CD8 T Cell Mediated Immunity to Neurotropic MHV Infection

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1. INTRODUCTION

Neurological disease induced by neurotropic coronaviruses has received considerable attention not only as a model for virus induced demyelination, but also of immune regulation and viral persistence within the central nervous system (CNS). The neurotropic JHM strain (JHMV) of mouse hepatitis virus (MHV) produces an acute CNS infection characterized by encephalomyelitis and demyelination. Several strains and variants have been derived from the lethal, parental JHMV to better study the demyelinating process as a subacute disease resembling the human CNS disease, Multiple Sclerosis. A concise overview of historical aspects, derivation of neurotropic coronavirus strains and variants, together with types of neurological disease and immune responses associated with these viruses is provided in the previous volume of this series (Perlman, 1998) and in several other recent reviews (Houtman and Fleming 1996, Lane and Buchmeier 1997, Stohlman *et al* 1999). Advances in molecular immunology techniques, combined with the use of selective exclusion of immune components, either using antibody (Ab) mediated depletion or genetic disruption, has resulted in significant progress in dissecting the interactions between virus, CNS cells, and immune responses and their role in demyelination. This chapter therefore focuses on advances of the past 3-4 years in elucidating interactions between CNS infection and immune responses leading to persistence. As MHV variants

The Nidoviruses (Coronaviruses and Arteriviruses).
Edited by Ehud Lavi *et al.*, Kluwer Academic/Plenum Publishers, 2001.

differ vastly in tropism, spread, clinical symptoms and mortality, this review focuses largely on a murine model of infection using the neuroattenuated 2.2v-1 variant of JHMV (Fleming *et al* 1986). This mAb selected variant establishes a persistent infection associated with extensive, subacute, ongoing CNS demyelination in the absence of infectious virus. During acute infection this virus replicates primarily in microglia, astrocytes and oligodendrocytes. Although the immune response of immunocompetent hosts generally eliminates infectious virus within 14 days post infection (p.i.), survivors remain persistently infected as evidenced by the detection of viral RNA (vRNA). Despite suffering from varying degrees of encephalitis and paralysis during acute infection, mice generally recover from clinical symptoms and remain asymptomatic during viral CNS persistence.

2. IMMUNITY DURING ACUTE INFECTION

2.1 CD8 T cell activation and recruitment into the CNS

Many acute infections are controlled by $CD8^+$ T cells, which are primed in the draining lymph nodes and home to the site of infection, where they execute effector functions (Ahmed and Gray 1996, Doherty *et al* 1994). However, the CNS provides a suitable environment for persistence due to the absence of classical lymphatic drainage, the presence of the blood brain barrier, low levels of MHC expression and relative resistance of resident CNS cells to apoptosis, factors all contributing to inefficient immunity (Cserr and Knopf 1997, Lipton and Gildon 1997). Acute JHMV infection of the CNS is nevertheless associated with potent T cell responses, which are critical for the clearance of infectious virus and survival of the host (Stohlman *et al* 1995, Williamson and Stohlman 1990). The strength of the $CD8^+$ T cell response is documented by class I restricted virus specific cytolytic activity of mononuclear cells isolated from the acutely infected CNS (Stohlman *et al* 1993, Castro and Perlman 1996). By contrast, cytolytic activity in peripheral lymphoid organs can only be found following *in vitro* stimulation with antigen (Castro and Perlman 1996), suggesting that most virus specific T cells are either recruited to the CNS or that effector function is predominantly acquired in the CNS due to high levels of viral antigen. The development of class I tetramer reagents has provided a powerful technology to phenotypically monitor virus specific $CD8^+$ T cell expansion and trafficking independent of functional differentiation (Flynn *et al* 1998, Murali-Krishna *et al* 1998). This technology takes advantage of tetramer formation via avidin binding to specifically biotinylated class I chains

