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IFN-γ Is Required for Viral Clearance from Central Nervous System Oligodendroglia

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Infection of the central nervous system (CNS) by the JHM strain of mouse hepatitis virus (JHMV) is a rodent model of the human demyelinating disease multiple sclerosis. The inability of effective host immune responses to eliminate virus from the CNS results in a chronic infection associated with ongoing recurrent demyelination. JHMV infects a variety of CNS cell types during the acute phase of infection including ependymal cells, astrocytes, microglia, oligodendroglia, and rarely in neurons. Replication within the majority of CNS cell types is controlled by perforin-dependent virus-specific CTL. However, inhibition of viral replication in oligodendroglia occurs via a perforin-independent mechanism(s). The potential role for IFN-γ as mediator controlling JHMV replication in oligodendroglia was examined in mice deficient in IFN-γ secretion (IFN-γ−/− mice). IFN-γ−/− mice exhibited increased clinical symptoms and mortality associated with persistent virus, demonstrating an inability to control replication. Neither antiviral Ab nor CTL responses were diminished in the absence of IFN-γ, although increased IgG1 was detected in IFN-γ−/− mice. Increased virus Ag in the absence of IFN-γ localized almost exclusively to oligodendroglia and was associated with increased CD8+ T cells localized within white matter. These data suggest that although perforin-dependent CTL control virus replication within astrocytes and microglia, which constitute the majority of infected CNS cells, IFN-γ is critical for control of viral replication in oligodendroglia. Therefore, different mechanisms are used by the host defenses to control virus replication within the CNS, dependent upon the phenotype of the targets of virus replication.

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A major responsibility of innate and adaptive immunity is to limit infections before the accumulation of sufficient damage to result in death or permanent sequelae. Most tissues are continuously sampled for the presence of foreign Ag; however, the central nervous system (CNS) appears to be at a distinct disadvantage in responding to infection. First, there is no specialized lymphatic drainage, potentially limiting or delaying Ag recognition. Second, the normal CNS is devoid of dendritic cells and must rely upon microglia, endogenous cells of the macrophage lineage, for initial Ag recognition and presentation. Finally, MHC molecules are not normally expressed by neurons or glial cells, although they appear to be rapidly up-regulated under the influence of IFN-α/β (1). CNS viral infections result in vigorous immune responses; however, these inherent limitations appear to contribute to viral persistence or latency (2). Indeed, infection by a variety of both RNA and DNA viruses ultimately results in persistent CNS infections. Although many viruses predominantly infect single CNS cell types, each of the major cell types can provide a reservoir for persistent or latent viral infection. For example, herpes simplex virus and measles virus infect and subsequently persist in neurons (3, 4). Other viruses, such as lymphocytic choriomeningitis virus (LCMV) and the neurotropic coronavirus strain JHM (JHMV) initially infect a variety of cell types (5–8) but subsequently persist in only a subset of CNS cells (9–12). Immunity within the CNS appears to arrive at a balance that eliminates infectious virions and minimizes damage by either allowing or actually facilitating viral persistence. A remaining unanswered question is how immune effector mechanisms influence viral infections of the diverse cell types that comprise the CNS, resulting in persistent infection within specific cell types.

Infection of the rodent CNS by JHMV provides a model of acute viral infection that progresses to a chronic infection associated with ongoing CNS demyelination (9, 13, 14). The immune response contributes to both viral clearance (15, 16), but also to viral-induced primary demyelination (13, 17). Virus-specific Ab and T cell responses (15, 16, 18) have been implicated in limiting infectious virus. Although these immune effector mechanisms suppress virus replication, they are unable to completely eliminate virus resulting in persistent infection (19). JHMV replication in oligodendroglia may contribute to demyelination (8); however, increasing evidence suggests that demyelination is immunopathologically mediated and possibly distinct from the immune mechanisms that control virus replication (13, 14, 17). Defining the roles of the immune effectors that contribute to elimination of infectious virus is clarifying the strategies of JHMV persistence within the CNS. Neutralizing Ab are generally detected only after JHMV clearance (20, 21) and are suspected of playing a role in establishing or maintaining chronic infection without directly contributing to viral clearance. By contrast, both CD4+ and CD8+ T cells accumulate within the CNS during acute JHMV infection and are associated with viral clearance (20, 22, 23). Virus-specific CD4+ T cells may (15) or may not (24) contribute directly to viral clearance, possibly via the secretion of soluble mediators. The CTL
response limits virus replication in astrocytes and microglia but only to a lesser extent in oligodendrocytes (16) via a perforin-dependent mechanism (20). Indeed, CTL escape mutants of JHMV are associated with persistent CNS infectious virus (25, 26). The CTL-mediated reduction of virus replication in oligodendroglia was suggested to be a consequence of the overall successful CTL-mediated elimination of virus producing cells and not due to direct cytolyis of oligodendroglia (16).

