# MHC Class I Expression and CD8 T Cell Function: Towards the Cell Biology of T-APC Interactions in the Infected Brain

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## 1 Introduction

Antigen presentation by major histocompatibility complex class I (MHC-I) and class II (MHC-II) molecules is a prerequisite for T cell engagement during the activation as well as the effector phase. The central nervous system (CNS) is unique in that cells resident in the parenchyma, glia and neurons, do not constitutively (or very sparsely at best) express MHC molecules (Aloisi et al., 2000; Sedgwick and Hickey, 1997; Xiao and Link, 1998), making them invisible to T cells. Additional restrictions on T cell surveillance are imposed by the absence of classical lymphatic drainage, the blood–brain barrier (BBB), and the unique anatomy of the brain microvasculature (Bechmann et al., 2007; Galea et al., 2007; Hickey, 2001; Xiao and Link, 1998; Lowenstein, 2002). Infiltrating cells not only have to cross the vascular wall to penetrate into the perivascular space, but more importantly overcome the barrier formed by the glia limitans to access the CNS parenchyma. While the first step is generally not associated with pathology, penetration from the perivascular space of postcapillary venules into the parenchyma is more restricted and once overcome, associated with clinical consequences (Bechmann et al., 2007). In the resting state, perivascular macrophages are maintained by replacement with circulating monocytes. However the glial limitans is not breached, and thus, these cells are considered to be located outside the confines of the BBB. While diffusion of soluble factors and antibodies is restricted by the BBB, especially by the tight, continuous, unfenestrated capillary epithelium, leukocyte infiltration preferentially occurs at distal sites in postcapillary venules (Bechmann et al., 2007). BBB permeability and leukocyte infiltration are thus not necessarily functionally nor physically linked. The barriers separating CNS parenchyma from the circulation explains the rare presence of T cells in the normal CNS parenchyma, despite the ability of peripherally activated and memory T cells to traffic to non lymphoid tissues independent of antigen presentation (Masopust et al., 2004). Nevertheless, T cells activated during an infection or auto-immune response, are able to enter into, and migrate within the brain parenchyma, even in the presence of an intact, non-inflamed BBB (Cabarrocas et al., 2003; Chen et al., 2005; Evans et al., 1996; Hickey, 2001). However, although entry of activated T cells into the CNS is independent of their

T.E. Lane et al. (eds.), *Central Nervous System Diseases and Inflammation*.

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antigen specificity, only those T cells that recognize antigen are retained. Thus, barriers limiting T cell surveillance of the brain are rapidly overcome following CNS infections and other inflammatory conditions (Griffin, 2003; Ransohoff et al., 2003). Under such conditions, it is also likely that a number of non-activated, bystander T cells, as well as other leukocytes, including B cells are able to penetrate into the brain parenchyma. Mechanisms propagating protective versus pathogenic potential of T cells in varying disease models are complex and require more in depth exploration. This chapter highlights recent advances relating to antigen presentation functions by resident CNS cells and effects exerted by CD8 T cells in vivo with an emphasis on anti-viral functions.

## 2 Requirement of MHC-I Antigen Recognition for CD8 T Cell Induction and Function

CD8 T cells are present in the CNS during many inflammatory diseases including infections and autoimmune diseases (Dorries, 2001; Neumann et al., 2002). They are primary mediators in clearing viral infections, in anti tumor defense, and transplant rejection. However, they are also associated with a number of pathologies, including demyelinating and degenerative diseases. Physiological and pathological consequences are sometimes inextricably intertwined. Examples of T cell induced pathology are multiple sclerosis (Neumann et al., 2002; Sospedra and Martin, 2005), as well as experimental models of demyelination (Brisebois et al., 2006; Evans et al., 1996; Huseby et al., 2001; Ip et al., 2006), while examples of viral clearing and associated pathology are brain atrophy in Borna virus encephalitis (Bilzer and Stitz, 1994), Rasmussen's encephalitis (Bauer et al., 2002; Bien et al., 2002), and HTLV-1 myelopathy (Nakamura, 2000). Trafficking of CD8 T cells into the CNS is antigen independent and regulated by both chemokines and activation state of the T cells. However, their accumulation and retention is dependent on MHC-I restricted antigen recognition (Cabarrocas et al., 2003; Carson et al., 1999; Chen et al., 2005; Reuter et al., 2005). As constitutive MHC-I expression is restricted to the meninges, choroid plexus, and perivascular spaces, engagement of CD8 T cells in the parenchyma requires induction of MHC-I on parenchymal cells. This is generally thought to be transcriptionally regulated resulting in de novo expression of MHC-I molecules.

### *2.1 Class I Antigen Processing Pathways*

Class I surface expression involves numerous proteolytic, transport, and assembly events, which are prominently influenced by type I and type II IFNs and TNFα (Garbi et al., 2005; Koch and Tampe, 2006; Strehl et al., 2005; Trombetta and Mellman, 2005). In the quiescent, constitutive state, the major pool of peptides are

derived from short lived nascent proteins in the cytoplasm, which are ubiquitinated and targeted to a multicatalytic protease complex, designated the 26S proteasome (Strehl et al., 2005). The proteasome complex, located within the cytoplasm, releases peptide precursors with a length and composition suitable for transport into the endoplasmic reticulum (ER). Transport is conducted by members of the ABC transporter family, TAP1 and TAP2, which form a heterodimer. However, 99% of peptides are degraded by aminopeptidases resident in the cytosol prior to transport into the ER (Reits et al., 2003). In the ER, nascent class I heavy chains, β2 microglobulin (β2 m), and peptides for presentation are assembled in a complex process involving further N-terminal peptide trimming for optimal class I binding and cellular chaperones, including tapasin (Garbi et al., 2005). Once a stable tripartite complex is formed, it is transported through the secretory pathway to the cell surface. The quantity and quality of peptides presented by MHC molecules thus reflects cellular protein turnover, proteolytic specificities of the proteasome and activity of aminopeptidases (Strehl et al., 2005; Yewdell et al., 2003).

The 26S proteasome is composed of a catalytic 20S core complex and two 19S regulator complexes (Strehl et al., 2005). The building blocks for the 20S proteasome are four rings, each formed by seven α or β subunits, respectively. Proteolytic activities and specificities are conferred by three β subunits localized to the two inner rings. The 19S regulator serves as a gate controlling entry into the proteolytic chamber. A second regulator is the IFNγ inducible PA28 complex, which also forms a ring composed of PA28α and PA28β subunits. Antigen processing and presentation is significantly increased in the presence of IFNγ, which enhances transcription of multiple genes associated with the processing machinery. Importantly, IFNγ induces a distinct subset of 20S proteasomal β immunosubunits, which can replace their β1, β2 and β5 homologs during assembly to form immunoproteasomes. Altered cleavage site preferences of IFNγ-induced immunoproteasomes result in enhanced formation of peptides carrying an optimal C-terminus compatible with class I binding, and an extended N-terminus that facilitates TAP transport. Independent of immunoproteasome β subunits, P28 can also modulate presentation of epitopes, albeit in a more restricted, epitope dependent manner. Additional IFNγ upregulated modulators of the antigen processing machinery are POMP, a facilitator of proteasome maturation, the transporters TAP1 and TAP2, and a subset of cytosolic and ER-specific aminopeptidases. The relative expression of all these components thus determines the efficiency and epitope diversity presented by MHC-I.

Not surprisingly, different tissues and cell types display a distinct array of constitutive and immunoproteasomes in the quiescent state, which is altered during inflammation. Lymphoid organs, e.g. thymus, lymph nodes and spleen, in normal adult mice contain high levels of immunoproteasomes and reduced levels of constitutive subunits, while constitutive subunits predominate in the brain, with little expression of immunosubunits (Stohwasser et al., 1997). Although beneficial for presentation of microbial epitopes, immunoproteasomes appear strictly controlled. Their half life is ~5-fold shorter than that of constitutive proteasomes, and they rarely completely replace constitutive proteasomes (Heink et al., 2005). Nevertheless,

at sites of infection subunit exchange can be very prominent, leading to an almost complete exchange as shown during hepatotropic lymphocytic choriomeningitis virus (LCMV) and bacterial *Listeria monocytogenes* infection (Khan et al., 2001). Replacement of constitutive by immunoproteasomes serves to focus immune reactivity towards anti-infectious CD8 T cell responses.