refolded with peptide and β_2m to detect antigen specific T cell receptors (TCR) on $CD8^+$ T cells using flow cytometric analysis (Altman *et al* 1996). Analysis of CNS infiltrating cells during acute JHMV infection using class I tetramers comprising the immunodominant epitopes from the nucleocapsid (N) and spike (S) proteins, revealed that the $CD8^+$ T cell compartment comprised up to 50-60% virus specific cells, independent of responder mouse strain (Bergmann *et al* 1999, Marten *et al* 2000a, Pewe *et al* 1999). However, tetramer staining in $CD8^+$ splenocytes and lymph nodes was at most 2-5% (Bergmann *et al* 1999). Cytolytic function in the CNS thus correlated with higher frequencies of virus specific T cells within the CNS. This is supported by ~20 fold higher frequencies of CNS mononuclear cells secreting IFN-γ in response to peptide stimulation in ELISPOT assays compared to splenocytes or lymph node cells (Bergmann, unpublished). These results support $CD8^+$ T cells as vital contributors in mediating viral clearance (Stohlman *et al* 1995). The inability to provide sterile immunity within the CNS can therefore not be attributed to poor induction, or inefficient recruitment of effector $CD8^+$ T cells. Persistence may rather be due to resistance of some infected CNS cell types to $CD8^+$ T cell effector functions.

2.2 Mechanisms of virus clearance

As demonstrated by adoptive transfer studies, virus specific $CD8^+$ T cells limit replication in astrocytes and microglia, and to a lesser extent in oligodendrocytes; however, they do not inhibit persistent infection (Stohlman *et al* 1995). Infection of perforin deficient ($P^{-/-}$) and IFN-γ deficient (IFN-$\gamma^{-/-}$) mice with 2.2v-1 revealed that different effector mechanism are utilized to control replication in a cell type specific manner. Whereas $CD8^+$ T cells eliminate virus from microglia and astrocytes via a perforin dependent mechanism (Lin *et al* 1997), IFN-γ is critical for controlling replication in oligodendroglia (Parra *et al* 1999). The lack of either component alone resulted in delayed viral clearance; this was more pronounced in IFN-$\gamma^{-/-}$ mice, although perforin-dependent cytotoxicity was not compromised (Parra *et al* 1999). IFN-$\gamma^{-/-}$ mice infected with 2.2v-1 also exhibited dramatically increased clinical disease and mortality compared to control mice. Similarly, 2.2v-1 infected $P^{-/-}$ mice recovered poorly from acute disease compared to control mice and developed a progressive paralytic disease despite initial improvement. More extensive demyelination in $P^{-/-}$ mice suggested that demyelination is independent of perforin mediated mechanisms (Lin *et al* 1997). Overall the pathogenesis in $P^{-/-}$ and IFN-$\gamma^{-/-}$ mice indicated that IFN-γ is more critical for virus clearance and disease recovery than perforin-mediated cytotoxicity. Interestingly, IFN-γ is

dispensable for virus clearance from neurons as shown by elimination of the neuronotropic OBLV-60 JHMV variant from the CNS of IFN-$\gamma^{-/-}$ mice (Lane *et al* 1997). Nevertheless, the primary source and mechanism of protective IFN-γ is yet to be identified, as NK, $CD4^+$ and $CD8^+$ T cells are all found in the CNS during acute infection (Williamson *et al* 1991). The relative expression of class I and class II MHC molecules on distinct CNS cell types is also likely to determine differential susceptibility to T cell produced IFN-γ at distinct phases of the infection (Sedgwick and Hickey 1997).

Efforts to define a role of Fas-FasL interactions as a perforin independent lytic mechanism contributing to viral clearance or immunopathology were unsuccessful using Fas-deficient (lpr) mice (Parra *et al* 2000). Infection of lpr mice also indicated that Fas-mediated cytotoxicity did not contribute to virus induced inflammation, mononuclear cell infiltration, extent of demyelination or frequencies of apoptotic cells. However, uncontrolled virus replication in the simultaneous absence of perforin and Fas uncovered the potential use of alternate cytolytic pathways in viral clearance (Parra *et al* 2000). These results, obtained using chimeric mice generated by reconstitution of lethally irradiated wt or lpr mice with splenocytes from $P^{+/+}$ or $P^{-/-}$ mice, suggested the possibility of compensation for perforin via Fas under conditions in which perforin mediated cytotoxicity is diminished, i.e. after the majority of infectious virus is cleared.