IFN-γ plays important roles in many antiviral immune responses and is predominantly secreted by CD8+ T cells (27, 28). Therefore, the reduction of JHMV-infected oligodendrocytes in perforin-deficient mice (20) could have occurred via IFN-γ secretion. Consistent with this interpretation, inhibition of IFN-γ that enhances the severity of viral infections (29–34) is not correlated with defects in the generation of other antiviral immune effectors (33, 34). The notion that IFN-γ plays a critical role in the pathogenesis of JHMV with tropism for microglia, astrocytes, and oligodendroglia is suggested by several observations. Analysis of the kinetics of cytokine mRNA accumulation within the CNS of mice infected with JHMV, including infection with a neuronotropic variant of JHMV (OBLV-60), suggested a relationship between IFN-γ and inhibition of viral replication (21, 35). T cell-dependent IFN-γ inhibits vaccinia virus replication in meninges but not peripheral organs (36), suggesting that IFN-γ secretion within the CNS may be a critical component in controlling virus replication within specific CNS cell types. However, as shown by the clearance of the OBLV-60 variant of JHMV from the CNS of IFN-γ−/− mice (7), IFN-γ is not critical for virus clearance from neurons. An antiviral role of IFN-γ is also supported by the elimination of JHMV from the CNS of perforin-deficient mice (20) and after adoptive transfer of either JHMV-specific CD4+ T or CD8+ T cells (15, 16). Virus-specific T cells all reduced virus in infected CNS cells including oligodendrocytes potentially via secretion of IFN-γ (15, 16). Finally, the expression of the IFN-γ receptor (37), but not MHC molecules on oligodendroglia (2), suggest that IFN-γ may reduce JHMV replication in this cell type. These observations provided the basis for examining the potential contribution of IFN-γ in limiting JHMV infection of oligodendroglia. Infectious virus was not completely cleared from the CNS of IFN-γ−/− mice, although CTL and neutralizing Ab responses were induced. Consistent with a vigorous but ineffective CTL response, CD8+ T cells were selectively recruited to white matter areas of the CNS. Survivors showed persistence of viral Ag in oligodendroglia consistent with CTL-mediated clearance from astrocytes and microglia, but not oligodendrocytes (16). These data suggest that although IFN-γ is not required for the inhibition of replication in neurons (7), it either directly or indirectly controls viral replication within oligodendroglia.

Materials and Methods

**Mice and virus**

C57BL/6 mice heterozygous for a nonfunctional IFN-γ gene (IFN-γ−/+) were obtained at N9 from Genentech (San Francisco, CA) (38) and maintained by homozygote mating. Syngeneic wild-type (wt) C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Male and female 6–8-wk-old IFN-γ−/− and wt mice were used in all experiments. Mice were infected by intracerebral injection with 25 pfu of the 2.2v-1 mAb derived neutralization escape variant of JHMV (39) and monitored for 21 days postinfection (p.i.). This strain produces a subacute encephalomyelitis with primary CNS demyelination and paralysis that progresses to chronic infection (13, 19, 39). Clinical scores were read daily and graded as described previously (20, 21, 39).

