Chapter C4

THE ROLE OF T CELLS IN CORONA-VIRUS-INDUCED DEMYELINATION

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Abstract: Mice infected with neurotropic strains of coronavirus develop acute encephalomyelitis and eliminate infectious virus. However, control of acute infection is incomplete resulting in persistence of viral RNA in the central nervous system (CNS) associated with ongoing primary demyelination. A high prevalence of virus specific CD8 and CD4 T cells within the CNS correlates with *ex vivo* cytolytic activity and IFN-γ secretion, which are both required for virus reduction during the acute infection. Although most infected cell types are susceptible to perforin mediated clearance, IFN-γ is required for controlling infection of oligodendrocytes. Furthermore, by enhancing class I expression and inducing class II expression within resident CNS cells IFN-γ optimizes T cell receptor dependent functions. In addition to its direct anti viral activity, these multifactorial effects make IFN-γ more essential than perforin for viral control. CD4 T cells enhance CD8 T cell expansion, survival and effectiveness. Although both CD8 and CD4 T cells are retained within the CNS during persistence, they cannot control viral recrudescence in the absence of humoral immunity. Demyelination can be mediated by either CD8 or CD4 T cells; however, although a variety of effector molecules have been excluded, a dominant common denominator remains elusive. Thus concerted efforts to control infection coincide with a variety of potential mechanisms causing chronic demyelinating disease.

Key words: CNS, demyelination, IFN-γ, perforin, T cells, mouse hepatitis virus

INTRODUCTION

Neurotropic strains of mouse hepatitis virus (MHV) produce an acute demyelinating encephalomyelitis in rodents (reviewed in 1-3).

Following infection with the JHM strain of MHV (JHMV) survivors have no detectable infectious virus by 2 weeks p.i., but viral antigen (Ag) and RNA persist within the CNS, predominantly in spinal cords up to 2 years p.i. Although persistently infected mice exhibit few clinical abnormalities, histological examination shows ongoing primary CNS demyelination consistent with the pathological changes characterizing the human demyelinating disease multiple sclerosis. Experimental CNS infection by MHV thus provides an excellent model to study the role of inflammatory cells during ongoing demyelination associated with persisting virus.

The majority of research focusing on the role of T cells in MHV pathogenesis has used the JHMV 2.2v-1 monoclonal antibody (Ab) neutralization escape mutant (1-3). This variant has several advantages in studying virus-induced demyelination when compared to parental JHMV and other variants: i) Most mice survive the infection, with demyelination observed in all survivors. ii) The cellular tropism of most JHMV strains for microglia, astrocytes and oligodendroglia is retained; neurons are only rarely infected. iii) Hepatitis, which is transient following infection with the MHV-A59 strain and may confound analysis of CNS specific immune responses, is rare following i.c. infection. Much of the data discussed within this chapter pertain to this variant.

Several independent studies demonstrate that both CD4 T cells and CD8 T cells are required to control acute virus replication within the CNS, thereby protecting from an otherwise lethal infection (1-3). However, T cell-mediated protection against JHMV is generally provided at the cost of immune-mediated demyelination (1-7). Demyelination also occurs in mice with T cell deficiencies following infection with either JHMV (5) or MHV-A59 (8). Nevertheless, mice defective in B cells or anti-virus antibody secretion all develop severe demyelination (9,10), negating a prominent role of humoral immune responses. Irrespective of the controversies regarding mediators and perpetuators of demyelination, the final effector cells are believed to be activated macrophages/microglia (1,2,6,11). This chapter focuses on mechanisms of viral clearance and how activated T cells may contribute to immune pathology in JHMV-infected mice with an emphasis on the complexity of interactions involved.

CONCERTED ANTI-VIRAL EFFORTS OF T CELLS

Although neutrophils, macrophages, NK cells and B cells constitute early infiltrates, T cells, most prominently the CD8 subset, provide the most

critical anti-viral functions (3). A protective role for T cells in acute JHMV pathogenesis was demonstrated by the failure of both SCID and nude mice, genetically deficient in T cells, to clear JHMV resulting in lethality within 12-16 days (5,6). Selective depletion of either CD4 or CD8 T cells both diminished viral clearance, implicating a contribution of both T cell subsets (1-3). In addition, adoptive transfer of either virus specific CD8 T cells or CD4 T cells protected mice from acute encephalomyelitis (1-3). However, whereas CD8 T cells afforded protection by clearing infectious virus, distinct CD4 T cell donor populations varied in their ability to mediate direct anti viral function (1). The precise role that T cells have in viral clearance and MHV-induced demyelination is an area of active investigation, as discussed below.

