonspecific binding. Cells were stained with PE- or FITC-labeled mAb specific for B220 (RA-6-6B2), CD3 (145-2C11), CD19 (ID3), or polyclonal goat anti-mouse Ig (all obtained from BD Pharmingen) in PBS containing 1% BSA (Sigma-Aldrich, St. Louis, MO), and analyzed on a FACScan flow cytometer (BD Biosciences, San Jose, CA) using CellQuest software (BD Biosciences).

Ab-secreting cells

JHMV-specific Ab were detected by ELISPOT. Clarified serum-free supernatant collected from JHMV-infected DBT cells (∼5 × 10^7 PFU/well) was used to coat 96-well plates (Millipore, Bedford, MA) overnight at 4°C. Control wells were coated with virus-free medium. To examine ASC specific for the JHMV S protein or JHMV nucleocapsid protein (N protein), wells were coated with lysates of HeLa cells infected with recombinant...
vaccinia viruses encoding either the full-length JHMV S protein or the JHMV N protein (42). Control wells were coated with a lysate prepared from HeLa cells infected with a recombinant vaccinia virus encoding β-galactosidase (VSC8; Ref. 42). Wells were blocked with MEM containing 2% BSA (Sigma-Aldrich) for 30 min at 37°C and washed twice with RPMI 1640 medium. Mononuclear cells isolated from spleen, CLN, and CNS were added at various dilutions in RPMI-HEPES containing 10% FCS. Following a 4-h incubation at 37°C, plates were washed four times with PBS-Tween. Biotinylated goat anti-mouse IgM, IgG (Jackson Immunoresearch Laboratories), IgA, IgG1, IgG2a, IgG2b, or IgG3 (Southern Biotechnology Associates, Birmingham, AL) diluted in PBS-Tween containing 10% FCS was added. Following incubation at room temperature for 5–10 min, wells were washed with water and air dried, and the spots were counted using a stereo dissecting microscope.

The frequency of total IgM, IgG, and IgA ASC was determined by ELISPOT using the protocol described above, except plates were coated with goat anti-mouse polyclonal Ig (ICN Pharmaceuticals, Aurora, OH). Bound Ig was detected by biotinylated goat anti-mouse IgG and IgM (Jackson Immunoresearch Laboratories).

Results

JHMV clearance precedes the serum Ab response

JHMV titers within the CNS of wt C57BL/6 mice and virus-specific Ab in serum were measured to establish the temporal correlation between virus replication and emergence of virus-specific humoral immunity. CNS virus replication peaked at day 5 p.i. and was reduced to undetectable levels by day 14 p.i. (Fig. 1A), consistent with previous studies (18, 21–23). Infected mice initially showed clinical signs of disease at day 5 p.i., which peaked between days 10 and 14 p.i. and then resolved slowly following viral clearance (Fig. 1B). This contrasts with virus recrudescence after day 14 p.i. in JHMV-infected syngeneic B cell-deficient mice and their inability to recover from clinical disease (19). To establish the time at which antiviral Ab emerged, virus-specific IgM and IgG Ab were measured in the sera of infected mice. Virus-specific IgM was initially detected at day 7 p.i. and antiviral IgG was initially detected at day 10 p.i. (Fig. 1C). Two biological activities of the antiviral Ab response potentially important in virus clearance, i.e., neutralization and inhibition of viral-induced cell fusion, were also examined. These activities are both directed at determinants within the viral S protein (44, 45). Both JHMV-neutralizing and fusion-inhibiting mAb also inhibit the potential Ab-independent innate cytolytic function of B cells (36). Coincident with the detection of antiviral IgG, neutralizing Ab was first detected at day 10 p.i. and increased until day 21 p.i., the last time point analyzed (Fig. 1D). However, fusion inhibition Ab were not detected before day 21 p.i. (data not shown). These data are consistent with a primary role of cell-mediated immunity in JHMV clearance from the CNS and suggest that neutralizing, but not fusion-inhibiting, Ab may participate in clearance of infectious virus late during acute infection and in prevention of virus reactivation within the CNS of B cell-deficient mice (19).