**Virus titration**

Brains were divided into thirds in the sagittal plane and processed for virus titration, histopathology, and RNA extraction. Viral titers were determined by plaque assay on monolayers of DBT cells as described previously (16, 20, 21). Briefly, tissue was homogenized in 4 ml of Dulbecco’s PBS (pH 7.4) and centrifuged (1500 × g for 7 min at 4°C), and supernatants assayed immediately or stored at −70°C. Average titers of groups of at least three mice per time point are presented.

**Virus-specific Ab response**

JHMV-specific IgG1 and IgG2a serum Ab were quantitated by ELISA as described (20, 21) and expressed as the log of the highest dilution with OD values three times above background level. Rabbit anti-mouse IgG1 or IgG2a were used as secondary Ab (Cappel Laboratories, Costa Mesa, CA). Neutralizing Ab titers were determined by plaque reduction assay (20, 21).

**Cytokines**

For analysis of cytokine production, CLN cells from days 3, 5, and 7 days p.i. were cultured in 96-well plates (8 × 103 cells/well) in the presence or absence of JHMV Ag. Supernatants were harvested at 24 h for IL-2 and at 48 h for IL-4, IL-10, and IL-5 secretion. Cytokine concentrations were determined by ELISA and calculated from standard curves using commercially obtained recombinant cytokines as described previously (40). Detection limits were 75 pg/ml for IL-2, IL-5, and IL-10 and 125 pg/ml for IL-4.

**Virus**

RNA was isolated from brains by homogenization in guanidine isothiocyanate and centrifugation through cesium chloride as described previously (21, 40). cDNAs were prepared using avian myeloblastosis virus reverse transcriptase and oligo(dT) primers (Promega, Madison, WI) at 42°C. Cytokine mRNAs was determined by semiquantitative PCR as described previously (20, 21, 40) using specific primer pairs for IL-5 (5′-oligoprimmer, 5′-GAT TAC AGA CAT GCA CCA TTG CCA CTA GTT-3′ and 3′-TGACGTTGATTTGAATTAAAATTAGTAGTATTGAG-3′ (41), IL-4, and IL-10 (21).

PCR products were quantitated by hybridization with [32P]ATP-labeled internal oligoprobes in a dot blot assay. Membranes were exposed to an imaging screen (Molecular Dynamics, Sunnyvale, CA) and analyzed using a PhosphorImager scanner (Molecular Dynamics). Cytokine cDNAs were normalized to the housekeeping gene HPRT to adjust for cDNA variations (21, 40).

**Cytolytic activity**

CTL activity was measured using CLN cells obtained at 6 days p.i. and spleen cells at various days p.i. (days 7, 9, and 14 p.i.) in a 51Cr release assay as described previously (16, 20). Cells were incubated for 7 days in the presence of the 1 μM SS10 peptide (20, 42, 43). EL-4 (H-2b) target cells were coated with 1 μM SS10 peptide before use as targets (20, 42). Effector cells were added to the target cells at various E:T ratios and 51Cr release measured after 4 h incubation at 37°C. Data are expressed as specific 51Cr release. Spontaneous release values were ≤20% of the total release values.

**Sequence analysis of Spike protein CTL epitope**

Viruses from brain samples obtained at 21 days p.i. were propagated once on confluent monolayers of DBT cells. When >80% of the cells exhibited cytopathology (24–48 h), cells were lysed by addition of guanidine thiocyanate solution and RNA isolated by phenol/chloroform extraction. RNA prepared from DBT cells infected with parental 2.2v-1 virus and uninfected DBT cells were used as controls for mutations incorporated by Taq polymerase and for PCR contamination respectively. RNA (5μg) was reverse transcribed using avian myeloblastosis virus reverse transcriptase and random-hexanucleotide primers (Promega). A 27-nucleotide cDNA encompassing the S protein CTL epitope (viral bases 1528–1554) plus surrounding 500 bases (1420–1890) was amplified for 45 cycles using AmpliTaq Gold Polymerase (Perkin–Elmer, Branchburg, NJ) and primers JS 1895, 5′-GCA TGC TAC GAT TTG TCC AGG CTG AGT C3′; and JS 1390, 5′-GAT GTC GCC TAC GCC CAG C-3′. Excess primers were removed using the Magic PCR Prep (Promega), and the cDNA was sequenced on an ABI Prism automated sequencing apparatus (Applied Biosciences, Foster City, CA) using JS 1390 as primer.