T Cell Priming, CNS Recruitment, and Effector Function

Mapping of T cell epitopes has greatly facilitated phenotypic and functional analysis of both CD8 and CD4 T cell expansion and recruitment into the CNS (3; see Chapter C6). Virus specific CD8 T cells are detectable in the CNS by day 6 p.i. and accumulate to high frequencies within the CNS by day 8 p.i. as demonstrated using MHC class I/peptide tetramers, intracellular IFN-γ staining, as well as ELISPOT technology (3,12-16). During acute infection, up to 40% of CD8 T cells in the CNS respond to a single immunodominant epitope in both BALB/c and C57BL/6 mice (3,12-16). By contrast, the frequency of virus specific CD8 T cells in the periphery is ~10-20 fold lower (3,12,13). Detection of virus specific CD8 T cells in cervical lymph nodes (CLN) and spleen 2 days prior to the CNS supports priming, expansion, and differentiation in the periphery, rather than the CNS (17). The high prevalence of virus specific T cells in the CNS correlates with virus specific *ex vivo* cytolysis by CNS derived cells, a property not detected in unfractionated spleen or CLN cells (3). Although these observations suggested acquisition of effector function within the CNS, virus specific cytolytic effectors can be demonstrated following CD8 T cell enrichment from splenocytes at day 7 p.i. (17). The rapid accumulation of armed effector CTL results in a potent regional response which efficiently reduces viral titers, but does not prevent persistence. Furthermore, despite protection and

enhanced viral clearance achieved by transfer of JHMV specific CD8 T cells, virus could still be detected in oligodendrocytes (18), a major target of replication for the JHMV 2.2v-1 variant (1-3,18-20).

Distinct Anti-Viral Functions of IFN-γ and Perforin

The suggestion that oligodendrocytes are more resistant to CD8 T cell mediated anti-viral function compared to other glial cell types prompted investigation of T cell effector functions utilized for protection. Anti-microbial T cell mediators include perforin, granzymes, and Fas-FasL, as well as soluble agents such as IFN-γ, TNF and selected chemokines (21;22). A unique aspect of JHMV pathogenesis is that the immunological mechanisms clearing infectious virus are cell type specific (19,20). Studies in genetically deficient mice revealed that replication in astrocytes and microglia is controlled via perforin mediated cytolysis, but not Fas/FasL interactions (19,23). Furthermore, abrogation of TNF-α by mAb treatment did not affect viral clearance (24). By contrast, IFN-γ controls replication in oligodendrocytes (20). Both cytolytic and IFN-γ effector mechanisms peak between days 8-10 p.i., coincident with maximal T cell infiltration (13,14,25). The inability to achieve sterile immunity, despite concerted action of these anti-viral mediators, suggested downregulatory mechanisms or viral evasion from T cell function. Although CTL escape variants play a prominent role in disease progression of weanling mice protected by neutralizing Ab, they appear to play no role in immune competent adult mice (3; see Chapter C6). However, the number of CNS T cells gradually decrease after day 10 p.i., despite the continued presence of Ag positive cells and viral RNA (13-15). Furthermore, Ag specific cytolytic activity at a single cell level rapidly declines by day 14 p.i. and remains absent thereafter (13). Loss of cytolytic activity is independent of either demyelination (14) or viral recrudescence in Ab deficient mice (9,12). The apparent inability to enhance T cell recruitment/function even during virus recrudescence (9,12) suggests restricted access or Ag driven apoptosis; however, there is no evidence to support increased apoptosis by high Ag levels *in vivo* (26). Whether CD8 T cells are downregulated by release of inhibitory factors within the local CNS environment or by the presence of suppressor cells is unknown.

Contrasting the loss of cytolysis, mRNA and ELISPOT analysis suggests that IFN-γ secretion in the CNS appears to continue after clearance of infectious virus (13,25). Furthermore, unlike the permanent loss of perforin mediated cytolysis, IFN-γ mRNA levels increase during JHMV reactivation in the CNS of B cell deficient μMT mice (26). The contradictory finding of reduced numbers of IFN-γ secreting cells in μMT

mice (12) may reside in potential apoptosis of μMT derived CD8 T cells upon *in vitro* stimulation, as apoptosis is not increased *in vivo* (26). Irrespective of the extent of IFN-γ secretion, the balance between loss of cytolytic activity but continued cytokine secretion appears to reflect an attempt to control infection while reducing CNS immunopathology.

