IFN-γ Secreted by Virus-Specific CD8$^+$ T Cells Contribute to CNS Viral Clearance

BEATRIZ PARRA,[1] CORNELIA C. BERGMANN,[1,2] DAVID R. HINTON,[3] ROSCOE ATKINSON,[2,3] AND STEPHEN A. STOHLMAN[1,2]

Departments of Molecular Microbiology and Immunology[1], Neurology[2], and Pathology[3]. Keck School of Medicine, University of Southern California, Los Angeles, CA 90033

1. INTRODUCTION

Replication of mouse hepatitis virus strain JHM (JHMV) in the central nervous system (CNS) is controlled by CD8$^+$ T cells. However, persistent infection and subsequent chronic demyelination are established. Understanding effector mechanisms during acute infection may help understand viral persistence. CD8$^+$ T cells respond to viral infections via two different cellular mechanisms, lysis of infected cells and secretion of anti-viral cytokines (Kaki *et al* 1995, Ruby *et al* 1991, Young *et al* 1995). JHMV replication in astrocytes and microglia is controlled by perforin-dependent cytolysis (Lin *et al* 1997) whereas replication in oligodendrocytes is controlled via IFN-γ (Parra *et al* 1999). The contributions of IFN-γ and perforin-dependent CD8$^+$ T cell function in viral clearance were examined in SCID mice following adoptive transfer of CD8$^+$ T cells deficient in IFN-γ secretion.

2. MATERIAL AND METHODS

Mice: BALB/c SCID and wild type (wt) BALB/c mice were obtained from NCI (Frederick, MD). Homozygous IFN-$\gamma^{-/-}$ BALB/c mice were provided by Robert Coffman, DNAX Research Corporation (Palo Alto, CA).

The Nidoviruses (Coronaviruses and Arteriviruses).
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Virus infection: SCID mice were infected with the 2.2v-1 strain of JHMV (Fleming *et al* 1986). Wt mice and IFN-$\gamma^{-/-}$ donors were immunized i.p. with 10^6 pfu of JHMV and 4 weeks later spleens were removed to purify CD8$^+$ T cells.

CD8$^+$ T cell purification: Spleen cells from immunize mice were depleted of B cells and macrophages by panning. CD4$^+$T cells were depleted by adsorbance to magnetic beads (Miltenyi Biotec Inc. Auburn, CA.). Recoveries of CD8$^+$ T cells were approximately 80% as determined by flow cytometry analysis.

Adoptive Transfers: Recipient SCID mice were adoptive transferred with CD8$^+$ T cells from either IFN$\gamma^{-/-}$ or wt immunized mice intravenously with 1-2x10^7 CD8$^+$ T cells and infected 5 h later. Viral replication and pathogenesis were determined at d 10 and 14 p.i., when the control SCID mice began to succumb.

CNS mononuclear cell populations: Mononuclear cells were isolated as previously described (Bergmann *et al* 1999). Virus specific CD8$^+$ T cells were identified by anti-CD8 and a tetrameric L^d-N-318 reagent and assayed for *ex vivo* CTL activity as described (Bergmann *et al* 1999). Microglial cells (CD45 ^{low} CD11b$^+$) were characterized by staining with anti-CD45 and anti-CD11b$^+$ mAbs (Pharmingen, San Diego, CA). MHC class I L^d expression was determined by the mean fluorescence intensity with an anti-H2L^d specific mAb (PharMingen).

Histopathology: Tissues were prepared for paraffin and frozen sections as described (Parra *et al* 1999). Viral antigen (Ag) was detected with mAb J3.3. CD8$^+$ cells were identified in frozen sections using rat anti-CD8a mAb (PharMingen). Sections were stained with mAb J3.3 and Ab specific for astrocytes (GFAP) or microglia (CD11b) as described (Parra *et al* 1999).

3. RESULTS

3.1 IFN-$\gamma^{-/-}$ CD8$^+$ T cells reduce virus replication less efficiently than IFN-$\gamma^{+/+}$ CD8$^+$ T cells.

Immunodeficient SCID mice reconstituted with CD8$^+$ T cells from immune wt (IFN-$\gamma^{+/+}$) donors reduced CNS viral replication at 10 and 14 days p.i. compared with control SCID mice (Fig. 1). CD8$^+$ T cells derived from immune IFN-$\gamma^{-/-}$ donors were less effective than IFN-$\gamma^{+/+}$ CD8$^+$ T cells (Fig 1). These results demonstrate that IFN-γ derived from CD8$^+$ T cells alone influences JHMV clearance from the CNS. Nevertheless, virus was reduced by IFN-$\gamma^{-/-}$ CD8$^+$ T cells compared to control SCID mice (Fig 1),