**Histopathological analysis**

Brain and spinal cord tissues were fixed in Clark’s solution for 3 h and prepared for parafin sections as described previously (16, 20). Sections were stained with hematoxylin and eosin or Luxol fast blue for routine examination. To examine viral Ag distribution, sections were incubated with the anti-HMNV mAb 13.3.5 specific for the nucleocapsid protein of JHMV (44) and immunoperoxidase-labeled anti-mouse mAb as secondary Ab (Vectastain-ABC kit, Vector Laboratories, Burlingame, CA). Multiple

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sial-strep frozen sections of the brain tissue of 2 mice at day 14 p.i. were stained with mAb J.3.3 Ab. Ag-positive cells were counted to compare the number of infected cells in both study groups. Similarly, five serial-strep paraffin sections of the spinal cord of three to four mice per group at day 21 p.i. were stained with Luxol fast blue, and the number of demyelination plaques was counted per unit area.

To examine CD4<sup>+</sup> and CD8<sup>+</sup> cells, immunoperoxidase staining was performed in acetone fixed frozen sections as described previously (45). In brief, rat anti-CD4 (L3T4, PharMingen, San Diego, CA) and rat anti-CD8 (Ly-2, PharMingen) were used as primary Ab. Visualization was achieved using biotinylated rabbit-anti-rat Ab, the Vectastain ABC kit and peroxidase Vectastain ABC kit for CD11b and peroxidase Vectastain ABC kit for AEC substrate for detecting J.3.3 mAb. Viral Ag in CD11b-positive cells was detected in acetone-fixed frozen sections, using alkaline phosphatase Vectastain ABC kit for Rip Ab and the peroxidase Vectastain ABC kit for RIp Ab and AEC substrate kits (both from Vector Laboratories), respectively. Viral Ag in oligodendroglia was detected in frozen sections from paraformaldehyde-perfused tissue and detected using the alkaline phosphatase Vectastain ABC kit for Rip Ab and the peroxidase Vectastain ABC kit with AEC substrate for detecting J.3.3 mAb. Viral Ag in CD11b-positive cells was detected in acetone-fixed frozen sections, using alkaline phosphatase Vectastain ABC kit for CD11b and peroxidase Vectastain ABC kit with AEC substrate for J.3.3 mAb.

**Results**

**Pathogenesis of JHMV infection in IFN-γ<sup>−/−</sup> mice**

Morbidity and mortality were compared in IFN-γ<sup>−/−</sup> and wt mice infected with JHMV until 21 days p.i. at which time infected wt mice recovered from acute disease (Fig. 1) (20, 21, 39). Animals in both groups began to exhibit encephalitis at 7 days p.i., which progressed to a paralytic disease (clinical score = 2.0 ± 0.2 for wt and 2.7 ± 0.2 for IFN-γ<sup>−/−</sup> mice at 14 days p.i.) (Fig. 1A). Wt mice exhibited almost total clinical recovery by 21 days p.i. (clinical score = 1.2 ± 0.2). By contrast, infected IFN-γ<sup>−/−</sup> mice rapidly progressed to a paralytic disease by 14 days p.i. and exhibited both slower clinical recovery and higher mortality (clinical score = 2.6 ± 0.6 at 21 days p.i.). Only 34% ± 8% of IFN-γ<sup>−/−</sup>-infected IFN-γ<sup>−/−</sup> mice survived infection compared with 89% ± 8% of control mice indicating that IFN-γ influences both survival and clinical course of JHMV-induced encephalomyelitis (Fig. 1B).

Higher viral titers were found in the CNS of infected IFN-γ<sup>−/−</sup> mice compared with controls at all times examined (Fig. 1C).