While the role of IFNγ in enhancing class I Ag processing and presentation efficacy is well established, other factors regulating the cell-type specific and/or processing kinetics are less well studied. For example, IFNα/β does not significantly impact immunoproteasomes in liver during murine LCMV infection, but does stimulate generation of immunoproteasomes in hepatocytes in vitro and in vivo following hepatitis C virus (HCV) infection (Shin et al., 2006). TNFα is also an inducer of immunoproteasomes in vitro and may contribute to IFNγ independent induction of immunoproteasomes early during infection (Khan et al., 2001).

In addition to the endogenous class I antigen processing pathway used by infected cells to present microbial epitopes, antigen can also be processed and presented following cellular uptake from exogenous sources, a process termed cross-presentation (Rock and Shen, 2005; Trombetta and Mellman, 2005). The mechanisms can involve both TAP-dependent, or endocytic loading of class I molecules. The capture and subsequent presentation of exogenous antigen may be an effective pathway for dendritic cells to prime naïve CD8 T cells if they are not directly infected (Chen et al., 2004). Peptides can also access the cytoplasm of adjacent cells through gap junctions formed by connexins (Neijssen et al., 2005). These intercellular channels are used for transport of nutrients and second messengers, but also allow diffusion of 4–10 amino acid peptides. Neighboring cells connected by gap junctions may thus provide an alternative peptide source for dendritic cells or activated monocytes to present peptide during the priming or the effector phase.

## 2.2 MHC Class I Regulation on Parenchymal CNS Cells

The paucity of class I surface expression on quiescent CNS cells in adult rodents, non-human primates and humans, suggests strict control of class I heavy chains and components of the antigen processing machinery compared to nucleated cells in other non lymphoid tissues and the vasculature. However, despite extensive characterization of the molecules involved in the regulation of expression of class I antigen presentation in other cell types, little is known about regulation of MHC-I expression in vivo in mature resident CNS cells, and their consequent capacity to present antigens to CD8 T cells. The mechanisms regulating MHC expression and T cell engagement have largely been studied using glial or neuronal cultures derived from neonatal rodents or immortalized cell lines (Aloisi et al., 2000; Dong and Benveniste, 2001; Sedgwick and Hickey 1997). Even when the cells studied are obtained from normal animals, placing glial cells in culture already acts as a stimulus for their activation even in the absence of any further stimuli, and this is likely

to determine their reactivity with immune cells. Furthermore, the majority of these data pertain to MHC class II presentation based on the association of demyelinating autoimmune disease with CD4 T cells (Bailey et al., 2006; Dong and Benveniste, 2001). Nevertheless, there is ample evidence for a role of CD8 T cells in demyelinating and neurodegenerative disease, as well as microbial infection (Neumann et al., 2002; Dorries, 2001). In vitro studies have revealed that resident CNS cells including microglia, astrocytes, oligodendrocytes and neurons are susceptible to CD8 T cell mediated killing (Neumann et al., 2002; Sedgwick and Hickey 1997; Xiao and Link, 1998). CD8 T cells are thus considered prime suspects in propagating CNS pathology. However, in vitro killing assays necessitate the use of cells derived from neonatal brains. CNS cells in their natural environment are more stringently regulated in their ability to express MHC and this is age dependent. Furthermore, regulation of MHC-I antigen presentation as well as susceptibility to CD8 T cell function is distinct among parenchymal CNS cell types in vitro and in vivo. Neurons are the most restricted in their capacity to express MHC-I (Neumann et al., 1995, 1997a, 1997b; Sedgwick and Hickey, 1997). Furthermore, even overexpression of MHC-I in the CNS did not trigger detectable killing in vivo, although cell death of cultured neonatal neurons in vitro could be demonstrated (Rall et al., 1995). Thus, the conditions under which cell killing in vitro has been detected, does not allow to draw conclusions on the potential immune-killing in the adult brain.

The capacities of glia and neurons to present antigens and engage CD8 T cells in vivo are not only dictated by MHC expression, but also by processing components and activating and inhibitory interactions. The nature of the insult, cell types infected, innate and bystander responses thus all contribute in shaping a protective or detrimental response. On one hand, the success of adaptive T cell responses in clearing intracellular pathogens is governed by processing and recognition of antigen in the context of MHC molecules and subsequent release of antimicrobial factors, including IFNγ, TNFα, perforin and granzymes (Harty et al., 2000). On the other side, excessive activation and dysregulation of CD8 T cell function contributes to pathology (Armstrong and Lampson, 1997; Barmak et al., 2003; Brisebois et al, 2006; Neumann et al., 2002; Stitz et al., 2002). Despite their capacity to act as targets for effector CD8 T cells in vitro, neuronal and glia susceptibility to "direct, not indirect destruction" by CD8 T cells in vivo remains to be determined beyond reasonable doubt. Concomitantly, the contribution of antigen processing components in antigen presentation and expression of MHC-I in vivo in different types of brain cells needs to be determined. A set of complementary experimental approaches (e.g. immunohistochemistry, flow cytometry, molecular) will be required, since single experimental approaches can be inconclusive.

### 2.2.1 Regulation of Antigen Processing in Glia

Among resident cells of the CNS parenchyma, microglia are implicated as the most potent efferent APC to brain infiltrating, activated CD8 T cells, with regard to MHC and costimulatory molecule expression. Quiescent mouse microglia in vivo do not

or only sparsely express surface MHC-I and do not express MHC-II (Sedgwick and Hickey, 1997). In vitro and in vivo activation of microglia by inflammatory cytokines upregulates expression of MHC-I and MHC-II molecules, as well as costimulatory molecules (Aloisi et al., 2000). During viral infection in vivo, surface MHC-I upregulation precedes MHC-II upregulation and does not require IFNγ (Bergmann et al., 2003).

Regulation of MHC-I at the level of processing genes following inflammation in vivo has not been explored. However, analysis of proteasomes from primary mouse microglia cultures revealed that expression of constitutive proteasomes resembles that of fibroblasts or lymphoblastoid cells (Stohwasser et al., 2000). The immunoproteasome subunits iβ1/LMP2 and iβ5/LMP7, but not iβ2/MECL1, were also detectable at low levels, contrasting with their absence from control cell lines. IFNγ as well as LPS treatment both induced immunoproteasome subunits concomitant with a decrease in incorporated constitutive subunits, although at distinct levels (Stohwasser et al., 2000). While LPS also induced TNFα, IL-6 and KC, the individual roles of these cytokines/chemokines in modulating proteasomes were not determined in this study. Analysis of the expression signature of either unstimulated or IFNγ stimulated primary rat microglia revealed expression of TAP1, class I heavy chains, constitutive proteasome subunits and the protease activator PA28a subunit in unstimulated cultures. Transcript levels of these genes were further upregulated by IFNγ, predominantly TAP 1 (Moran et al., 2004). De novo transcribed genes included the immunoproteasome subunits iβ1/LMP2 and iβ5/LMP7, the proteasome activator PA28 β chain and 26S components, heterologous MHC-I heavy chains and the MHC-II invariant chain.

Similar to microglia, expression of MHC-I and -II is undetectable in astrocytes from naïve animals. Whereas MHC-I is upregulated during inflammation, detection of MHC-II has been more controversial (Sedgwick and Hickey, 1997). In vitro, neonatal astrocytes spontaneously express MHC-I in culture and are readily induced to express MHC-II upon IFNγ treatment or virus infection (Massa et al., 1993; Sedgwick and Hickey, 1997). Similar to microglia, microarray analysis of primary murine astrocyte cultures revealed that a large proportion of genes upregulated by IFNγ were immune response genes (Halonen et al., 2006). Specifically, genes involved in MHC-I and MHC-II antigen processing, e.g. TAP1, proteasome components, class I heavy chains and β2 m, MHC-II molecules, invariant chains, and costimulatory molecules were most strongly induced.