consistent with expression of perforin mediated cytotoxicity. Viral Ag in recipients of IFN-$\gamma^{-/-}$ $CD8^+$ T cells was dramatically increased compared to IFN-$\gamma^{+/+}$ recipients at 14 days p.i. Viral Ag localized to multiple cell types in brain of both reconstituted groups; however, it was mainly localized to spinal cord oligodendrocytes (Fig. 3). This contrast to the multiple infected cell types in spinal cords of control SCID mice. In contrast to reduced viral Ag positive cells in the CNS of wt $CD8^+$ T cell recipients, an increase in infected oligodendrocytes was present in IFN-$\gamma^{-/-}$ $CD8^+$ T cell recipients (Fig. 2), consistent with $CD8^+$ T cells controlling JHMV replication in oligodendrocytes via IFN-γ (Parra *et al* 1999).

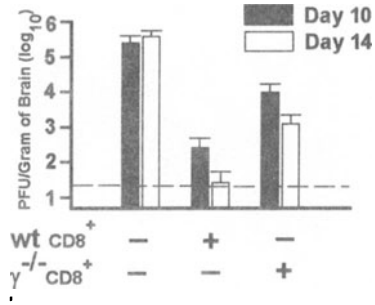

Figure 1. $CD8^+$ T cells reduce JHMV replication via IFN-γ. $CD8^+$ T cells from JHMV immune IFN-$\gamma^{-/-}$ or wt mice were transferred into SCID mice and infected with JHMV. Virus replication at 10 and 14 days p.i is expressed as Log_{10} PFU/gm tissue. Titers are means of at least 4 mice/group. Dashed line is the assay detection limit. Data are representative of 2 experiments.

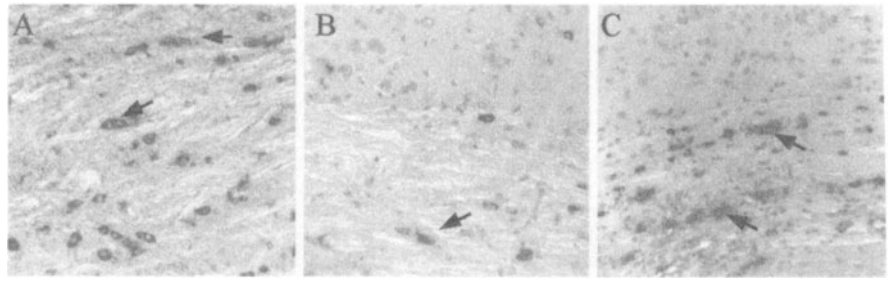

Figure 2. Viral Ag in oligodendroglia is reduced via $CD8^+$-derived IFN-γ. Viral Ag in spinal cords: A) unreconstituted; B) IFN-$\gamma^{+/+}$ $CD8^+$ reconstituted and; C) IFN-$\gamma^{-/-}$ $CD8^+$ reconstituted SCID mice at day 14 p.i. Arrowhead points indicate oligodendrocytes.

Functional virus specific IFN-$\gamma^{-/-}$ $CD8^+$ T cells are recruited to CNS. Brain infiltrating cells showed similar proportions of virus specific $CD8^+$ T cells recruited in both groups of reconstituted SCID mice (Fig. 3). IFN-$\gamma^{-/-}$ $CD8^+$ T cells are recruited by 4 d p.i. and accumulated with both the same

kinetic and to similar numbers in both groups (data not shown). Equal $CD8^+$ T cells within the brain parenchyma and spinal cord white matter tracts were in both groups (data not shown). $CD8^+$ T cells deficient in IFN-γ secretion within the CNS at d 10 p.i. retained *ex vivo* cytolysis (Fig. 3).

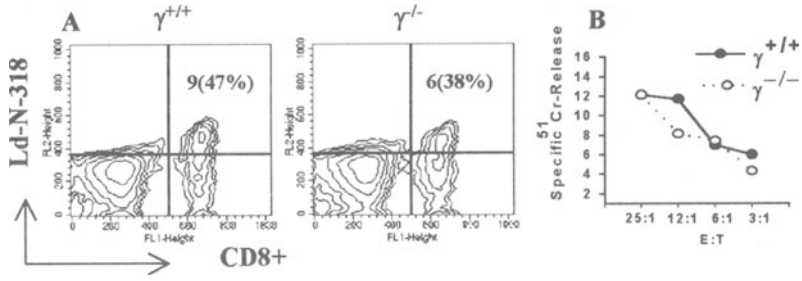

Figure 3. Virus-specific $CD8^+$ T cells recruited into the CNS of $CD8^+$ IFN-$\gamma^{-/-}$ T cell reconstituted SCID mice. CNS infiltrating cells were prepared from reconstituted mice at d 10 p.i. Cells were stained for $CD8^+$ and virus-specific TcR (L^d -N-318 tetramer) (Panel **A**). Numbers represent the percentage of total population. Data from two experiments. (Panel B). *Ex vivo* ^{51}Cr release from pN peptide coated targets. Nonspecific cytolysis was zero as determined with uncoated targets.