In contrast to wt mice, in which infectious virus was no longer detected after day 7 p.i., infectious virus (≥10<sup>5</sup> plaque forming units/g of brain) persisted in the CNS of all IFN-γ<sup>−/−</sup> mice until 21 days p.i., the last time point examined (Fig. 1C). To rule out reversion of the 2.2v-1 variant to wt JHMV, virus isolated from the brains of IFN-γ<sup>−/−</sup> mice at 21 days p.i. was assayed for plaque formation in the presence or absence of mAb J.2.2 (used for selection of JHMV neutralizing escape variant 2.2v-1-1) (39). No differences in either plaque numbers or morphology was detected, consistent with the absence of in vivo reversion (data not shown).

**Immune responses in infected IFN-γ<sup>−/−</sup> mice**

Infected IFN-γ<sup>−/−</sup> mice were analyzed for JHMV-specific CTL activity to determine whether the inability to clear infectious virus was due to altered effector function. CTL activity in CLN cells of JHMV-infected IFN-γ<sup>−/−</sup> mice was identical to the activity in wt mice during virus clearance from the CNS (Fig. 2), indicating that the absence of IFN-γ did not diminish CTL activity. JHMV isolated during infection of neonatal mice partially protected via maternal Ab, exhibit a high mutation rate within the viral H-2<sup>b</sup>-restricted CTL epitope allowing viral escape from CTL recognition (25, 26). To examine the possibility that CTL escape mutants contributed to the persistence of infectious virus within the CNS of IFN-γ<sup>−/−</sup> mice, the S protein mRNA encoding the CTL epitope (RNA bases 1528–1554) as well as ~500 bases (1420–1890) surrounding the epitope was analyzed for mutations. No dominant base substitutions or deletions within these S protein sequences were detected by sequencing of bulk PCR products for virus pools isolated from the CNS of persistently infected IFN-γ<sup>−/−</sup> mice at 21 days p.i. after infectious virus had been completely eliminated from the CNS of wt mice. These data are consistent with a direct antiviral effect of IFN-γ on JHMV replication within the CNS.

An alternative explanation for reduced virus clearance in IFN-γ<sup>−/−</sup> mice may reside in preferential induction of Th2-type responses. No differences in proliferative responses to either JHMV Ag or the CTL-specific S510 peptide were detected in either splenocytes or CLN cells from infected IFN-γ<sup>−/−</sup> mice compared with wt mice at 7 or 14 days p.i. (data not shown). However, CLN isolated from infected IFN-γ<sup>−/−</sup> mice at 3 and 5 days p.i. secreted more IL-2 in response to JHMV Ag compared with infected wt controls (Fig. 3). IL-4 secretion was not detected from cells derived from either group (data not shown). IL-5 and IL-10 were
secreted at higher levels from CLN derived from infected IFN-γ−/− mice at 7 days p.i. (Fig. 3). No differences were detected in the quantity of IL-10 mRNA; however, less IL-5 mRNA was detected in the CNS of infected IFN-γ−/− mice compared with wt controls (Fig. 3). The diminished expression of IL-5 mRNA and transient expression of IL-4 mRNA at day 7 p.i. (Fig. 3) in IFN-γ−/− mice suggest the absence of increased trafficking or retention of Th2 cytokine secreting cells within the CNS in the absence of IFN-γ. This contrasts with an initial increase in Ag-specific secretion of Th2 type cytokines (IL-5 and IL-10) by peripheral T cells.

Consistent with the increased secretion of Th2-type cytokines, a 5-fold increase in JHMV-specific IgG1 was detected in the serum of infected IFN-γ−/− mice compared with wt controls, whereas a 10-fold increase in IgG2a was initially detected in wt mice compared with IFN-γ−/− mice at day 9 p.i. (Fig. 4). Although the IFN-γ−/− mice continued to have increased serum JHMV-specific IgG1 at 14 days p.i., by 21 days p.i., the levels of both isotypes were equivalent in the IFN-γ−/− and wt mice (Fig. 4). As reported previously (20, 21), no serum neutralizing Ab was detected before day 9 p.i. and no differences were detected in JHMV neutralizing titers at 14 or 21 days p.i. (data not shown) suggesting that secretion of neutralizing Ab was not altered in the absence of IFN-γ.