The kinetics of B cell and T cell recruitment into the CNS were compared following JHMV infection by flow cytometry. Few CD3+ T cells were present in the CNS before day 7 p.i. (Table I). The frequency of T cells rapidly increased by day 7 p.i. and peaked at day 10 p.i., consistent with previous results in both JHMV-infected C57BL/6 × BALB/c F1 and BALB/c mice (18, 46, 47). Cells expressing the B cell markers B220, surface Ig (sIg), and CD19 were also initially detected at day 5 p.i. but increased less dramatically than T cells by day 7 p.i. CNS infiltration of mature B cells peaked at day 10 p.i., similar to T cells. The peak of B cell infiltration thus coincided with detectable neutralizing Ab in serum. The decrease in B220+sIg+CD19+ B cells after day 10 p.i. suggests either apoptosis, traffic into a peripheral compartment, or differentiation into plasma cells. The increased frequency of B220+ cells relative to CD19+ cells may reflect B220+CD19− plasmablasts, which secrete limited amounts of Ab (48). Although the frequency of B cells recruited into the CNS was substantially lower than the number of T cells recruited during JHMV-induced inflammation (Table I), B cells potentially secrete large quantities of effector molecules. Limited infiltration by mature B cells thus does not necessarily represent poor effector function.

JHMV-specific ASC are primed predominantly in CLN

The CNS does not support priming of naïve T cells (49, 50); however, it does appear to support differentiation of mature B cells into ASC (51). By contrast, during most peripheral viral infections B cell differentiation occurs in germinal centers (52, 53). To examine the activation kinetics of cells secreting anti-JHMV Ab following infection, the frequency of virus-specific IgM and IgG ASC were

<table>
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<th>Table I. B cells within the CNS of JHMV-infected mice*</th>
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*Values represent the number of cells expressing each marker per 1 × 10⁶ CMC cells prepared from the brains and spinal cords of infected mice. Data are representative of two separate experiments. Values obtained from mice injected with PBS were subtracted at days 3 and 5 p.i.
examined in CLN based on reports that CNS Ag preferentially activates B cells within the CLN (54). Although few in number, virus-specific IgM ASC in CLN were initially detected at day 5 p.i., peaked at day 10 p.i. and then declined to undetectable by day 30 p.i. (Fig. 2A). In contrast to IgM ASC, JHMV-specific IgG ASC did not emerge in the CLN before day 10 p.i. but increased sharply to a peak at day 14 p.i. Following day 14 p.i., the frequency of virus-specific IgG ASC in CLN gradually declined until day 30 p.i.

The kinetics and frequency of virus-specific IgM and IgG ASC were also determined in spleens of infected mice (Fig. 2B). Virus-specific IgM ASC in spleen were initially detected at day 3 p.i., before detection in CLN. However, similar to the CLN, the low frequency of virus-specific IgM ASC peaked at day 10 p.i. and then declined, reaching undetectable levels by 21 days p.i. Anti-JHMV-specific IgG ASC were initially detected in spleen at day 7 p.i., before detection of JHMV-specific serum IgG at day 10 p.i. (Fig. 1C). However, unlike IgM ASC in spleen and both IgM and IgG ASC in CLN, splenic JHMV-specific IgG ASC remained at relatively low, but stable, levels from day 10 p.i. until at least day 30 p.i. Consistent with the suggestion that plasma cells rarely traffic via blood (51), analysis of peripheral blood showed few virus-specific ASC at any time point p.i. with the exception of day 10 p.i. (data not shown). Therefore, although virus-specific IgG ASC in CLN peaked with a 4-day delay compared with spleen, maximal frequencies were 3- to 5-fold higher than in the spleen between days 14 and 21 p.i. However, the maintenance of low, but relatively stable, IgG ASC in the spleen following resolution of acute infection differs from the CLN, in which the IgG ASC frequencies declined substantially between days 14 and 30 p.i.