Overall the results suggest that IFN-γ is more vital than perforin in the hierarchy of immune effectors controlling JHMV infection and mortality. The nonredundant roles for both IFN-γ and perforin are more clearly evident by pathogenesis studies in mice deficient for both perforin and IFN-γ (27). These mice exhibit ongoing viral replication in all glial cell types coincident with a high mortality rate by day 14 p.i. Adoptive transfers demonstrate that wt memory CD8 T cells were most efficient in reducing virus, while perforin deficient T cells were slightly less efficient. IFN-γ deficient T cells had no affect on reducing viral titers. The inefficiency of IFN-γ deficient donor CD8 T cells to provide protection coincided with diminished class I upregulation and an absence of class II expression on resident microglia. IFN-γ mediated upregulation of class I Ag processing expression (28) is especially crucial within the CNS due to the paucity of MHC expression in the healthy, quiescent CNS (29,30). As both IFN-γ and TNF-α release as well as perforin mediated killing are strictly dependent on MHC–TCR contact (31), CD8 T cells initially recruited by chemokines (32) are likely to be less effective in exerting effector function *in vivo* in an IFN-γ deficient environment. In addition to its direct anti-viral role, IFN-γ thus enhances both CD8 and CD4 T cell function by upregulating MHC class I and class II presentation. The correlation between Ag recognition and elicitation of effector function has clear consequences for the efficiency of distinct effector functions *in vivo*. Whereas release of IFN-γ can be triggered by a subset of antigen presenting cells and act on a distal, non MHC expressing cell types, perforin mediated cytolysis can only be exerted on cells presenting viral Ag. The multifactorial effects of IFN-γ within the CNS thus shed light on the higher efficacy of IFN-γ compared to perforin in anti JHMV effector mechanisms.

Auxilliary and Direct Anti-Viral Functions of CD4 T Cells

The precise role of CD4 T cells as direct anti-viral mediators are less well understood, especially their contribution to anti-viral IFN-γ secretion. The majority of CD4 T cells accumulating within the CNS are virus specific (33), similar to CD8 T cells. Depletion of CD4 T cells prevents JHMV clearance from the CNS (1-3). Although transferred JHMV specific CD4 T

cells protect from acute disease, the mechanisms of protection may be unrelated to IFN-γ mediated control (1). CD4 T cells also provide crucial accessory function by enhancing expansion of virus specific CD8 T cells and maintaining CD8 T cell viability within the infected CNS (12,34). Although depletion of CD4 T cells does not prevent CD8 T cells from entering the CNS parenchyma, it compromises their viability, as indicated by increased apoptosis (34); the mechanisms involved remain obscure. Predominant localization of CD4 T cells in the perivascular and subarachnoid spaces during JHMV infection is consistent with recent data demonstrating that Fas/FasL interactions, a mechanism of CD4 T cell mediated cytolysis, does not contribute to JHMV clearance, encephalomyelitis or demyelination (23,34).

T CELL FUNCTION DURING PERSISTENCE

A hallmark of JHMV persistence and ongoing demyelination is significant CNS retention of virus specific CD8 and CD4 T cells (12-16). Persisting T cells appear tightly linked to persisting virus as a source for chronic activation, as shown by the disappearance of T cells from the CNS of mice infected with a JHMV variant that does not persist (15). However, the percentages of virus specific cells within the CD8 T cell compartment remain remarkably similar throughout infection, negating selective enrichment or loss of cells with virus specificity (13-16). Despite an apparently dormant T cell state, several observations support an active turnover process: i) Persisting CD8 T cells exhibit restricted fine-specificity (35); ii) A reversed pattern of immunodominance emerges comparing acute and persistently infected CB6F1 (H-2^{dxb}) mice (13); and iii) CD69 expression is sustained during persistence, indicative of chronic CD8 T cell activation (13). Sustained CD69 expression has been observed on persisting, yet activated CD8 T cells in the CNS, lung and liver, following other viral infections (36-38).

Despite an apparent role for continued Ag presentation in T cell survival within the CNS, there is also evidence to support the contrary. CD8 T cells are readily maintained in the CNS following challenge of influenza virus immune mice with a heterologous neurotropic influenza virus (36). Unlike the JHMV model, there was no evidence for persistent infection and these cells retain *ex vivo* cytolytic capacity. The phenotype of these cells is reminiscent of peripheral memory CD8 T cells found in nonlymphoid organs (39), suggesting that T cell retention within the CNS may in part reflect

peripheral memory T cells. However, in the JHMV model ultimate disappearance of all T cells in the absence of JHMV RNA in both brains and spinal cords suggests persisting RNA supports active T cell maintenance by ongoing chemokine release and/or continued Ag presentation. The source and renewal of such persisting T cells is under investigation.

The sufficiency of T cells for protection during MHV persistence has been challenged by the discovery of viral recrudescence in mice deficient in Ab (8,9,26). The necessity of Ab in controlling persistent infection is supported by the accumulation of virus specific Ab secreting cells within the CNS of wt mice (40). T cells may nevertheless contribute to control during the chronic phase, perhaps by secreting IFN-γ and supporting B cell differentiation and/or survival.