The regulation of antigen processing and MHC-I expression on oligodendrocytes is also important in elucidating their role as targets of antimicrobial or autoimmune CD8 T cell cytolysis during demyelinating diseases, but also in glial graft rejection during remyelination therapies. Nevertheless, studies on MHC regulation and processing capabilities in this glia subset are limited. Oligodendrocytes in naïve adult mice do not express detectable surface MHC-I. However, IFNγ stimulates MHC-I expression dramatically in vitro and in vivo (Popko and Baerwald, 1999; Sedgwick and Hickey, 1997). Interestingly, transgenic expression of class I heavy

chains beyond a certain threshold leads to their accumulation in the ER and severe defects in myelination (Baerwald et al., 2000; Popko and Baerwald, 1999). This may be due to low basal levels of proteins involved in antigen processing, thus impeding assembly and transport of MHC molecules to the cell membrane. The concerted regulation of antigen processing genes may thus be particularly important in oligodendrocytes to allow continued deployment of MHC-I at the cell membrane, while securing a functional secretory pathway for the myelination process. However, regulation of components of the antigen processing machinery have only been sparsely studied in oligodendrocytes.

Analysis of Schwann cells from sciatic nerve revealed no MHC surface expression (Tsuyuki et al., 1998). Although mRNA encoding class I heavy chains was faintly detected, mRNA for β2m, TAP-1, and LMP2 was undetectable (Tsuyuki et al., 1998). Similar to neonatal derived microglia and astrocytes, IFNγ predominantly induced transcripts associated with genes of the MHC-I antigen processing machinery. Consistent with these data, IFNγ also induced strong MHC-I surface expression. It is interesting that no induction of MHC expression was mediated by TNFα in Schwann cells. Unlike MHC-I expression, MHC-II remained undetectable both at the level of surface expression as well as mRNA after IFNγ treatment. Consistent with the IFNγ inducible MHC-I expression, mouse Schwann cells are susceptible to CD8 T cell mediated cytolysis in vitro (Steinhoff et al., 1990). Of note, CD8 T cells primed to mycobacterial antigen displayed crossreactivity to host determinants displayed by Schwann cells, suggesting a possible role for molecular mimicry. Supporting MHC-I upregulation under conditions of IFNγ supplementation, MHC-I is also upregulated on oligodendrocytes and Schwann cells during virus induced inflammation (Pereira et al., 1994; Ramakrishna et al., 2006; Redwine et al., 2001). However, similar to microglia and astrocytes, the relative roles of IFNα/β and IFNγ in MHC-I regulation in vivo have not been elucidated. Thus, rather than focusing exclusively on MHC expression, future studies may have to address the concerted expression of components of the antigen presenting machinery, as well as their kinetics of induction, since the quality, quantity and longevity of presented antigens is likely to determine microbial control and disease outcome.

MHC-I expression in vivo is also observed on oligodendrocytes in adult mice mildly overexpressing the myelin component proteolipid protein (PLP) (Ip et al., 2006). These mice develop a late onset progressive demyelination associated with axonopathic changes and infiltrating CD8 T cells and CD11b macrophage like cells. Whereas MHC-I expression was undetectable in wt mice, PLP mutants exhibited substantial increase in MHC-I expression in white matter. Closer analysis co-localized MHC expressing structures with myelin markers but not neuronal markers. Importantly, as the vast majority of T cells were $CD44^{+}$ $CD62L^{-}$ $CD69^{+}$ effector CD8 T cells, localization of ~25–30% of T cells in close proximity to MHC–I suggested direct interaction of CD8 T cells with targets (Ip et al., 2006). Whether CD69 expression on T cells reflects MHC-I driven activation or provides a signature for CD8 T cells that have entered non lymphoid parenchymal organs is unclear (Bergmann et al., 1999; Hawke et al., 1998). Furthermore, the

mechanisms underlying MHC-I surface expression and a role of T cell receptor (TCR) mediated CD8 contact and/or stress responses also remain to be elucidated in this model.

Relevant to oligodendrocyte transplant mediated remyelination, both the haplotype combination and cellular graft composition determined graft survival and efficacy of remyelination (Tepavcevic and Blakemore, 2005, 2006), suggesting a role for MHC mediated rejection. Using a rat model of toxin induced demyelination, grafts enriched for oligodendrocyte precursor cells (OPCs) persisted longer in allogeneic recipients than unenriched glial cultures. Persisting remyelination was attributed to undetectable MHC-I expression on OPC cultures under basal conditions. MHC-I was only induced on OPC following IFNγ treatment, whereas MHC-II was never detected on OPC or oligodendrocytes. By contrast, MHC-I expression was constitutive on astrocytes and microglia in the implant cultures, reducing graft survival and remyelinating potential.

Another intriguing role for MHC-I expression in brain synaptic function has been proposed by a number of authors (Boulanger and Shatz, 2004; Corriveau et al., 1998; Goddard et al., 2007; Huh et al., 2000; Oliveira et al., 2004). MHC-I expression has been found in neurons of the visual, and olfactory system, and in dorsal root ganglion neurons. Results from β2 m deficient mice have shown that these animals have alterations in the development of visual connectivity, synaptic remodeling in response to insults in dorsal root neurons, and hippocampal neuron synaptic function. In addition, MHC peptides have been shown to modulate the function of olfactory system neurons (Leinders-Zufall et al., 2004). Taken together, these results suggest the hypothesis that MHC-I expression in the CNS may regulate wider aspects of brain physiology and behavior. If so, MHC-I expression may provide a further link between immune reactivity and brain function through structural and synaptic effects of MHC-I expression. Although the precise mechanisms on how this may occur remains to be explored, how MHC-I expression in normal brain physiology relates to the function of MHC-I during inflammatory and immune brain diseases opens up further exciting avenues for future research.

## 3 Priming of Adaptive Responses and CD8 Effector Function in Response to Viral CNS Infection

### *3.1 Location Determines Brain Immune Reactivity*

There are two fundamentally different immune compartments in the brain. The brain ventricles, meninges and choroid plexi contain all cellular, vascular and lymphatic components necessary for immune function that are also associated with most other organs. This includes the dendritic cells (DC), the major cell type capable of inducing primary T cell responses, which can be found within the meninges,

choroid plexus and cerebrospinal fluid (CSF) under non inflammatory conditions (McMenamin, 1999; McMenamin et al., 2003; Pashenkov et al., 2003). These anatomical sites are strategic for capture of foreign or self antigens, which can trigger their migration to deep cervical lymph nodes (CLN), the primary lymph nodes draining the brain and CSF (Cserr and Knopf, 1992). However, the brain parenchyma itself is devoid of DC in its naive state, lacks classical lymphatic drainage, and its endothelial cells form a tight diffusion barrier between the vascular and brain compartment (Bechmann et al., 2007). In addition to these structural differences a number of molecular mechanisms intervene in the brain parenchyma to display a very peculiar type of 'dampened' immune reactivity.

Administration of antigens into either CNS immune-compartment demonstrated profound differences in immunogenicities. Injection of a particulate antigen or infectious agent (e.g. live influenza virus, BCG, non-replicative adenoviral vectors) exclusively and selectively into the brain parenchyma only causes innate inflammatory responses, but fails to stimulate systemic adaptive immune responses (Cartmell et al., 1999; Lowenstein, 2002; Matyszak and Perry, 1996; Stevenson et al., 1997; Thomas et al., 2001). By contrast, injection of the same type of antigen into the ventricular system, nonetheless, induces both an innate inflammatory and a systemic adaptive immune response (Matyszak, 1998; Matyszak and Perry, 1996; Stevenson et al., 1997). However, injection of a soluble diffusible antigen (e.g. OVA) into either compartment, does induce a systemic B cell response (Knopf et al., 1998).