Virus-specific ASC within the CNS accumulate to maximum frequencies following viral clearance

In the absence of humoral immunity, JHMV is cleared from the CNS with kinetics similar to those found in wt mice (19). However, clearance is incomplete in B cell-deficient mice, and JHMV reactivates within the CNS reaching levels similar to those found during acute infection (19). To determine the kinetics of virus-specific ASC accumulation at the site of infection, the frequency of anti-JHMV ASC within the CNS was determined. No anti-JHMV ASC were found within the CNS at days 3 or 5 p.i. Low frequencies of both IgM and IgG ASC were initially detected at day 7 p.i., which increased only slightly by day 10 p.i. (Fig. 3A). In contrast to the periphery, in which the frequencies of virus-specific IgM and IgG ASC peaked between days 10 and 14 p.i., the frequencies within the CNS continued to increase substantially until day 21 p.i., with no significant decline by day 30 p.i. The continued presence of relatively high frequencies of virus-specific ASC throughout days 21–30 p.i. contrasts sharply with the low to undetectable frequencies in both the CLN and spleen (Fig. 2). Therefore, detection of both antiviral IgM and IgG ASC in the periphery precedes detection of serum antiviral Ab and ASC within the infected CNS. This adds support to the notion that B cells are activated in the periphery and subsequently accumulate within the CNS, where they mature into ASC (51).

Preferential recruitment and activation of virus-specific ASC secreting different IgG isotypes into the CNS following JHMV infection were also examined. At day 10 p.i. the majority of virus-specific ASC in the CNS secreted IgG2a (Fig. 3B). No virus-specific IgG1 or IgG3 ASC and only a small number of plasma cells secreting IgG2b were detected. By day 14 p.i., IgG2b ASC had increased from 10% at day 10 p.i. to ~50% of virus-specific ASC. In addition, virus-specific IgG1-secreting ASC were detected at both days 14 and 21 p.i. Overall, the frequencies of IgG2a, IgG2b, and IgG1 ASC within the CNS remained essentially equivalent between days 14 and 21 p.i. Few virus-specific IgG3 ASC were also detected at days 14 and 21 p.i. Therefore, ASC of all four IgG isotypes (IgG1, IgG2a, IgG2b, and IgG3) were present within the CNS of infected mice at days 14 and 21 p.i. However, while IgG2a ASC dominated at day 10 p.i., IgG2b and IgG1 ASC appeared to be preferentially expanded or recruited only following virus clearance from the CNS. The switch to an increased frequency of the IgG2b isotype ASC by day 14 p.i. (Fig. 3B) correlates with peak expansion of IgG ASC in CLN (Fig. 2A).

Small numbers of JHMV-specific IgA ASC were detected in the CNS at days 5–10 p.i. and increased between days 10 and 14 p.i. (Fig. 3C). Nevertheless, only a small fraction of the total plasma cells secreted virus-specific IgA. Presentation of data derived from Fig. 3, A and C, to reflect total numbers of the ASC subpopulations per brain (Fig. 3D), shows that the low frequency of ASC within

![FIGURE 2.](http://www.jimmunol.org/)

JHMV-specific IgM and IgG ASC in peripheral lymphoid organs. The frequency of JHMV-specific IgM and IgG ASC in CLN (A) and spleen (B). Mice were infected with JHMV and the virus-specific IgM and IgG ASC were detected by ELISPOT at various times p.i. Data represent the average of two to five separate experiments per time point ± SEM.

![FIGURE 3.](http://www.jimmunol.org/)

JHMV-specific ASC within the CNS. Frequency of JHMV-specific ASC in the CNS was determined by ELISPOT using CMC prepared from the brains and spinal cords of infected mice. A, Virus-specific IgG1 or IgG3 per 10⁶ CMC. B, Percentage of JHMV-specific ASC secreting IgG isotypes. C, Frequency of virus-specific IgA ASC within the CNS following infection. D, The number of ASC per mouse brain/spinal cord based on data represented in A and C. Data represent the average of three to five separate experiments per time point ± SEM.
the CNS of JHMV-infected mice at early time points p.i. was not masked by the influx of non-B cells recruited during inflammation. Furthermore, following day 14 p.i., JHMV-specific IgA ASC represented only a minor fraction of ASC compared with IgM and IgG ASC, which increased most predominantly between days 14 and 21 p.i.