T CELLS AS CORRELATES OF DEMYELINATION

T cells control viral replication in several models of viral CNS infection, but are also key mediators of the pathological changes associated with encephalitis and/or demyelination (1-7,41,42). During JHMV infection both CD4 or CD8 T cells alone are capable of inducing demyelination (7). Unlike TMEV induced demyelination, there is little evidence for involvement of an autoimmune CD4 T cell component or epitope spreading. A prominent role for CD4 T cells is however indicated by clinical improvement as well as remyelination of JHMV infected mice treated with anti-IP-10 Ab (32). IP-10 treatment specifically reduced CD4, not CD8 T cells within the CNS, suggesting CD4 T cell dependent pathology in persistently infected mice. Secretion of RANTES leading to increased macrophage recruitment may be one mechanism (32). Mice deficient in perforin, IFN-γ, TNF-α, NOS2 and IL-10 all develop demyelination similar to that in wt mice (19,20,24,43,44), suggesting none of these effector molecules alone dominate the demyelinating process. Recent findings demonstrating that CD4 and CD8 T cells contribute to demyelination via different mechanisms (45,46) further complicate understanding the network of effector molecules involved. Infection of mice deficient in recombination activation gene 1 activity (RAG$^{-/-}$) or SCID mice results in no demyelination. However, adoptive transfer of splenocytes from mice immunized with JHMV resulted in demyelination within 7 days post transfer (5-7). Splenocytes enriched for either CD4 or CD8 T cells mediated demyelination, although no demyelination was observed if both subsets were deleted. Further, reconstitution of infected RAG$^{-/-}$ mice using T cell subsets selectively deficient in T cell effector functions indicate that IFN-γ, but not perforin or TNF-α is crucial for CD8 T cell mediated demyelination (45).

By contrast, the absence of IFN-γ severely enhances demyelination mediated by CD4 T cells (46). Surprisingly, increased demyelination was not necessarily correlated with the amount of activated macrophages/microglia within the white matter. Furthermore, although virus replication was diminished to some extent after adoptive transfer of CD4 or CD8 T cells from wt or immunodeficient donors, only transfer of populations from immunocompetent B6 mice effected virus clearance (7). Contrasting a detrimental role of IFN-γ in demyelination by T cell transfers into infected H-2^b RAG$^{-/-}$ mice, adoptive transfers of memory CD8 T cells into H-2^d SCID mice suggest that both IFN-γ and perforin together propagate the demyelination process, but that CD8 T cell IFN-γ secretion alone does not increase pathology (Parra & Stohlman, unpublished). Finally, demyelination following JHMV infection can also be caused by non virus-specific bystander CD8 T cells, albeit only following activation by cognate Ag (47). Bystander CD4 T cells do not cause demyelination (Haring & Perlman, unpublished). In a recent study demyelination was shown to be γδ T cell-mediated in nude mice (48).

The complex linkages between IFN-γ, MHC class I and II expression, and chemokines have made it extremely challenging to delineate distinct pathways leading to demyelination. For example, impaired MHC and costimulatory molecule expression on resident CNS cells in the absence of IFN-γ are likely to ameliorate perforin and TNF mediated cell damage, but at the cost of increased virus load. Furthermore, macrophages/glia activation may be reduced, in turn affecting chemokine secretion (32). Together the current evidence suggests that JHMV induced demyelination is influenced by the balance between virus replication predominantly in oligodendroglia, distinct immune effector functions mediated by both CD4 and CD8 T cells and their effects on glia/macrophage activation.

CONCLUSIONS

T cell mediated immune responses within the CNS can be both beneficial by clearing infectious agents, and detrimental by destroying tissue, triggering autoimmunity, and activating resident cells. The CNS responds rapidly to coronavirus infection by chemokine mediated recruitment of specific as well as bystander T cells. Recognition of cognate Ag presented by MHC is crucial in triggering Ag-specific T cell function both during virus

and auto-Ag induced inflammation. However, prolonged T cell stimulation by foreign as well as self Ag promotes immune pathology, such as demyelination. The ubiquitous upregulation of class I during inflammation allows both IFN-γ and perforin mediated anti-viral functions. Whereas perforin secretion is directed, only affecting cytolysis of Ag presenting cells, IFN-γ can act on neighboring and distal cells not expressing MHC. Soluble mediators, i.e. IFN-γ and RANTES, may have detrimental effects by perpetuating chemokine secretion by astrocytes and recruiting activated macrophages. Efforts to link sustained immune activation to ongoing chemokine secretion and Ag recognition during viral persistence will provide clues for candidate factors promoting macrophage/microglia dependent demyelination and CNS pathogenesis.

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