This differential immune-reactivity is thought to reside, at least partly, in the distribution of DC. DC localize predominantly to lymphoid tissue, where they take up antigen and mature to potent antigen presenting cells (APC). Alternatively they acquire antigen at inflamed sites and traffic back to lymphoid tissue to activate T cells (Caux et al., 2000). Recent data has also suggested that monocytes recruited to sites of acute inflammation can acquire the phenotype of DC and present antigen to primed T cells thus propagating T cell responses (Leon et al., 2007). DC uptake of foreign antigen in the ventricular system is likely to trigger migration of DCs to CLN. Alternatively, antigens can drain directly into deep CLN. An explanation for differential priming of lymphocytes in the distinct CNS compartments may reside in the inability of particulate antigen to drain from the brain parenchyma, either through a cellular or diffusible route. Thus, particulate antigens injected into the brain parenchyma cause inflammation, but are never transported to the lymph nodes to prime a systemic immune response. Soluble antigen can diffuse from the brain to the ventricles, and thus, eventually reach the lymph nodes, where it will stimulate a systemic immune response.

Irrespective of the antigen transport and delivery mode, naïve T cells are primed in the CLN, expand and traffic to the site of insult, where they exert effector function upon antigen re-encounter. Thus, although DCs can enter the CNS parenchyma during inflammation to sustain T cell function, initial T cell activation preceding disease onset likely occurs in the CLN. The role of DCs during chronic inflammation is discussed in more detail in Chap. 13.

## *3.2 Cell Type Dependent CD8 Effector Functions During CNS Infection*

### 3.2.1 Immune Control of Neuronal Infections

T cells are critical anti-viral effectors in controlling replication of various RNA and DNA viruses infecting the CNS and PNS (Dorries, 2001; Griffin, 2003; Divito et al., 2006). However, evidence in the past decade revealed that anti viral mechanisms depend on viral tropism for neuronal as well as glia subsets. As in other tissues, viral clearance can be achieved via perforin or Fas ligand dependent lytic pathways resulting in cell death, or through the release of 'curative' antiviral cytokines which clear virus, while sparing the infected cells (Guidotti and Chisari, 2001). Although CNS recruitment of CD8 T cells is MHC-I independent, local expression of perforin mediated killing, as well as secretion of perforin, IFNγ, TNFα and CCL5 (Rantes), are dependent on MHC-I/T cell receptor (TCR) interactions (Slifka et al., 1999; Slifka and Whitton, 2000). Interruption of cell-cell interactions by physical separation leads to rapid down-regulation of IFNγ, and TNFα, but not perforin at the transcriptional level (Slifka et al., 1999). These data suggest that expression of T cell effector function also requires continuous TCR engagement in vivo. Successful control of CNS pathogens may thus depend on prolonged MHC dependent target cell contact. Whereas perforin/granzyme can only act directly on the infected target cells through physical contact with T cells, soluble mediators can potentially act distally on adjacent cells, irrespective of their ability to physically engage T cells directly (Guidotti and Chisari, 2001). At least in theory, the curative pathway is thus especially beneficial for the host in preserving non regenerative cells vital for host function such as neurons and the cells which synthesize and maintain myelin.

Both cytolytic and noncytolytic mechanisms are employed by T cells to control virus infected CNS cells (Dorries, 2001; Griffin, 2003). Nevertheless, noncytolytic mechanisms would be favored to avoid extensive cell destruction. This is most evident in non-lytic neuronal infections, which establish viral persistence without killing the host cell, e.g. LCMV or herpes simplex virus (HSV-1). Neurons persistently infected by LCMV can be cured by adoptive transfer of LCMV specific CD8 T cells without suffering detectable cell loss (Oldstone et al., 1986; Tishon et al., 1993). By contrast, infection of cells in the leptomeninges during the acute infection leads to severe neurological disease following CD8 T cell transfer (Mucke and Oldstone, 1992). In the LCMV study the acute phase infection was associated with class I expression, whereas persistence was not.

Class I surface expression has also been detected on neurons infected with Sindbis virus (Kimura and Griffin, 2000), and during acute, but not persistent HSV infection (Pereira and Simmons, 1999). CD8 T cells contribute to reduction of Sindbis viral RNA from neurons, although humoral immunity is credited with primarily clearing Sindbis virus from neurons (Kimura and Griffin, 2000). In antibody deficient mice Sindbis virus infection of neurons is also primarily controlled by T cell

derived IFNγ mediated noncytolytic mechanisms (Binder and Griffin, 2001). It is interesting that motor neurons were more sensitive to antiviral IFNγ mediated effects compared to cortical neurons, indicating regional differences in IFNγ susceptibility. Measles virus CNS disease is also controlled by non cytolytic, IFNγ mediated mechanisms without apparent neuronal loss (Patterson et al., 2002). CD8 T cells are also critical in controlling HSV-1 replication in a non-cytolytic manner in trigeminal ganglia sensory neurons and establishing latency (Divito et al., 2006). Latent infection is associated with ongoing CD8 T cell stimulation (van Lint et al., 2005) and reactivation from latency is inhibited in an MHC-restricted and antigen specific manner without notable neuronal loss or pathology (Khanna et al., 2003; Theil et al., 2003). In this case CD8 T cells require both perforin and IFNγ to block reactivation in TG cultures ex vivo. Nevertheless, the lytic pathway is tempered by expression of the inhibitory receptor CD94-NKG2a on CD8 T cells and its major ligand Qa-1b on a subset of neurons (Suvas et al., 2006). In addition to distinct regulation of CD8 T cell function by latently infected neuronal populations, protection in neurons is mediated by viral anti-apoptotic activity and IFNγ mediated blockade of HSV genes playing a role in reactivation (Divito et al., 2006).

Contrasting the preferential non cytolytic clearance in these infections, perforin is essential to clear a virulent West Nile virus from neurons in an MHC-I dependent manner (Shrestha et al., 2006a). IFNγ plays a role in limiting viral spread in the periphery and dampening CNS infection (Shrestha et al., 2006b). The fact that clearance of a less virulent strain of WNV is perforin independent supports the concept that damage may predispose infected neurons to an enhanced capacity for MHC-I surface expression and thus susceptibility to CD8 T cell mediated lysis (Neumann et al., 1995, 1997a).

A role for both perforin and IFNγ is evident during Theilers's murine encephalomyelitis virus (TMEV) infection, an experimental model of virus induced demyelinating disease (Drescher et al., 1997). Infection by TMEV, a picornavirus family member, is characterized by two types of diseases. In 'resistant' C57BL/6 mice the viral DA strain primarily infects neurons. These mice develop acute encephalitis and clear virus within 2 weeks with no pathological sequelae. In 'susceptible' SJL mice acute virus infection in gray matter is controlled, but persists in white matter of the spinal cord mainly in macrophages, but also oligodendrocytes and astrocytes (Drescher et al., 1997). MHC- I restricted CD8 T cells are the effectors providing protection from chronic disease in resistant mice. Perforin deficiency in resistant mouse strains is associated with mortality within 20 days, decreased viral clearance with virus spread to motor neurons in the spinal cords, and tissue destruction (Rossi et al., 1998). Lytic mechanisms targeting neurons thus play a dominant role in controlling early neuronal infection in the gray matter. Nevertheless, IFNγ plays an equally critical role in protection from chronic TMEV demyelinating disease, as evidenced by the failure to clear virus, extensive demyelination, and severe neurological symptoms in the absence of IFNγ signaling in mice normally resistant to chronic infection (Drescher et al., 1997; Pullen et al., 1994; Fiette et al., 1995; Rodriguez et al., 2003). Although a direct comparison is tentative due to administration of different virus

doses in different laboratories, survival rates were significantly enhanced in IFNγ deficient compared to perforin deficient mice (Rodriguez et al., 1995; Rossi et al., 1998). The protective IFNγ effects clearly reside in the ability of CNS resident cells to respond to IFNγ (Murray et al., 2002). Results from adoptive CD8 T cell transfers into $Rag^{-/-}$ mice were consistent with bone marrow chimeras harboring somatic cells intact for IFNγR reconstituted with bone marrow cells deficient in IFNγ signaling and vice versa. Although virus antigen positive cells were similar in $Rag^{-/-}$ recipients of IFNγ deficient CD4 or CD8 T cells, demyelination was significantly enhanced in the CD4 T cell recipient group (Murray et al., 2002). How prevention of IFNγ mediated downregulation of T cells or distinct T cell functions contribute to disease remains unresolved.