**Plasma cells with unknown specificities prevail over virus-specific ASC in the CNS**

B cells express the MHV receptor and interact with infected cells expressing the viral S protein (33–36). This interaction results in death of the infected cell via a fusion-dependent mechanism, which is independent of perforin, Fas/Fas ligand, IL-1, or TNF-α (36, 55). This Ab-independent antiviral mechanism has been suggested to contribute either to complete clearance of infectious virus or to suppression of JHMV reactivation (19, 36). B220<sup>-</sup>, CD19<sup>-</sup>, and slg<sup>B</sup> B cells are recruited into the CNS with kinetics similar to those of T cells following JHMV infection (Table I). Due to the 7-day delay in accumulation of virus-specific ASC (Fig. 3), the CNS was also examined for recruitment of plasma cells before detection of virus-specific Ab synthesis. Plasma cells secreting IgM, IgG, or IgA were quantitated using a 4-h ELISPOT assay similar to the one used to examine the frequency of virus-specific ASC. A small number of IgM, IgG, and IgA ASC were indeed detected at day 5 p.i. (Fig. 4A), consistent with the initial detection of both mature B cells and T cells (Table I). However, in contrast to maximum T cell infiltration (days 7 and 10 p.i.; Table I), the number of plasma cells increased continuously. IgM ASC were slightly higher than IgG ASC at day 14 p.i., whereas IgG ASC constituted the majority of the population by day 21 p.i. Comparison of the frequencies of total IgM and IgG ASC with those of virus-specific ASC indicated that only a small fraction of plasma cells recruited early into the CNS following infection are indeed virus specific, with the majority secreting Ab of unknown specificities (Fig. 4B). For example, at day 14 p.i. virus-specific IgM ASC comprised only ~15% of IgM ASC within the CNS, increasing to 83% at day 21 p.i. By contrast, virus-specific IgG ASC constituted ~25% of IgG ASC at day 14 p.i. and remained at this percentage until day 21 p.i. Thus, while the virus-specific IgM population switched from a minority to the vast majority of total IgM ASC following clearance of infectious JHMV, virus-specific IgG comprised a constant 25% of IgG ASC between 14 and 21 days p.i. Although the frequency of virus-specific IgG ASC increased ~6-fold between days 14 and 21 p.i. (see Fig. 3A), total IgG ASC also increased, indicating no further enrichment of virus-specific IgG ASC.

**ASC alter their composition and antiviral specificities during persistence**

Mature B cells are found in the JHMV-infected CNS (Table I) before virus-specific plasma cells (Fig. 4). The presence of B cells and nonvirus-specific plasma cells within the CNS, before differentiation of antiviral ASC, may contribute to both the complete clearance of JHMV from the CNS and/or prevention of virus reactivation (19) by two distinct mechanisms: innate Ig may act by virus trapping (56), while B cells themselves may use an Ab-independent cytolytic mechanism (33–36). B cell-mediated cytolytic activity is inhibited by S protein-specific virus-neutralizing and cell-cell fusion-inhibiting Ab (34–36). To assess a possible role for B cell-mediated cytolyis of virus-infected CNS cells, recruitment kinetics of IgM and IgG ASC specific for the S protein were examined. Anti-S protein ASC in spleen and within the CNS were barely detectable at day 7 p.i. and peaked within the CNS between days 14 and 21 p.i. (Fig. 5). Initial recruitment of N protein-specific IgM ASC was slightly delayed compared with S protein-specific ASC but followed similar kinetics and magnitude (Fig. 5A). Few ASC specific for both the S and N proteins (Fig. 5B) were detected within the CNS at day 10 p.i. and increased by day 14 p.i., consistent with the kinetics of IgM and IgG ASC specific for all virus structural components (Fig. 3). The low frequency of S protein-specific ASC recruited into the CNS during acute infection supports the possibility that B cells rapidly recruited into the CNS may contribute to JHMV pathogenesis via an innate effector function.

Table II summarizes the relative numbers of virus-specific, including N and S protein-specific ASC with respect to total ASC during the course of infection. Whereas N and S protein-specific ASC account for most virus-specific ASC until day 14 p.i., they constitute only 50% by day 21 p.i. and even fewer by day 30 p.i. A 2-fold excess of N plus S protein-specific IgG ASC relative to IgM ASC at day 21 p.i. mimics the virus-specific IgG:IgM ASC ratio. However, the relative increase of total virus-specific ASC suggests the emergence of novel ASC specificities to other viral proteins during persistence. Ongoing differentiation within the virus-specific B cell compartment after clearance of infectious JHMV, in addition to the increasing proportion of nonviral ASC in the CNS, suggests diversification in the humoral response driven by persistent infection.