Histopathology

Spinal cords and brains from infected IFN-γ−/− mice were compared with infected wt mice to determine the influence of the absence of IFN-γ on viral Ag distribution and extent of pathological changes. Prominent demyelination was found within white matter tracts of both groups after 21 days p.i. Semiquantitative analysis showed no significant differences in demyelination plaque numbers between IFN-γ−/− (2.4 ± 1.4) and wt (3.4 ± 2.1) mice suggesting that IFN-γ does not influence development of JHMV-induced demyelination. Similarly, no differences in the overall amount of mononuclear inflammation were noted; however, an increase in the amount of CD8+ T cells was detected in the white matter tracts of infected IFN-γ−/− mice at 7 and 14 days p.i. compared with wt mice. Semiquantitative estimation of CD8+ T cells infiltrating the brain parenchyma of infected mice showed a 4-fold increase in the number of CD8+ T cells in IFN-γ−/− mice at days 7 and 14 p.i. (200 ± 80 and 750 ± 210) compared with wt mice (30 ± 10 and 110 ± 50). Interestingly, increased CD8+ infiltration in the white matter regions correlated with increased Ag-positive cells in IFN-γ−/− mice suggesting that the lack of IFN-γ results in increased viral Ag promoting local infiltration of CD8+ T cells.

Differences in the amount and type of Ag-positive cells were observed beginning at day 7 p.i. (Fig. 5, A and B). Decreasing numbers of viral Ag-positive cells were observed in wt mice, consistent with the clearance of infectious virus (Fig. 1C). By contrast, subsequent to day 7 p.i., significant Ag persisted until day 21 p.i. in IFN-γ−/− mice (Fig. 6, A and B). By day 14 p.i. the IFN-γ−/− mice had a 10-fold increase in numbers of viral Ag-positive cells (IFN-γ−/− mice = 1500 ± 780 vs wt mice = 75 ± 60) compared with wt mice. Consistent with the presence of infectious virus in infected IFN-γ−/− mice, oligodendroglia, astrocytes, microglia, and occasional neurons contained viral Ag; however, double staining for viral Ag in astrocytes, microglia, and oligodendrocyte revealed

**FIGURE 2.** Cytotoxic activity in JHMV-infected IFN-γ−/− and control wt mice. CLN cells were obtained from infected mice at 6 days p.i. and cultured in vitro for 7 days in the presence of 1 μM SS10 peptide. Cytolytic activity was measured using SS10 peptide coated EL-4 target cells. Similar results were obtained using recombinant vaccinia virus SS10 infected EL4 and IC-21 target cells. Spleen cells at days 7, 9, and 14 p.i. were also examined, and no significant differences in CTL activity were detected in splenocytes from infected IFN-γ−/− mice compared with wt mice (data not shown).

**FIGURE 3.** Specific cytokine secretion from CLN cells and cytokine mRNA expression in brains of 22v-1-infected IFN-γ−/− and wt mice. At various time points p.i. CLN cells were cultured in the presence or absence of JHMV Ag and cytokine secretion determined by ELISA assay. Supernatants were tested at 24 h for IL-2 (A) and at 48 h for IL-5 (B) and IL-10 (C). The levels of cytokine mRNA in infected brains were determined by RT-PCR and semiquantitative dot blot and the data expressed as a relative value obtained for comparison.

**FIGURE 4.** Kinetics of JHMV-specific Ab in infected IFN-γ−/− and wt mice. Ab titers were measured in sera by ELISA assay as described in Material and Methods.
that oligodendroglia accounted for the majority of Ag-positive cells within the CNS of IFN-γ0/0 mice at day 21 p.i. (Fig. 7A). These data are consistent with the inability of CTL to mediate clearance from oligodendrocytes (16, 20) and demonstrate that IFN-γ is indeed an important immune effector in controlling JHMV infection of oligodendroglia.