Establishment of TMEV persistence in $H\text{-}2D^b$ MHC-I deficient mice, in addition to the detection of MHC-I molecules on infected neurons in TMEV infected brains of resistant mice supports MHC-I dependent viral clearance mechanisms (Azoulay-Cayla et al., 2000; Fiette et al., 1993; Njenga et al., 1997a, b). Surprisingly, in contrast to MHC-I sufficient susceptible mice, class I deficiency results in the absence of neurological deficits despite extensive demyelination, implicating a detrimental role of MHC-I in development of neurological deficits (Rivera-Quinones et al., 1998).

CD8 T cells also contribute to TMEV clearance and control of acute disease in susceptible SJL mice (Begolka et al., 2001). The inability of CD8 T cells to effectively control acute infection in β2m deficient SJL mice not only results in an earlier onset of acute disease, but also a more rapid onset of chronic demyelinating disease, and epitope spreading associated with the autoimmune nature of the chronic disease (Begolka et al., 2001). In both resistant and susceptible strains of mice, virus specific CD8 T cells comprise over 50% of CD8 T cells within the CNS (Johnson et al., 1999; Kang et al., 2002). The inability to prevent persistent infection and disease in susceptible mice, despite similar magnitudes of CD8 responses has been speculated to reside in less efficient presentation of $K^s$ vs $D^b$ restricted epitopes in the respective hosts (Kang et al., 2002).

Borna disease virus (BDV) is another highly neurotropic virus causing persistent CNS infection associated with severe tissue destruction (Planz et al., 1993; Stitz et al., 2002). Similar to LCMV, BDV is a noncytolyic virus in which immune pathology is mediated by CD8 T cells. Neurons and astrocytes are primary targets for infection. MHC-I is expressed in BDV infected rat brains (Stitz et al., 1991) and on infected neurons propagated in vitro from infected rats (Planz et al., 1993). CD8 T cells isolated from infected rat brains during acute infection have vigorous cytolytic activity ex vivo (Planz et al., 1993); however, similar to other CNS infections, the CD8 T cells loose cytolytic activity and ability to suppress infectious virus during persistence (Sobbe et al., 1997). Therefore, viral persistence appears to be related to the inability of CD8 T cells to exert anti-viral function rather than the lack of MHC-I target structures on infected cells. Following transfer of T cells isolated from brains of infected rats into immunosuppressed BDV infected recipients, degenerative disease corresponded to CD8 T cell entry into the parenchyma and detection of CD8 and perforin RNA (Sobbe et al., 1997). By contrast, CLN and splenic T cells

only induced neurological symptoms if re-stimulated in vitro with BDV antigen. Irrespective of the implication of CD8 T cells in BDV-induced brain atrophy in rats, similar pathogenesis in BDV infected perforin deficient and wt mice indicates perforin plays no role in viral control nor development of neurological disease in the mouse model (Hausmann et al., 2001). Nevertheless, adoptive transfer of activated CD4 T cells prior to infection mediated protection and virus clearance. Protection coincided with earlier and enhanced parenchymal infiltration of CD8 T cells (Noske et al., 1998). An antiviral and protective role for CD8 T cell secreted IFNγ was also demonstrated in mice vaccinated with vectors expressing the nucleocapsid protein to establish CD8 memory T cells (Hausmann et al., 2005). IFNγ not only eliminated virus from neurons, but also limited pathological damage mediated by immune responses in unvaccinated animals.

These data highlight the diverse responses of T cells to primarily neuronotropic infections and consequences of ineffective early control resulting in viral persistence and pathology. In conclusion, susceptibility of neurons to perforin mediated lysis appears to be dependent on virulence, neuronal subpopulations affected, and the presence of IFNγ. Neuronal damage mediated by virus replication itself and high IFNγ make infected neurons more susceptible to CD8 mediated cytolysis rather than curative mechanisms of clearance. This balance is beneficial to the host to block rapidly spreading virulent viruses at the sacrifice of few neurons. Nevertheless, rapidly spreading viruses such as BDV may not be susceptible to CD8 effector function after a threshold of viral spread has been exceeded. The curative process during LCMV persistence is rather slow (Oldstone et al., 1986), suggesting a paucity in IFNγ production in vivo, or low sensitivity to the effects of IFNγ. Irrespective of the mechanisms, the signals triggering IFNγ secretion by CD8 T cells in the absence of detectable class I expression in persistently infected neurons remains an enigma. Possible explanations may reside in class I independent IFNγ secretion observed in unconventional CD8 T cells (Braaten et al., 2006) or dysregulated CD8 T cells as observed in anti-tumor responses (De Geer et al., 2006; Maccalli et al., 2003). Alternatively, viral epitopes may be presented by cell types other than the primary targets of infection via crosspriming (Rock and Shen, 2005; Neijssen et al., 2005; also see Chapter 13). This mechanisms has been demonstrated for CNS recruitment of tumor-specific CD8 T cells (Calzascia et al., 2003). An examination of this problem at the cellular level will allow to determine whether IFNγ is being secreted by T cells in direct contact with infected neurons or by T cells contacting other brain cells. The recent demonstration of the existence of immunological synapses between CD8 T cells and infected astrocytes during the clearance of virally infected astrocytes provides a direct cellular approach to the questions discussed above (Barcia et al., 2006, 2007). From a teleological point of view it would make sense that the immune system clears brain infections by a non-cytolytic mechanism. It will be important to examine this hypothesis more thoroughly utilizing detailed combined anatomical and molecular approaches. If virus is truly cleared by non-cytolytic mechanisms, stimulating anti-viral brain immune responses would be acceptable as therapeutic intervention; however, if the immune system were to indeed kill infected brain cells, a therapeutic approach would have to take

potential direct brain toxicity into account. Also, it is possible that the mechanisms of clearance may differ for RNA vs. DNA viruses, depending on viral virulence or potential for latent infection, level of T cell activation, antigen presentation capacity of infected CNS cells, as well as infected cell type and/or neuroanatomical location. While strong evidence exists in favor of non-cytolytic clearing mechanisms, we believe that the potential existence of brain cytotoxicity ought to be reexamined in detail.

### 3.2.2 Immune Control of Glial Infections

Cell type dependent and pathogen specific mechanisms of CD8 T cell effector function are also evident in infections primarily targeting glial cells in the acute phase of infection. Glial tropic coronaviruses, represented by neurotropic mouse hepatitis virus strains, are controlled by CD8 T cells during the acute phase (Bergmann et al., 2003, 2004; Lin et al., 1997); nevertheless virus persists in the form of RNA for the life of the mouse, indicating escape from T cell effector function (Bergmann et al., 2006). Is it not known how the RNA genomes persist. Persisting infectious virus cannot be recovered from CNS explants or from immunosuppressed mice perfused to remove neutralizing antibody prior to explant. Nevertheless, persistence in a replication competent form is indicated by the necessity of intrathecal humoral immune responses to maintain infectious virus below detection thresholds (Bergmann et al., 2006; Ramakrishna et al., 2002, 2003; Tschen et al., 2006). The cell types harboring persisting virus are astrocytes and oligodendrocytes (Perlman and Ries, 1987; Ramakrishna et al., 2003; Gonzalez et al., 2006). Virus-specific CD8 T cells are enriched in the CNS comprising at least up to 50% of total CD8 T cells (Bergmann et al., 1999), similar to acute TMEV infection. The vast majority express a $CD44^{hi}$, $CD62L^{-/lo}$, $CD11a^{hi}$, and $CD49d^{+}$ (VLA-4) activation/memory phenotype. Consistent with their activated effector phenotype, virus-specific CD8 T cells from the acutely inflamed CNS exert cytolytic effector function and produce IFN-γ and to a lesser degree TNFα upon short term antigen stimulation ex vivo (Bergmann et al., 1999; Ramakrishna et al., 2004, 2006). Expression of granzyme B and high levels of CD43 are consistent with expression of effector function in vivo. Studies in mice genetically deficient for CD8 effector molecules demonstrate that antiviral activity is mediated by both a perforin-dependent and an IFNγ dependent pathway (Lin et al., 1997; Parra et al., 1999). However, IFNγ played a more prominent protective role compared to perforin, as demonstrated by diminished virus control and enhanced mortality in the absence of IFNγ function. Mice deficient for both functions were even more susceptible to viral-induced disease and were unable to control infection, despite increased expansion and recruitment of CD8 T cells to the CNS (Bergmann et al., 2003). Immunohistochemical analysis revealed that perforin sufficed to control replication in astrocytes and microglia, while oligodendrocytes appeared insensitive to perforin mediated cytolysis in the absence of IFN-γ (Parra et al., 1999). By contrast, mice deficient in perforin controlled replication in oligodendrocytes, but could not eliminate virus from astrocytes

