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# MECHANISMS OF VIRAL CLEARANCE IN PERFORIN-DEFICIENT MICE

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## 1. ABSTRACT

The roles of CD4+ T cells, IFN-γ and TNF-α in viral clearance from the central nervous system (CNS) were examined in perforin gene deficient (PKO) mice. Depletion of CD4+ T cells from the PKO mice resulted in a significant 1 $\log_{10}$ PFU/gm increase in viral titer over control-treated PKO mice. PKO mice treated with anti-IFN-γ mAb also had a significant 1 $\log_{10}$ increase in infectious virus whereas inhibition of TNF-α did not alter viral clearance or clinical disease in the PKO mice. These data suggest, in addition to perforin-mediated cytolysis, CD4+ T cells and IFN-γ, but not TNF-α could contribute to JHMV clearance from the CNS.

## 2. INTRODUCTION

Infection of the CNS by JHMV (MHV4), a neurotropic strain of mouse hepatitis virus (MHV) induces primary demyelination and persistent infection in susceptible strains of mice (Lampert, Sims, and Kniazeff, 1973; Weiner, 1973). Although the mechanisms of viral persistence remains unclear, the complete elimination of virus from the CNS by the immune response during the acute infection appears to be the critical parameter in preventing viral persistence and chronic demyelination (Houtman and Fleming, 1996b). Viral clearance of JHMV from the CNS is influenced by both cellular and humoral responses (Compton, Barthold, and Smith, 1993; Fazakerley and Buchmeier, 1993; Kyuwa and Stohlman, 1990); however, since T cell recruitment and viral clearance occur prior to de-

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tectable serum neutralizing antibodies, cellular immunity appears to be most critical. Consistent with this concept, adoptive transfer of JHMV-specific CD8+ cytotoxic T lymphocytes (CTL) provides protection from both acute and chronic infection by limiting viral replication in astrocytes and microglia, but not major histocompatibility complex class I-negative oligodendrocytes (Stohlman et al., 1995a). The exact mechanisms involved in immune mediated clearance are not yet clear. Anti-viral effects of CD8+ CTL appear to be predominantly due to direct lysis of infected cells via perforin-mediated cytolysis since clearance of JHMV from the CNS in mice genetically deficient in perforin (PKO) is delayed, but not abolished (Lin, Stohlman, and Hinton, 1997). Moreover, these data suggest the coexistence of other anti-viral mechanisms along with perforin-mediated cytolysis such as CD4+ T cells or cytokines. CD4+ T cells participate in viral clearance by providing help for CD8+ CTLs (Williamson and Stohlman, 1990). However, adoptive transfer of some, but not all JHMV-specific CD4+ T cells also results in clearance of virus from the CNS (Korner et al., 1991; Stohlman et al., 1986; Wijburg et al., 1996; Yamaguchi et al., 1991). Furthermore, cytokine secretion by both CD8+ and CD4+ T cells may exert anti-viral activity either directly or indirectly. During JHMV infection, tumor necrosis factor (TNF)-α and interferon (IFN)-γ mRNA expression increased at the time of maximal decrease in virus replication (Parra et al., in press; Pearce et al., 1994). Consistent with IFN-γ as an anti-viral agent, mice treated with anti-IFN-γ are more susceptible to MHV infection, while mice treated with IFN-γ (Smith et al., 1991)or adoptively transferred with a CD4+ Th1 line secreting IFN-γ are protected (Pope et al., 1996). Unlike IFN-γ, anti-TNF-α treatment of mice infected with JHMV did not show any alteration in encephalitis or demyelination (Stohlman et al., 1995b). In this report, the presence of anti-viral mechanisms other then perforin-mediated cytolysis were examined in PKO mice depleted of either CD4+ T cells, IFN-γ or TNF-α.

## 3. MATERIALS AND METHODS

Breeding pairs of perforin +/+ and -/- mice of 129 x C57BL/6 background were kindly provided by Dr. W. Clarke (UCLA). Mice were breed and maintained SPF at University of Southern California vivaria. Groups of seven to eight week old mice were infected intracranially with 100 PFU of the DM strain of JHMV which inducs a uniformly fatal encephalomyelitis. JHMV replication in the CNS was determined by plaque assay as previously described (Stohlman et al., 1986). Data presented are the average of triplicate determinations for groups of three to seven mice. For anti-cytokine mAb treatments, mice were treated with 1 mg of anti-IFN-γ (XMG1.2) or anti-TNF-α (XT22.11) at -2, 0, +2 days post-infection (p.i.). To deplete CD4+ T cells, mice were treated with 200 ug of anti-CD4+ (GK1.5) mAb using the same time course. Control mice experiments were treated with isotype-matched rat-anti-galactosidase mAb (GL113). CNS penetration of these mAb's were confirmed using goat-anti-rat antibodies conjugated with peroxidase and visualized with AEC chromagen (Stohlman et al., 1995b). Flow cytometry analysis of splenocytes from anti-CD4 treated mice showed > 98 % depletion (data not shown).