Discussion

Immune responses to viral infection of the CNS contribute to both virus clearance and demyelination (2, 9, 13, 14, 47–49). JHMV infects all major CNS cells types, resulting in an acute encephalomyelitis with demyelination (7–11, 13, 14). During acute infection, the immune mechanisms required for viral inhibition appear distinct from those that contribute to primary demyelination (13, 17). It is the inability of the immune response to eliminate virus that results in persistent infections associated with ongoing demyelination. CTL eliminate infectious JHMV from infected microglia and astrocytes via a perforin-dependent cytolytic mechanism (16, 20). However, virus is still eliminated from the CNS in the absence of perforin-mediated cytolysis (20), suggesting that other mechanisms play important roles in viral elimination, especially from oligodendroglia that appear at least partially refractory to CTL-mediated clearance (16). IFN-γ is produced by both CD4+ and CD8+ T cells in response to viral infections (49, 50). However, it is essential for virus elimination during some (29–32, 48) but not all (7, 51) infections. Direct antiviral effects of IFN-γ appear to be not only dependent upon the type of infection but also the tissue or cell type infected. For example, IFN-γ contributes to viral clearance from the periphery but not CNS (52), or from the CNS but not peripheral organs (36). Although JHMV was partially cleared from the CNS of IFN-γ0/0 mice, infectious virus persisted. Consistent with the ability of the IFN-γ0/0 mice to exert partial control of JHMV replication, CTL effectors were not inhibited in the absence of IFN-γ. These data support previous observations that other cytokines, including IL-2, IL-4, IL-6, and IL-10, in addition to IFN-γ contribute to CTL induction (50). Thus, it appears that CTL play a role in local viral inhibition even in the absence of IFN-γ probably via direct perforin-mediated cytolysis of infected astrocytes and microglia (16, 20). Infection of IFN-γ0/0 mice with a JHMV variant whose replication is predominantly restricted to neurons demonstrated that IFN-γ plays little or no role in the elimination of JHMV from neurons (7), similar to the inability of CTL to mediate the clearance of LCMV from neurons (12). By contrast, elimination of measles virus from neurons is mediated by a CD4+ T cell population (53), possibly due to the local secretion of IFN-γ (30).

The mechanism(s) of JHMV clearance from neurons is not clear;
IFN-γ contributes to viral clearance but that it does not directly influence macrophage-mediated demyelination. Nevertheless it is possible that the equivalent demyelination in JHMV-infected IFN-γ0/0 mice occurs by an effector mechanism that differs from autoimmune demyelination (56, 57) or alternatively it may be due to direct effects of JHMV replication in oligodendroglia (8) associated with necrotic, rather than apoptotic cell death (20).

The immune-mediated encephalomyelitis induced by JHMV infection is comprised of NK cells, virus specific CTL and CD4 T cells and monocytes. The emerging picture of this complex infection that results in viral persistence and chronic demyelination is that specific immune effector mechanisms contribute to the control of virus replication within specific subsets of the major CNS cell types. Although NK cells are rapidly recruited, they appear to play little or no role in this infectious process (6, 23). Similarly, neutralizing Ab is detected only after the majority of virus is cleared (20, 21), suggesting that it plays little or no role in limiting virus replication. By contrast, the virus-specific CTL response appears to limit acute infection of both astrocytes and microglia (16). JHMV-specific induction of CTL was initially reported to be dependent on CD4 T cells (22); however, recent data demonstrate that CTL are induced and traffic normally into the CNS during infection of CD4-depleted hosts (45). The inability to limit virus in the absence of CD4 T cells is related to a requirement for the maintenance of CTL viability within the CNS. Therefore, CD4 T cells play a critical role in CTL effector function within the CNS, in addition to their potential direct role in virus clearance (15, 53). This report demonstrates that IFN-γ contributes to the overall inhibition of virus replication specifically in oligodendroglia. Thus, both virus-specific cytolytic activity and IFN-γ appear to differentially inhibit JHMV replication within the CNS by exhibiting cell type specific effector function. The inability of CTL to inhibit replication in oligodendroglia may reflect the absence of MHC class I expression, similar to the inability of CTL specific for LCMV to limit virus replication in neurons (12). Expression of the IFN-γ receptor on oligodendroglia (37) and the recent suggestion that IFN-γ may be critical in limiting measles virus expression in neurons (30) both support a vital role for this cytokine in limiting virus infections within specific CNS cell types.

References