and microglia (Lin et al., 1997). Adoptive transfer of virus specific memory CD8 $^{+}$ T cells deficient in either cytolytic activity or IFN-γ secretion into infected immunodeficient hosts confirmed distinct susceptibilities of glial cell types to T cell antiviral functions in vivo (Bergmann et al., 2003, 2004). Even though detection of class I expression on astrocytes during MHV infection has been inconsistent (Redwine et al., 2001; Hamo et al, 2007), perforin-mediated control of virus replication in astrocytes and microglia supports direct MHC-I/TCR interactions in these cells.

Mechanisms of T cell mediated control of intracerebral murine cytomegalovirus (MCMV) replication are controversial. Adoptive transfer studies using splenocytes from MCMV primed donors revealed that CD8 T cell mediated perforin, but not IFNγ function, is critical in reducing virus from adult, immune-compromised SCID mice (Cheeran et al., 2005). In this experimental model MCMV replicates productively in astrocytes, but other CNS cell types have not been ruled out. By contrast, reconstitution of SCID mice with CD4 T cells, but not CD8 T cells from immunized mice reduced virus and provided protection from disease, without preventing establishment of latency in another study (Reuter et al., 2005). These distinct outcomes have not been reconciled, but may reside in distinct virus strains utilized. Brains from fetal and perinatal mice are most susceptible to MCMV with the predominant infected cells localized to the subventricular zone (Tsutsui et al., 2005). This region primarily harbors undifferentiated neural stem/progenitor cells, giving rise to neuronal and glial cells during brain development. Prominent susceptibility of neural stem cells to MCMV infection may thus lead to differential expression of viral genes in the developing brain, depending on differentiation into immature glial cells or neurons. Both glial cells and neurons may be sources of latent infection.

So far, interactions between immune cells and target brain cells have been studied at the population level. Thus, there is relatively little information on the in vivo cell biology of T cell interactions with infected brain cells studied at the single cell level (Barcia et al., 2006; McGavern et al., 2002). Over the last 10 years immunological synapses have been characterized as the cellular substrate of intercellular communication in the immune system. Immunological synapses that form at the junction between T cells and antigen presenting cells consist of a rearrangement of membrane proteins (i.e., intercellular adhesion molecules [e.g. ICAM-1], TCR), intracellular TCR downstream signaling tyrosine kinases, as well as cytoskeletal structures, and intracellular organelles of the secretory pathway of the T cells (Cemerski et al., 2007; Davis et al., 2007; Dustin and Cooper, 2000; Friedl et al., 2005; Grakoui et al., 1999; Huppa and Davis, 2003; Huse et al., 2006; Lee et al., 2002; Monks et al., 1998). Although various types of arrangements of T cell proteins have been found at these intercellular junctions, a canonical structure, known as the mature immunological synapse has been described as consisting of the following arrangement: a peripheral supramolecular activation cluster (pSMAC), consists of a ring of adhesion molecules that anchor the membrane of the T cell to the membrane of the APC, while a central-SMAC (cSMAC), consists of a higher concentration of TCR and signaling molecules. Immunological synapses have been described for both CD4 and CD8-T cells, and NK cells in contact with various types of APCs, e.g. dendritic cells, B cells, or target cells.

Evidence for multi-cellular CD8 T cell engagement has been provided in the LC MV-infected CNS (McGavern et al., 2002), although target cells were not identified. Furthermore, it was recently shown that anti-adenoviral CD8 T cells infiltrating the brain form classical mature immunological synapses with class I expressing astrocytes (Barcia et al., 2006, 2007). These immunological synapses were characterized through the formation of the classical supramolecular activation clusters (SMACS), which constitute the hallmark of immunological synapses (Fig. 14.1). In this model a non-replicating adenoviral vector was used to predominantly target astrocytes in the rat brain, resulting in a fixed number of astrocytes harboring viral genomes. This virus is replication-defective and thus unable to directly kill infected cells. As the parenchymal CNS infection itself does not induce significant inflammation nor a systemic anti-adenoviral immune response, systemic anti-adenoviral immunization was induced with a different Ad vector injected systemically. Systemic

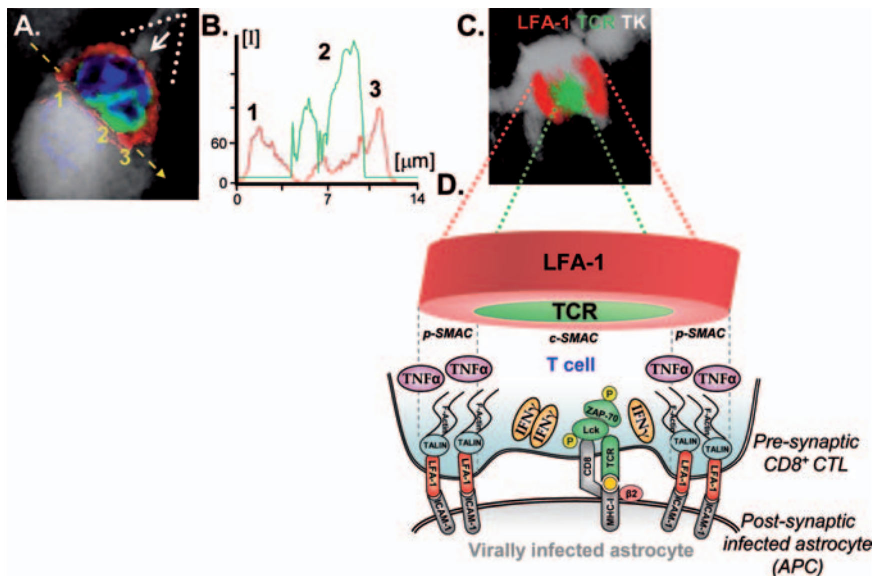


**Fig. 14.1** Immunological synapses in the brain in vivo during an antiviral immune response. This figure shows an immunological synapse formed between CD8 T cells and an adenovirally infected astrocyte. (**a**) shows the synapse as seen under the confocal microscope; the infected cell is detected through its expression of a marker gene expressed from the viral genome (i.e. HSV1-TK; white), and the CD8 + T cell is detected through its expression of LFA-1 (red) and TCR (green). (**b**) shows the relative quantification of fluorescence across the yellow arrow in (**a**); 1,2, 3 are the areas indicated in the fluorescence intensity graph in (**b**). Notice the typical distribution of increased intensity of LFA-1 at the p-SMAC (in red), and the peak of TCR intensity at the c-SMAC. (**c**) shows the view from the 3-D reconstruction of the image stack illustrated in (**a**). The view shown in (**c**) is viewed from the white triangle and white arrow in (**a**). (**d**) illustrates schematically the distribution of molecules at the p-SMAC and c-SMAC, and also illustrates the intracellular re-distribution and potential secretion of T cell effector molecules either towards the immunological synapse (i.e. IFN-γ), or outside the immunological synapse (i.e. TNF-α). (**d**) is based on results from Huse et al. (2006), and Lowenstein et al. (2007) (*See Color Plates*).

anti-adenoviral immunization triggered a systemic anti-adenoviral immune response, which led to brain inflammation. Brain inflammation consisted mainly in an infiltration of the brain parenchyma of CD8 T cells and macrophages, and a perivascular infiltration of CD4 T cells.