## 4. RESULTS

It has been suggested that CD4+ T cells are involved in the clearance of JHMV from the CNS by providing help to CTL since depletion of CD4+ T cells prevented JHMV

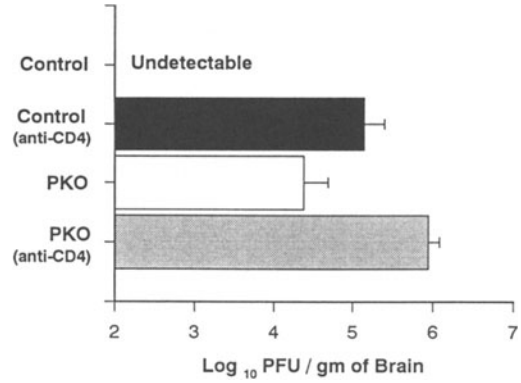


**Figure 1.** Depletion of $CD4^+$ T cells results in decreased viral clearance. Perforin wild-type (control) and perforin-deficient mice (PKO) were either treated with isotype-matched anti-β-galactosidase mAb or with anti-$CD4^+$ mAb and CNS virus titer assayed at 10 days post-infection.

clearance from the CNS (Williamson and Stohlman, 1990). However, viral clearance data from PKO mice indicated that, in addition to perforin-mediated anti-viral mechanism, CD4+ may participate directly in viral clearance (Lin, Stohlman, and Hinton, 1997). To determine whether CD4+ T cells contribute to viral clearance in the absence of perforin, viral replication in CD4 depleted PKO and control mice was compared. Figure 1 shows that, in agreement with previous experiments (Williamson and Stohlman, 1990), anti-CD4 treatment significantly ($P < 0.05$) increased virus titer in the control mice from undetectable to over 4 $\log_{10}$ PFU/gm at day 10 p.i.. Interestingly, depletion of CD4+ T cells from the PKO mice also resulted in a further increase of 1 $\log_{10}$ of infectious virus as compared to the isotype-treated control PKO mice ($P < 0.05$). These data suggest that in addition to its role in providing help for CTL, CD4+ T cell may contribute to viral clearance either directly or via secretion of anti-viral cytokines.

TNF-α has antiviral activity and can synergize with interferons in the induction of resistance to both RNA and DNA virus in diverse cell types (Wong and Goeddel, 1986). Since both CTL and CD4+ T helper cells secret TNF-α, the contribution of TNF-α to viral clearance in the absence of perforin-mediated cytolysis was addressed by examining JHMV clearance in control and PKO mice treated with anti-TNF-α mAb. In agreement with previous experiments, virus titers were undetectable in moribund control mice at day 10 p.i. Anti-TNF-α mAb did not inhibit viral clearance nor improve clinical disease (data not shown). Similarly, inhibition of TNF-α in JHMV infected PKO mice resulted in only a slight increase in virus titer over the untreated PKO mice. The ability of transferred mAb to cross the blood brain barrier was confirmed by the diffuse presence of rat immunoglobulin within the CNS (Figure 2). These data support previous data by showing that TNF-α does not contribute to JHMV clearance from the CNS even in the absence of perforin.

IFN-γ is secreted by CD8+ and CD4+ T cells and has been suggested to contribute to viral clearance. Although NK cells are thought to mediate anti-viral response via secretion of IFN-γ, the kinetics of IFN-γ mRNA expression in the CNS correlates with the infiltration of T lymphocytes into the CNS (Parra et al., in press). In addition, nude mice with intact NK response were unable to clear JHMV from the CNS (Houtman and Fleming, 1996a). To determine the potential anti-viral effect of IFN-γ, virus replication was compared in PKO and control mice were treated with anti-IFN-γ mAb (Figure 3). Similar to mice depleted of CD4+ T cells, treatment with anti-IFN-γ resulted in a significant ($P < 0.05$) delay in viral clearance in control mice. Anti-IFN-γ treatment of PKO mice also re-

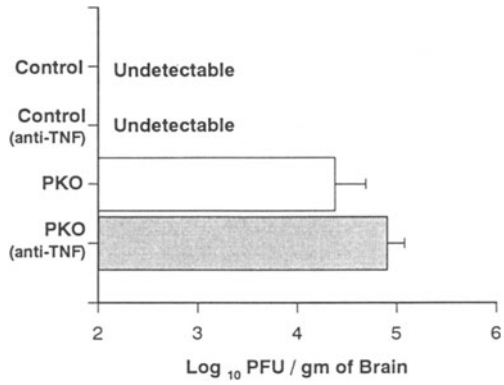


**Figure 2.** Inhibition of TNF-α do not inhibit viral clearance. Perforin wild-type (control) and perforin-deficient mice (PKO) were either treated with isotype-matched anti-β-galactosidase mAb or with anti-TNF-α mAb and CNS virus titer assayed at 10 days post-infection.

sulted in a significant ($p < 0.05$) 1 log increase in virus titer over the isotype-treated PKO mice. These data suggest that IFN-γ can provide anti-JHMV function in addition to perforin-mediated cytolysis.