In addition to $CD8^+$ T cells, $CD4^+$ T cells exert crucial anti-viral activity. Adoptive transfer and selective depletion of $CD4^+$ T cells indicate that the mechanisms by which $CD4^+$ T cells provide protection are also multifactorial. Although $CD4^+$ T cells were not required for induction of $CD8^+$ T cell responses following infection with lethal JHMV, they enhanced $CD8^+$ T cell expansion and/or activation in splenocytes (Stohlman *et al* 1998). More importantly, although the absence of $CD4^+$ T cells did not inhibit entry of $CD8^+$ T cells into the CNS parenchyma, their accumulation was reduced, coincident with an increase in apoptotic cells. Thus, despite the paucity of $CD4^+$ T cells to enter the CNS parenchyma during acute JHMV infection, they contribute to the maintenance of $CD8^+$ T cell function and viability in the CNS (Stohlman *et al* 1998). However, $CD4^+$ T cells are found within the CNS parenchyma following 2.2v-1 infection (Lin et al 1997). Although there is no evidence for $CD4^+$ T cell mediated cytotoxicity in the CNS, their ability to modify viral persistence via secretion of chemokines or cytokines, especially IFN-γ, is unknown. The notion that soluble mediators may contribute directly to protection is supported by adoptive transfer of $CD4^+$ T cells into infected recipients (Stohlman *et al* 1999). On the other hand $CD4^+$ T cells may be detrimental by accelerating CNS inflammation and enhancing demyelination (Lane *et al* 1997).

3. IMMUNITY DURING PERSISTENT INFECTION

3.1 Retention of T cells during persistence

$CD4^+$ and $CD8^+$ T cell infiltration into the CNS both peak between days 8 and 10 p.i. and gradually decline thereafter. However, even after infectious virus is eliminated, a high percentage of T cells remains in the CNS (Bergmann *et al* 1999, Marten *et al* 2000a,b). This observation, together with T cell retention in a model of neurotropic influenza virus infection (Hawke *et al* 1998) suggested that the CNS environment may retain T cells for prolonged periods even after initial viral clearance. Analysis of $CD8^+$ T cell functional activity revealed that cytolytic function is rapidly downregulated even before infectious JHMV is completely cleared. Furthermore, direct *ex vivo* cytotoxicity remained undetectable in persistently infected mice (Bergmann *et al* 1999, Marten *et al* 2000a). In contrast to impaired antigen responsiveness at the cytolytic level, IFN-γ secretion was maintained, indicating differential regulation of distinct effector functions (Bergmann *et al* 1999).

Interestingly, the relative percentage of virus specific cells within the $CD8^+$ population, as determined by tetramer staining, remains constant throughout infection (Bergmann *et al* 1999, Marten *et al* 2000a, b). This apparent lack of enrichment of virus specific cells indicated a stable steady state among T cell populations residing in the CNS. The specificity of non-tetramer staining $CD8^+$ T cells remains to be resolved. As T cell retention in the CNS is thought to require cognate antigen recognition (Hickey *et al* 1997), T cells specific for as yet unidentified JHMV epitopes, possibly located within the nonstructural proteins, cannot be ruled out. The potential existence of autoantigen-specific T cells is less likely as persistently infected mice show no symptoms of autoimmune disease. However, infections are generally carried out in mouse strains relatively resistant to CNS autoimmune disease. Host genetic factors may therefore contribute to the suppression of T cell reactivity or overt clinical symptoms associated with autoimmune disease.

T cell retention despite barely detectable vRNA levels, in addition to persistence of T cells in the influenza model, suggested that residual antigen is redundant for T cell maintenance in the CNS. This is indirectly supported by the abundant presence of D^b-S510 tetramer binding $CD8^+$ T cells in the CNS of infected mice, in which the wild type (wt) epitope encoding vRNA sequence is no longer detectable due to the exclusive emergence of CTL escape mutants (Pewe *et al* 1999). T cells may thus be trapped in the CNS or recruited by an antigen independent mechanism. The requirement of viral

persistence for retention of T cells was addressed by taking advantage of a nonpathogenic 2.2v-1 variant designated 2.2/7.2v-2 (Fleming *et al* 1986). Despite a deletion in the hypervariable region comprising the immunodominant S510 epitope, infection was associated with similar replication patterns and peak virus titers compared to parental 2.2v-1 in both C57BL/6 (H-2^b) and BALB/c (H-2^d) mice (Fleming *et al* 1986, Marten *et al* 2000a). In contrast to subacute paralysis and extensive demyelination typical of 2.2v-1 infected mice, 2.2/7.2v-2 infection was clinically silent and caused little to no demyelination. Nevertheless, phenotypic and functional analysis of CNS infiltrating cells demonstrated that this infection induced potent $CD8^+$ T cell responses similar to those induced by the pathogenic variant (Marten *et al* 2000a, b). Furthermore, although spread of 2.2/7.2v-2 to the spinal cord was delayed, transient replication was associated with an influx of T cells similar in magnitude to 2.2v-1 infected mice. At 8-9 weeks p.i. vRNA was no longer detectable in 2.2/7.2v-2 infected mice, indicating complete resolution of infection, whereas vRNA was still evident in spinal cords from 2.2v-1 infected mice (Marten *et al* 2000b). Despite transient infiltration during the acute phase, the lack of vRNA coincided with the absence of both $CD8^+$ and $CD4^+$ T cells. T cell retention is thus tightly linked to the presence of vRNA and presumably antigen expression, indicating that viral persistence is a driving force for T cell maintenance within the CNS. Whether chronic T cell activation is a result of direct MHC-TCR dependent or cytokine/chemokine mediated bystander mechanisms remains to be elucidated. Potential candidates mediating ongoing recruitment may be the chemokines CRG-2 and RANTES (Lane *et al* 1998).