![FIGURE 4.](http://www.jimmunol.org/) Rapid recruitment of plasma cells in the CNS of JHMV-infected mice. A. Frequency of total ASC within the CNS following infection determined by ELISPOT using polyclonal anti-Ig capture Ab. Data are the average of three experiments per time point ± SEM. B. Percentage of virus-specific IgM and IgG ASC within the total ASC populations as derived from A and Fig. 3.

![FIGURE 5.](http://www.jimmunol.org/) IgM and IgG ASC specific for the viral S and N proteins within the CNS following JHMV infection. IgM ASC (A) and IgG ASC (B) frequencies specific for the viral S and N protein in the CNS were determined using lysates of cells infected with recombinant vaccinia viruses expressing either the JHMV S or N proteins. Background spots that developed on lysates prepared from cells infected with vS8C were subtracted. Data are derived from four to seven experiments at each time point with four to six mice per experiment. Mean ASC ± SEM.
Table II. Recruitment, retention, and specificity of ASC within the CNS

<table>
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<th>ASC</th>
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<tbody>
<tr>
<td>IgM</td>
<td></td>
</tr>
<tr>
<td>Total (^b)</td>
<td>1,300 (\pm) 0</td>
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<tr>
<td>Virus-specific (^c)</td>
<td>106 (\pm) 58</td>
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<tr>
<td>S protein-specific (^d)</td>
<td>45 (\pm) 24</td>
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<td>40 (\pm) 10</td>
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<td>N protein-specific (^d)</td>
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\(^a\) C57BL/6 mice were infected intracerebrally with 250 PFU of JHMV and the CMC isolated and ASC enumerated at the days p.i. indicated. Frequencies are presented per \(10^8\) CMC.

\(^b\) Total ASC were determined by ELISPOT using anti-mouse Ig as capture and mouse-anti-IgM or anti-IgG as detection Ab.

\(^c\) Virus-specific ASC were enumerated using JHMV as capture Ag.

\(^d\) S and N protein-specific ASC were determined using lysates of HeLa cells infected with recombinant vaccinia viruses expressing either the JHMV S or N proteins.

**Discussion**

Humoral immunity protects the CNS from infection by preventing viral dissemination from peripheral infection sites (14, 38, 39, 57). However, once the CNS is infected, antiviral Ab may play a direct role in eliminating infectious virus (7) or in viral persistence (4, 6, 14, 19). JHMV infection produces an acute demyelinating encephalomyelitis predominantly controlled by cellular immunity (10, 11). Despite elimination of infectious virus to undetectable levels, virus persistence is characterized by viral RNA and Ag (10, 11, 18, 36), suggests an unknown mechanism (36), its presence would directly eliminate infectious virus. Nevertheless, it is not clear whether virus-neutralizing Ab alone is responsible for preventing virus reactivation.

Plasma cells and intrathecal Ab play a diverse role in viral CNS infections (61) and are hallmarks of CNS autoimmune disease (62). However, the mechanisms of B cell activation, recruitment, and retention within the CNS remain elusive. A small population of B cells (51), similar to T cells (63), are present in the resting CNS. Although B cells primed in the periphery traffic into the CNS slowly in the absence of inflammation (49, 51), they are rapidly recruited during inflammatory responses (13, 16, 17, 54, 61). Following JHMV infection, not only is the accumulation and/or continued differentiation of IgM ASC delayed relative to activation in the peripheral compartments, but maximal frequencies occur well after viral clearance at a time when Ag load is low, relative to acute infection. In addition, retention of virus-specific ASC within the CNS showed dramatically different kinetics compared with peripheral sites. Peripheral virus-specific IgM ASC declined after day 10 p.i., while the numbers within the CNS increased until day 21 p.i. and then remained stable. Similarly, following viral clearance, JHMV-specific IgG ASC increased only within the CNS but not in the peripheral compartments. Accumulation and/or continued differentiation of ASC within the CNS following JHMV clearance from glial cells is similar to CNS accumulation of virus-specific ASC following Sindbis virus infection of neurons (7, 61).
However, in contrast to JHMV infection, splenic Sindbis virus-specific ASC continued to increase following virus clearance (61), possibly due to the T cell-independent role of Ab in Sindbis virus clearance (7).