## 5. DISCUSSION

Unlike many viral infections where a single anti-viral mechanism is sufficient for protection and clearance of virus, JHMV infection of the CNS had been shown to involve participation of many types of immune effector mechanisms (Kyuwa and Stohlman, 1990). This report attempts to further define the mechanisms involved in JHMV clearance from the CNS. The data presented are in agreement with previously published reports on the effect of anti-CD4, anti-IFN-γ and anti-TNF-α treatment in JHMV pathogenesis (Smith et al., 1991; Stohlman et al., 1995b; Williamson and Stohlman, 1990). Depletion of CD4+ T cells from mice resulted in significantly decreased viral clearance in the treated control mice over the untreated control mice while a smaller, but significant decrease in clearance was observed in the treated PKO mice over the untreated PKO mice. This data

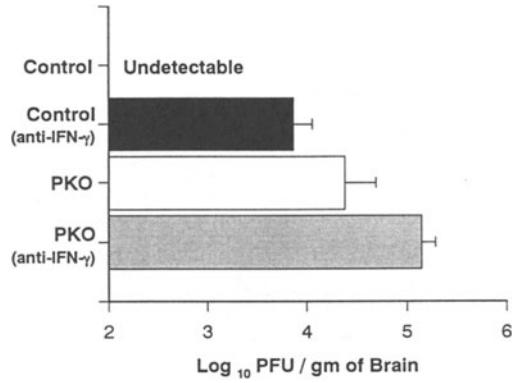


**Figure 3.** Anti-IFN-γ treatment increased JHMV replication in CNS. Perforin wild-type (control) and perforin-deficient mice (PKO) were either treated with isotype-matched anti-β-galactosidase mAb or with anti-IFN-γ mAb and CNS virus titer assayed at 10 days post-infection.

confirms the importance of CD4+ T cells in JHMV clearance from the CNS. Moreover, reduced virus clearance in the CD4-depleted PKO group support the hypothesis that CD4+ T cells have direct anti-viral roles in addition to providing help to CD8+ T cells. However, it remains unclear whether depletion of CD4+ T cells alters CTL development, trafficking into the CNS, cytokine secretion, or survival in the CNS.

CD4+ T cells could help eliminate virus from the CNS through direct lysis of infected MHC class II positive CNS cells via perforin, Fas/FasL, or TNF-mediated cytolysis. These data analyzing CD4-depleted PKO mice suggest the possibility of Fas/FasL or TNF-mediated involvement in viral clearance. Fas/FasL-mediated cytolysis has been implicated in the down-regulation of immune responses but has not been shown to mediate viral clearance (Hahn et al., 1995; Kagi et al., 1995; Scott, Grdina, and Shi, 1996; Van Parijs, Ibraghimov, and Abbas, 1996). TNF-α is a pleuripotent pro-inflammtory cytokine that has anti-viral activity and is expressed by many cell types, including CD4+ T cells, CD8+ T cells, microglia, astrocytes, and macrophages. In the absence of perforin, anti-TNF-α mAb treatment did not influence viral clearance or clinical disease, consistent with previous reports showing no effect of TNF-α inhibition on JHMV-induced demyelination or encephalitis (Stohlman et al., 1995b). TNF-α mRNA expression are associated with acute encephalitis and it's level increased in the CNS of JHMV-DM infected mice until death when no virus could be isolated (Parra et al., in press). Further, TNF-α mRNA is present in astrocytes of persistently infected mice (Sun et al., 1995). Although, TNF-α mRNA is not translated in JHMV-infected cells, it may be secreted by adjacent non-infected reactive CNS cells (Stohlman et al., 1995b). Together, these data suggest TNF-α has no direct role in the pathogenesis of JHMV-induced CNS disease and that its detection in the CNS may be a result of an on-going reactive gliotic process during viral infection.

Like TNF-α, IFN-γ is a pleuripotent cytokine that possesses direct anti-viral activity; however, the anti-viral activity of this cytokine is confounded by it's participation in the regulation of cell-mediated immunity. This may explain the discrepency in the amount of virus titer increases observed between the anti-IFN-γ treated control and PKO mice. Nonetheless, a direct but minor anti-viral role for IFN-γ is suggested in the PKO mice. These results are consistent with the in vitro anti-JHMV activity of IFN-α (Zhang et al., in press). As well, they are in agreement with the delayed clearance observed in IFN-γ deficient mice infected with a OBLV-60 strain of JHMV (Lane, Paoletti, and Buchmeier, 1997) and the kinetics of IFN-γ mRNA expression during infection (Parra et al., in press; Pearce et al., 1994). Further experiments are needed for clarification of IFN-γ's ability to influence cellular immunity.

In summary, the present study showed that CD4+ T cell are important for viral clearance either by a direct anti-viral effector mechanism or by secretion of IFN-γ. Although not directly demonstrated, these data indicate that CD4+ T cells may in fact utilize each of these mechanisms to help eliminate JHMV from the CNS.

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