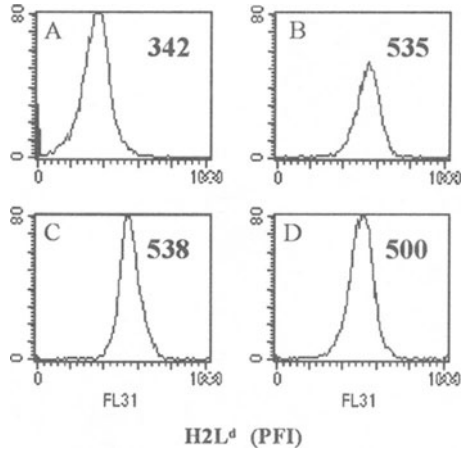

Figure 4. MHC class I expression on microglia during JHMV infection is upregulated independently of CD8-derived IFN-γ . CNS cells were isolated from (A) naïve, (B) JHMV infected and infected (C) IFN-$\gamma^{+/+}$ or (D) IFN-$\gamma^{-/-}$ $CD8^+$-reconstituted SCID mice. Cells were gated on microglia ($CD45^{low}$ and $CD11b^+$). L^d expression was determined as peak mean fluorescence intensity (PFI). Data represent two independent experiments.

$CD8^+$ T cell derived IFN-γ independent increased MHC class I expression. To determine if the lack of IFN-γ from $CD8^+$ T cells influenced JHMV clearance, MHC class I L^d, the immunodominant N epitope restriction element was examined on microglia/macrophages. L^d expression

was found on microglia of control SCID mice after infection (Fig. 4A, 4B). Microglia in both $CD8^+$ T cell recipient groups have similar levels of increased L^d expression (Fig. 4C, 4D).

4. DISCUSSION

JHMV infection of mice with perforin or IFN-γ deficiencies suggested that both effector mechanisms played critical roles in viral clearance (Lin *et al* 1997, Parra *et al* 1999). Although the previous data suggested a predominant role of the $CD8^+$ T cell response in clearance, analysis of gene deleted mice is complicated by additional cell types. These experiments were conducted by reconstitution of immunodeficient SCID mice to eliminate contributions of $CD4^+$ T cells and B cells. The results demonstrate that in addition to cytotoxicity, IFN-γ is a key $CD8^+$ T cell effector important for the control of the acute CNS infection. JHMV is highly oligotropic and $CD8^+$ T cells may have an absent or diminished cytotoxic action on this low MHC class I expressing cell type. However, in wt mice virus is eliminated from oligodendrocytes by a vigorous CTL response (Stohlman *et al* 1995). $CD8^+$ T cells competent for IFN-γ secretion efficiently reduced virus from oligodendrocytes of infected SCID mice compared to partial elimination in recipients of IFN-$\gamma^{-/-}$ $CD8^+$ T cells. IFN-γ is not required for CTL induction (Parra *et al* 1999, Graham *et al* 1993) or for homing to the site(s) of infection (Nansen *et al* 1998). Indeed, IFN-$\gamma^{-/-}$ $CD8^+$ T cells with intact perforin-dependent cytotoxicity trafficked normally into the CNS and reduced virus from most MHC class I expressing cells.

It is possible that IFN-γ is required for increased expression of MHC class I on targets, thereby promoting effective recognition (Young *et al* 1995). Class I was expressed at similar levels on microglial of SCID mice reconstituted with $CD8^+$ T cells from both groups. Increased expression occurred after infection, independent of $CD8^+$ T cells, consistent with early NK cell recruitment (data not shown) and the IFN type I (α/β) mediated increase in MHC expression following viral infection (Njenja *et al* 1997). These data support the concept that $CD8^+$ T cells inhibit virus in oligodendrocytes via a IFN-γ dependent mechanism. However, IFN-γ may also inhibit viral spread from other cell types by limiting susceptible cells. Therefore, virus accumulation in oligodendrocytes during an infection in which $CD8^+$ T cells are unable to secrete IFN-γ could result from both increase viral spread, in addition to limited cytotoxicity. These data are consistent with the increase in CNS viral Ag in SCID mice reconstituted with IFN-$\gamma^{-/-}$ $CD8^+$ T cells. However, it is equally likely that an inability to lyse infected oligodendroglia, in addition to the lack of a direct IFN-γ

mediated anti-viral activity, results in infection of other CNS cell types seeded by uncontrolled oligodendroglia infection. Although the precise role of CD8$^+$ T cell effector mechanisms is complicated by the dynamics of an ongoing infection, these data support the hypothesis that separate effector mechanisms are functioning at the single cell type level within the CNS.

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