The systemic anti-adenoviral immune response resulted in a significant reduction in the number of brain astrocytes that express adenoviral proteins, and a concomitant reduction in the number of viral genome copy numbers present in the CNS. Loss of infected cells was dependent on both CD4 and CD8 T cells. The presence of CD8 T cells within the brain parenchyma suggested the operation of direct cytolytic mechanisms in the elimination of infected cells. Although no direct evidence for apoptotic astrocytes was obtained, macrophages containing remains of infected astrocytes were found throughout the area of the brain that had been cleared of infected cells. This suggested that the formation of immunological synapses may represent the microanatomical substrate underlying CD8 T cell effector functions in the CNS, and mediate the anti-viral clearing of CD8 T cells. The importance of these studies is the demonstration that immunological synapse do form indeed in vivo in the brain during the clearing of virally infected astrocytes by the adaptive immune response. Their in vivo description in the context of an anti-viral immune response highlights their physiological role as the structure underlying neuroimmune interactions in vivo. Also, the existence of immunological synapses in the brain during the clearing of virally infected brain cells opens up the examination of neuroimmune interactions at the single cell level.

### 3.2.3 Immune Clearing of Infected Oligodendrocytes

The apparent resistance of oligodendrocytes to perforin mediated clearance mechanisms in coronavirus-brain infections is not resolved. Nevertheless, glial tropic coronavirus infection of mice with an IFN-γ signaling defect selectively in oligodendroglia directly confirmed the importance of IFN-γ signaling in this cell type for controlling oligodendroglial viral clearance (Gonzalez et al., 2005, 2006). Virus clearance was delayed and viral antigen almost exclusively localized to oligodendrocytes resulting in at least tenfold higher antigen load compared to wt mice in the waning period of acute infection. These data implied either differences in antigen presentation by glial cell subsets and/or inherent resistance of oligodendrocytes to contact dependent CD8 T cell function. Although previously controversial, MHC-I upregulation on oligodendrocytes has recently been demonstrated in two independent models of neurotropic MHV infection (Redwine et al, 2001; Ramakrishna et al., 2006) and in mice expressing IFNγ as a transgene in the CNS (Horwitz et al., 1999). However, the kinetics of class I upregulation during infections may be delayed compared to other glia. Both IFNα/β and IFNγ can favor MHC-I antigen processing by inducing expression of proteasomal subunits and peptide transporters TAP1 and TAP2. Microglia upregulate surface MHC-I as early as 3–4 days following glial tropic MHV infection, prior to detection of significant amounts of IFNγ (Bergmann et al., 2003; Zuo et al., 2006). Expression on microglia is indeed reduced and

more transient in the absence of T cell produced IFNγ (Bergmann et al., 2003). Although IFNγ independent MHC upregulation can be mediated on cultured glial cells by type I IFNs, TNFα or IL-6 in vitro, their contribution in vivo is unknown. Similar to neurons, oligodendrocytes may have more stringent regulation of class I presentation to sustain their vital function in maintaining myelin. Preliminary analysis indeed suggests that class I is not upregulated on oligodendrocytes in IFNγ deficient mice (Bergmann, unpublished). Furthermore, surface expression is associated with vigorous de novo induction of mRNA species encoding antigen processing genes (Malone et al., 2006). More detailed studies are required to assess the possibility of impaired antigen processing, distinct thresholds of oligodendrocytes to initiate and sustain antigen presentation, or regulation by inhibitory receptors.

An antigen specific model of chronic CD8 T cell inflammation is provided by transgenic mice constitutively expressing a viral protein in oligodendrocytes (Evans et al., 1996). Transgenic mice expressing the LCMV protein nucleoprotein or glycoprotein under the MBP promoter exhibit no clinical or pathological abnormalities. Peripheral LCMV infection is cleared similar to wt mice, with no apparent involvement of CNS infection. However, although transient accumulation of CD8 T cells in meninges and ventricular linings was similar in both infected mice, CD8 T cells accumulated to significantly higher levels in the CNS of transgenic mice compared to wt mice, in which CD8 T cells waned by 3 weeks p.i. CD8 T cells persisted at constant levels throughout 1 year p.i. and was associated with MHC-I expression. A second viral challenge resulted in enhanced CD8 and CD4 T cell inflammation, and upregulation of proinflammatory cytokines including IFNγ, leading to demyelination and enhanced clinical disease. These data implied chronic CD8 T cell inflammation was triggered by class I mediated oligodendrocyte CD8 T cell interactions.

### *3.3 Long Term CD8 T Cell Survival in the CNS*

Following successful T cell mediated control of acute viral replication, T cells decline but significant numbers are nevertheless maintained in the CNS for many months or life span of infected mice. This is observed both following primary responses or recall responses (Hawke et al., 1998; Marten et al., 2000b; van der Most et al., 2003). After infectious virus is eliminated in the glial tropic coronavirus model, both CD4 and CD8 T cells persist concomitantly with detectable, persisting viral mRNA, but undetectable viral protein or infectious virus titers (Bergmann et al., 1999, 2006; Marten et al., 2000b). Despite the drop in total T cell numbers, the percentages of virus specific T cells within the CD8 T cell compartment within the CNS remain remarkably stable throughout infection (Bergmann et al., 1999; Marten et al., 2000b; Ramakrishna et al., 2004). This suggests that survival/retention signals do not discriminate between virus specific CD8 T cell populations and populations of unknown specificities potentially recruited as bystander cells.

There is no evidence that T cells exert antiviral function during coronavirus persistence. Cytolytic activity by primary virus-specific CD8 T cells is readily detected ex vivo during acute virus replication, but barely, if at all detectable by 2 weeks p.i., after clearance of infectious virus (Bergmann et al., 1999; Marten et al., 2000a; Ramakrishna et al., 2004). IFN-γ mRNA levels also decline, as virus is cleared (Parra et al., 1997; Zuo et al., 2006). Loss of cytolytic function is independent of demyelination associated factors (Marten et al., 2000a). Ex vivo cytolysis can also not be recovered during virus recrudescence in B cell deficient mice (Ramakrishna et al., 2002), despite MHC-I expression on microglia and oligodendrocytes (Ramakrishna et al., 2006). However, there is no evident impairment of IFN-γ secretion upon antigen re-exposure in vitro, suggesting CD8 T cells are not anergized (Bergmann et al., 1999; Ramakrishna et al., 2002). Comparison of coronavirus-specific primary versus memory CD8 T cells in response to CNS challenge revealed that CNS derived CD8 T cells from immunized mice exhibit enhanced cytolysis at a single cell level as well as increased IFN-γ and granzyme B production, compared to naïve mice after challenge (Ramakrishna et al., 2004). Importantly, reactivated memory CD8 T cells retained cytolytic function coincident with increased granzyme B levels for prolonged periods compared to primary CD8 T cells. Retention of cytolytic activity in reactivated memory cells persisting in the CNS was first demonstrated in a model of neurotropic influenza virus challenge (Hawke et al., 1998). The lethality of this influenza virus infection in naïve mice prevented longitudinal analysis of primary CD8 T cell function. Loss of virus-specific cytolytic function thus reflects distinct differentiation states of primary compared to memory CD8 T cells rather than an intrinsic property of the inflamed CNS environment. Importantly, despite retention of a cytolytic phenotype, there was no evidence for ongoing pathology in either challenge model.