CLN are major sites of B cell activation following deposition of nonreplicating Ag within either the ventricles or parenchyma (51, 52) and in the presence of an intact blood brain barrier. It is not clear how parenchymal infection with a neurotropic virus, resulting in loss of blood brain barrier integrity, secretion of a variety of chemokines and cytokines, and rapid accumulation of a wide range of inflammatory cells influence the initial site(s) of B cell activation. The sharp peak in IgM ASC in spleen at day 10 p.i., contrasting with the modest increase in CLN, suggests that IgM ASC induced within the spleen traffic only transiently, if at all, through the CLN during JHMV infection. By contrast, IgG ASC accumulation to higher frequencies in CLN between days 10 and 21 p.i., despite an apparently delayed onset in expansion and/or retention compared with the spleen. These data suggest transient activation of virus-specific IgM ASC predominantly in the spleen, with delayed IgG ASC accumulation preferentially in the CLN. Little evidence was found to indicate that virus-specific ASC migrate via the blood between these two B cell maturation sites. IgM and IgG ASC may thus be activated in both the spleen and CLN with different kinetics relating to Ag drainage and load. The kinetics and distribution of JHMV-specific ASC confirm observations during alphavirus-induced encephalitis: virus-specific B cells are activated in the periphery and subsequently migrate into the CNS (7, 61), where they undergo terminal differentiation (51, 61). Recruitment followed by terminal differentiation into IgG ASC is consistent with the higher frequency of antiviral IgM ASC and retention of both virus-specific and nonreactive IgG ASC. It remains unclear whether the resident or the recruited B cell populations differentiate into ASC in a T cell-dependent manner within the CNS (51, 54, 61); however, CNS retention of both T cells and B cells appears to require the continued presence of Ag (47, 49, 51, 54).

The kinetics of T cell and B cell accumulation within the JHMV-infected CNS provides a unique insight into the host’s immune response to a neurotropic viral infection, which persists following initial immune control. Mature B cells, plasma cells secreting Ab to nonviral Ag, and T cells are rapidly recruited into the CNS following infection. Whereas virus-specific T cells peak during initial viral clearance, then decline and lose cytolytic activity (8, 18, 46), virus-specific ASC are prominent only after infectious virus has been cleared, and their frequency continues to increase during viral persistence. The delayed accumulation of virus-specific ASC and their prolonged retention are consistent with a role of local Ab in preventing reactivation (19). Interestingly, while ASC specific for the viral S and N proteins appear to decline with time, total virus-specific ASC do not (Table II). In addition, IgG ASC with apparently nonviral specificities accumulate to high, stable frequencies following virus clearance and return of blood brain barrier integrity. The large number of apparently nonvirus-specific IgG ASC in the CNS suggests continued recruitment and/or differentiation of B cells is not solely driven by viral Ag; cytokine/chemokine secretion or stimulation by self Ag may contribute to this process. The decline of S protein ASC from the CNS without the loss of serum-neutralizing Ab, in addition to the inability to account for the nonstructural viral proteins, suggests a number of possibilities. Virus replication may be initially suppressed by neutralizing Ab but then controlled by Ab with a second specificity. Alternatively, Ab specific for other viral structural proteins may be critical in controlling virus reactivation. Lastly, anti-JHMV serum Ab may be maintained by plasma cells residing in the bone marrow following resolution of the acute infection (52, 53, 64). In-deed, preliminary analysis indicates that no ASC are present in bone marrow at day 21 p.i. and only few IgM and IgG virus-specific ASC are present in bone marrow at day 30 p.i. Therefore, serum Ab levels may be maintained by preferential ASC loss from the CNS vs the bone marrow. In summary, these data confirm that the inability of cellular immunity to achieve absolute viral clearance is compensated by a dominant role of humoral immunity in controlling viral persistence within the CNS.

References