The functional role of $CD8^+$ T cells during persistence is unclear; however, several lines of evidence support dynamic T cell populations associated with persistence: 1) Unlike memory $CD8^+$ T cells characterized by the $CD44^{hi}$, $CD62L^{lo}$, $CD69^-$ activation/memory phenotype, the majority of $CD8^+$ T cells in the persistently infected CNS express the very early activation marker CD69, independent of specificity (Bergmann *et al* 1999). This marker is typically only transiently upregulated early during T cell activation; however, CD69 expression on CNS derived $CD8^+$ T cells peaks coincident with clearance of infectious virus and remains upregulated, indicating ongoing stimulation. 2) In H-2^{bxd} mice responding to both the immunodominant N and S epitopes, ongoing chronic activation was evident by a switch in immunodominance from N to S specific $CD8^+$ T cells during the course of infection (Bergmann *et al* 1999); 3) $CD8^+$ T cells isolated from persistently infected mice exhibited highly focused reactivity to the wt N epitope sequence, compared to broader specificity characteristic of the acute $CD8^+$ populations (Marten *et al* 1999). While a broad polyclonal TcR specificity can thus potentially accommodate mutations arising during acute

replication, persistence is associated with recruitment or survival of T cell subsets with exquisite specificity for the wt epitope. This may serve to limit the potential for autoantigen crossreactive $CD8^+$ T cells.

The selective depletion of immune functions combined with highly sensitive methods to monitor individual T cell populations have thus been valuable tools to elucidate the mechanisms by which both $CD8^+$ and $CD4^+$ T cells provide protection during acute JHMV infection. However, their contribution in preventing viral recrudescence during persistence is an open question. Recent results from the analysis of JHMV infection in mice homozygous for disruption of the Ig μ gene ($IgM^{-/-}$) and thus genetically deficient in B cells and Ab, clearly demonstrated increased mortality associated with recrudescence of infectious virus (Lin *et al* 1999). Although replication was initially controlled by cell mediated immunity in the absence of Ab, T cells alone did not suffice to effectively clear infectious virus to below detection resulting in subsequent uncontrolled replication. Passive transfer of anti-JHMV Ab following initial clearance prevented reactivation of infectious virus within the CNS of $IgM^{-/-}$ mice. However, as these mice lack B cells and Ab, it is unclear if B cells themselves play an additional Ab independent role in suppressing virus, e.g. by supporting T cell activation peripherally or maintaining them in the CNS. Defects in T cell function are evident during LCMV infection of $IgM^{-/-}$ mice (Homann *et al* 1998). Nevertheless, these data suggest that humoral immunity plays no role in controlling virus during acute infection but is crucial in establishing and maintaining CNS viral persistence.

4. CONCLUSIONS

Virus infections of the CNS frequently result in persistence associated with subclinical disease and/or disease after a long latent period. To guarantee survival of both the host and the virus, an equilibrium must be established between virus replication, spread and the immune system to minimize virus- or immune-induced pathology. Control of JHMV infection in the CNS involves a well orchestrated dynamic immune response, in which distinct players take on critical roles at various time points. Whereas cellular effector mechanisms control acute infection in a cell type specific manner, the humoral immune response plays a critical role in preventing viral recrudescence. Although distinct molecular effector mechanisms have been defined, a number of important issues relative to the regulation of immunopathology and mechanisms of persistence within the CNS require further investigation: Contribution of persisting T cell subsets to the demyelinating process, factors regulating T cell recruitment and effector

function, direct antiviral role of $CD4^+$ T cells, and finally mechanisms via which antibody or B cells themselves suppress virus replication.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grants NS18146 and AI33314.

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