Enhanced effector function by reactivated memory CD8 T cells directly translated to more effective virus control upon challenge compared to naïve mice in both the coronavirus and neurotropic influenza virus models. Nevertheless, despite a nearly 3 week phase of apparent clearance during which infectious virus remained undetectable, CD8 T cell persistence at higher numbers and a pre-armed state was insufficient to prevent coronavirus reactivation in the absence of antiviral antibody (Ramakrishna et al., 2006). Interestingly, MHC expression was significantly downregulated during the period of apparent virus clearance. At the time of recrudescence MHC expression on microglia and oligodendrocytes was low compared to the acute response, suggesting too few if any target MHC molecules presenting viral epitope to re-initiate IFNγ mediated class I upregulation and trigger antiviral function. Despite being fully armed to exert anti-viral activity upon antigen exposure in vitro, memory T cells in the CNS in vivo may thus not be responsive in an environment in which too few cells are persistently infected to engage antiviral T cell function. These observations highlight the functional controversies encountered upon the comparative analysis of CNS CD8 T cell function in vitro and in vivo. Furthermore, they emphasize the complex regulation of MHC-I/TCR interactions and a potential feedback loop involving IFNs in sustaining or downregulating MHC-I. As viral clearance progresses fewer MHC- I microbial antigen complexes are recognized

during viral control, IFNγ levels drop, resulting in decreased transcription of genes associated with MHC-I antigen processing and presentation.

The mechanisms of prolonged T cell persistence in the CNS in the absence of overt chronic inflammation and CNS disease have not been extensively explored. Comparison of the fate of T cells following infection with a persisting and non persisting glial tropic coronavirus variant suggested a role for persisting viral RNA in sustaining T cell retention (Marten et al., 2000b). Active maintenance is also supported by selection of CD8 T cell populations with limited T cell receptor specificities during the persistent phase of coronavirus infection compared to the acute phase (Marten et al., 1999). By contrast, following challenge of T cell immune mice with a neurovirulent influenza virus infection, residual virus was undetectable, demonstrating that retention of CD8 T cells in the CNS was independent of persisting viral antigen as measured by PCR analysis for viral RNA (Hawke et al., 1998). MHC expression within the CNS was not analyzed in either study. It is possible that immunological synapses provide an anatomical substrate to sustain long term T cell survival in the CNS even after MHC expression drops. The longevity of immunological synapses in vivo during the clearing of viral infections is currently being examined.

The contribution of local homeostatic proliferation or ongoing recruitment to T cell maintenance in the CNS remains unclear. Memory cells traffic poorly into the quiescent CNS (Masopust et al., 2004) and activated T cells are only retained within the CNS upon cognate antigen recognition (Hickey, 2001; Chen et al., 2005). In the influenza virus model, memory CD8 T cells retained in the CNS after viral clearance had very slow turnover compared to peripheral memory CD8 T cells (Hawke et al., 1998). The memory T cell survival factor IL-15, which regulates homeostasis of CD8 memory cells in lymphoid organs (Masopust and Ahmed, 2004), does also not appear to be required for prolonged CD8 T cell survival in the coronavirus persistently infected CNS (Zuo and Bergmann, unpublished), supporting low turnover.

Persisting infection is associated with prolonged detection of CXCR3 ligands, potentially mediating ongoing lymphocyte recruitment (Tschen et al., 2006; also see Chap. 12). However, blockade of CXCR3 signaling early during persistence selectively reduces CD4 but not CD8 T cells (Stiles et al., 2006). These observations suggest that neither peripheral chemokine-mediated recruitment nor local division contribute significantly to CNS CD8 T cell maintenance. These findings implied that a small subpopulation of T cells responding to infection are recruited into a long lived, persisting pool with enhanced survival in the CNS. It is unknown whether T cell retention within the CNS is associated with enhanced expression of anti-apoptotic factors and/or survival factors in the CNS environment. Higher propensity for apoptosis by T cells in lymphoid compared to non lymphoid tissues (Wang et al., 2003) suggests that CD8 T cells surviving a selection process during the decline phase may have a prolonged lifespan in the CNS. Thus although T cells are the primary cells undergoing apoptosis during acute antiviral responses in the CNS (Gonzalez et al., 2006), those that do not become susceptible to antigen induced cell death may survive long term in the CNS.

## 4 Concluding Remarks

The communication between CD8 T cells and resident cells of the CNS appears highly sophisticated, yet our understanding is still very limited. Major limitations reside in the unique interactions and crosstalk between CNS cells amongst themselves, their fully differentiated state in mature individuals, and their anatomical location. This network amongst a very large number of different cells types makes it difficult to extrapolate results obtained from in vitro studies using primary cultures from neonatal tissue to events in vivo. Although glial cells and neurons are capable of expressing MHC-I in vitro and thus acting as targets for in vitro stimulated CD8 T cells, the mode of CD8 T cell function in vivo has largely been deducted from indirect evidence, such as viral clearance, loss of detection of viral genomes, demyelination or tissue atrophy. How T cells eliminate viral infections from the CNS remains contested. It is likely to depend crucially on the individual virus, its capacity to remain latent or persistent in CNS cells, the exact nature of the infected cell type, the anatomical region infected, and the level of T cell activation. Additional characteristics, such as sex and age are also likely to be crucial determinants of the outcome of T cell mediated clearing of viral infections. While teleologically, non-cytolytic clearing of non-dividing infected brain cells may be preferable to killing of postmitotic neurons, the alternative needs to be thoroughly examined. Direct contact or even proximity of class I expressing cells and CD8 T cells in situ has only been demonstrated in isolated reports. Similarly, the notion of curative rather than cytolytic virus clearance should be regarded critically, as demonstration of apoptotic or dead CNS resident cells in situ, especially if the numbers are sparse are technically challenging. The technical challenge of determining cell death in vivo is substantial. Immune-mediated killing cell assays rely on removing T cells from the target organ and exposing them to artificial target cells in vitro. While such studies have demonstrated the capacity of T cells isolated from infected brains to kill pre-selected target cells, this comes short of demonstrating that these cells indeed kill brain cells in vivo. In vivo killing assays using CFSE labeled target cells have been developed, yet their application to CNS tissue has not yet been achieved. While such assays come closer to demonstrating that T cells can kill within the animal, whether T cells can directly kill CNS target cells in situ, remains to be explored further. It is likely that novel methods will need to be developed to examine directly the capacity of T cells to do so.

The involvement of activating and inhibitory receptors on T cells and expression of their ligands in the CNS is not well understood. Similarly, regulation of anti-apoptotic factors requires further investigation. A better understanding of the relative kinetics of MHC presentation, T cell accumulation, and regulation of effector function at the target site during inflammatory reactions may lead to novel preventive and therapeutic intervention in enhancing, microbial clearance, while minimizing pathological damage. Finally, it is likely that a combination of novel microanatomical imaging techniques will encourage new analyses of T cell activity in the CNS. We believe that a novel examination of the interactions of T cells with

target cells at the individual cellular level in vivo, will allow new perspectives on T cell function in the CNS. Until now, even quantitative assays on T cell function in the CNS have relied on studies of the whole population of brain infiltrating T cells. Many times, due to unavoidable technical constraints, functional analysis has relied on the ex vivo functional analysis of brain infiltrating T cells. Equally, the examination of target brain cell function has relied on the analysis of large populations of cells, for which their past direct interactions with effector cells remained unknowable. New morpho-functional approaches, such as the study of immunological synaptic function in vivo utilizing confocal microscopy, or direct in vivo analysis of T cell – CNS cell interactions using two photon microscopical approaches will usher in further understandings of T cell function in the brain in vivo, either during the beneficial clearing of viral infections, immunopathology in response to brain infection, or autoimmune attack.

**Acknowledgements** We thank Stephen Stohlman for reading this review and providing helpful comments. The authors of this article were funded by NIH grants PO1 NS18146, RO1 AI47249 and NMSS grant RG 3808 (C. C. B) and NIH/NINDS grants R01 NS44556, 1 RO1 NS42893, U54 NS045309, R21 NS047298, and R01 NS054193, and The Bram and Elaine Goldsmith Chair In Gene Therapeutics (P.R.L.). PRL also thanks the generous support the BOG-GTRI receives from the Board of Governors at Cedars Sinai Medical Center and Kurt Kroeger for his assistance in preparation of Fig. 14.1.

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