CNS Neurons: The Basis and Benefits of Low Class I Major Histocompatibility Complex Expression

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1 Introduction: Host Cells That Restrict "Counterproductive" MHC Recognition

The host immune response is generally thought to consist of cells with "professional" immunologic functions, such as B and T cells, macrophages, and natural killer (NK) cells. However, differentiated cells which do not normally participate in immune surveillance may be recruited to serve an integral function in the immune-mediated elimination of foreign intracellular pathogens such as viruses. As discussed elsewhere in this volume, most cells have the ability to present immunogenic, "non-self" peptides (called epitopes) in association with "self" class I major histocompatibility complex (MHC) molecules. This cell surface complex is engaged by the T cell

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receptor (TCR) of cytotoxic T lymphocytes (CTL). Appropriate MHC-epitope-TCR interaction leads to the CTL-mediated lysis of the epitope-expressing target cell via the perforation of the plasma membrane, introduction of CTL-derived proteolytic enzymes (granzymes) into the target cell cytosol, and eventual cell death, presumably via apoptosis.

For the majority of virally infected tissues, this strategy is ultimately beneficial: the net gain (reduction in the number of virus-producing cells) outweighs the consequence of lysis of an infected differentiated cell. This is true because, for most tissues, immune-mediated cell loss is accompanied by increased cell proliferation, which restores the damaged tissue. For example, hepatitis B virus (HBV) infects between 5% and 40% of all hepatocytes within the livers of chronically infected humans (BIANCHI and GUDAT 1980). An estimated 7% of HBV-infected hepatocytes are lost each day (NOWAK et al. 1996), implying that between 0.3% and 3% of all hepatocytes must be replenished daily to maintain a stable liver cell mass. Thus, for many virally infected tissues, a balance exists between cell loss and cell renewal.

The presentation of immunogenic epitopes by virally infected cells can be seen as an altruistic event. Because the expression of these antigenic molecules often precedes production of infectious viral progeny, immune-mediated lysis of the infected cell destroys a "factory" of virus production and therefore decreases the total viral burden. Thus these unfortunate few die for the benefit of the common good: reduced viral load and recruitment of T cells to the site of a productive infection minimizes the opportunity of the virus to infect a neighboring cell.

However, altruistic antigen presentation may not always benefit an infected host. While expression of MHC molecules is highest in cells such as those of the lymphoid system, the gut, and the kidney, MHC class I is expressed to a low to undetectable degree in other cells, including those of the brain, sperm cells at certain stages of differentiation, and trophoblasts within the placenta (DAVID-WATINE et.al. 1990). In this chapter, we discuss recent findings concerning the molecular basis and potential benefits of low MHC expression, predominately focusing on class I MHC presentation in central nervous system (CNS) neurons and trophoblasts within the placenta. We also take a broader perspective concerning the lack of CTL recognition in the CNS and describe work which suggests that neurons may employ several mechanisms, low MHC expression being one of them, to avoid immune cell recognition.

1.1 Obligatory Haplotype Mismatch: The Maternal Fetal Interface

Pregnancy poses an unique challenge to the maternal immune response, since the developing fetus can be viewed as a natural graft. The placenta contains fetal and paternally derived histocompatibility antigens that are potentially immunogenic for the maternal immune system (VOLAND et.al. 1994); the activation of the maternal host defenses against what it perceives as "foreign" would obviously be detrimental to the developing fetus. One factor contributing to the lack of a maternal anti-fetus response is that the placental trophoblasts, which are in direct contact with maternal blood and tissues, express little or no classical MHC antigens (FAULK and TEMPLE 1976; HUNT

and ORR 1992). The deleterious consequences of trophoblast MHC expression on fetal survival were confirmed in transgenic mouse studies in which a mouse class I MHC gene (D^d) was expressed in murine trophoblasts under the transcriptional control of the c-fos promoter, known to be highly expressed in trophoblasts during development (DESCHAMPS et.al. 1985). Expression of this MHC molecule in vivo resulted in a higher incidence of spontaneous abortion, measured as a function of reduced transgene transmission to surviving pups. Thus, if the MHC expression had no effect on survival of the pups, one would expect approximately 50% transmission of the transgene in heterozygous (D^d transgenic×normal) crosses. However, in the litters born to these parents, transmission was reduced to 20%–30%, indicating in utero death of 20%–30% of the transgenic fetuses. Breeding of the c-fos-MHC male transgenic with β_2-microglobulin knockout females (who lack expression of the classical class I MHC and therefore fail to positively select $CD8^+$ T cells) restored transmission of the transgene to 50% of the progeny. Thus, while the expression of MHC molecules in the trophoblasts does not inexorably lead to spontaneous abortion (20%–30% of surviving neonates of (D^dnormal) crosses were transgenic) there does exist a correlation between transgene transcription and MHC expression on placental tissues (VOLAND et al. 1994).

The absence of MHC may not be the only way in which the fetus is protected from the maternal immune response. In humans, the highly polymorphic "classical" MHC class I molecules are encoded in the HLA-A, -B, and -C gene families. At least three additional class I genes, HLA-E, -F, and -G have been identified. These nonclassical (class Ib) genes are highly homologous to the classical HLA genes, and all associate with β_2-microglobulin. However, in contrast to the classical HLA genes, HLA-E, -F, and -G are nonpolymorphic: for example, HLA-G is derived from alternative splicing and a premature stop codon (reviewed in WOOD 1994), resulting in a protein that lacks a full cytoplasmic tail (GERAGHTY et.al. 1987). HLA-G is predominately expressed on trophoblasts in fetal placental tissues at the materno-fetal interface, where the classical class I and II genes are absent (CAROSELLA et al. 1996), implying that it plays a potentially important role in maternal tolerance to the developing fetus.

Whether this nonclassical MHC expression on trophoblasts can present peptides to the maternal immune response remains unresolved (SANDERS et.al. 1991; VINCE and JOHNSON 1995). However, a link between HLA-G expression and resistance to NK cell-mediated lysis has been demonstrated, suggesting that HLA-G acts as a "surrogate" cell surface MHC molecule to prevent NK-mediated lysis (LIAO et.al. 1991). HLA-G is capable of inhibiting the NK activity of decidual large granular leukocytes against the trophoblasts (FERRY et.al. 1991). Blocking the HLA-G antigen with antibody restored NK cytolytic activity (CHUMBLEY et al. 1994). Since HLA-G polymorphism is likely to be low, it is possible that HLA-G can serve as the public ligand for the NK cell receptor, protecting fetal trophoblasts from NK killing and conferring immunological tolerance to fetal tissue.

1.2 Essential and Nonrenewable: CNS Neurons

For many viral infections, lysis of infected cells by the class I MHC-mediated CTL response does not often result in long-term deficits since, following infection, many of these tissues can recover by upregulation of cell division. However, neurons of the CNS are an essential, but nondividing population; therefore, loss of these cells, either by virus-mediated cytolysis or CTL-mediated killing, would have obvious deleterious consequences to a host. Thus the immune response to a virus may be more damaging than the viral infection itself. Perhaps to provide protection against such an immune response, normal uninfected CNS neurons lack surface expression of class I MHC molecules. Whether neurons can be induced to express MHC is still debated, although recent reports have convincingly shown that MHC expression on CNS neurons can be detected under certain pathological circumstances. The debate centers around the "inability" versus "reluctance" of CNS neuron class I MHC expression. The inability of some neuronal culture systems to present epitopes has been attributed to an intrinsic defect in the synthesis of the antigen-presenting machinery. Alternatively, reluctant expression may depend on stimulating factors such as cytokines that are normally absent within the parenchyma; the presence of these inducers may allow for upregulation of MHC on neurons.

2 Constraints on Immune Cell-Target Cell Interaction in the Brain

The low level of class I MHC on all brain parenchymal cells implies that immune responses in the CNS differ significantly from those in the periphery. In addition to the low levels of MHC expression, may of additional features unique to the CNS contribute to the hypothesis that immune responses are suppressed within the brain. These features, including the presence of the blood-brain barrier and absence of lymphatic drainage, have been extensively reviewed elsewhere (WEKERLE et al. 1986; SEDGWICK and DORRIES 1991; CSERR and KNOPF 1992; RALL and OLDSTONE 1995). Here, we briefly discuss recent contributions to understanding the role of the blood-brain barrier, lymphatic drainage, and brain-derived gangliosides in restricting immune recognition in the brain and describe how the neuronal environment may restrict viral infections, indirectly altering the generation of immunogenic peptides.

2.1 The First Obstacle: The Blood-Brain Barrier Restricts Trafficking of Immune Mediators into the CNS

The interface between the circulation and the brain parenchyma, known as the blood-brain barrier, is comprised of endothelial cells, basal membranes, and astrocyte endfeet (RAPOPORT 1976; BRIGHTMAN et al. 1983). This complex barrier controls the exchange of cells and metabolites between the blood and the brain, predominately

due to tight junctions within the capillary endothelium. Consequently, many circulating lymphocytes are restricted from crossing the endothelial barrier and patrolling the brain parenchyma. It is this high degree of surveillance imposed by the tightly packed endothelial cells which has contributed to the belief that the CNS is immune privileged.

Continuous tight junctions are not the only feature that makes the capillaries of the CNS distinctive. Due to the need for blood-borne cells and metabolites to pass between endothelial cells to enter the brain, a decisive factor in determining how easily a molecule enters the CNS is lipid solubility (GOLDSTEIN and BETZ 1986). Lipid-soluble molecules, such as nicotine, ethanol, and heroin, can readily cross the barrier, while water-soluble molecules, including many of the immune-mediating proteins such as antibodies, cytokines, and complement components, cannot gain access to the parenchyma. For non-lipid-soluble molecules to enter the CNS parenchyma, specific transporter molecules present on the endothelial cells are required.

From an immunological perspective, the blood-brain barrier restricts, but does not prohibit, immune responses from occurring within the CNS. For example, lymphocytes are not completely blocked from gaining access to the CNS (WEKERLE et al. 1986; HICKEY and KIMURA 1987; TYOR et al. 1989; HICKEY et al. 1991). Hickey and coworkers have shown that activated T cells enter the CNS in a random manner (HICKEY et al. 1991), regardless of antigen specificity or MHC compatibility. Thus it appears that any T cell clone which is activated in the periphery will ultimately gain access to the CNS parenchyma. This condition, as Hickey points out, is supported experimentally by grafting studies in which an allograft, normally tolerated if grafted into the CNS, will be rejected rapidly when the host is exposed to alloantigens in the periphery (MASON et al. 1986). Furthermore, the barrier changes in response to viral infections and inflammatory responses by altering the profile of adhesion molecules expressed on the endothelial surface and by possible transient increases in blood-brain barrier permeability (LASSMANN et al. 1991; WEKERLE et al. 1991; RALL et al. 1995). Thus CNS "immune privilege" may be a constantly changing state, influenced both by intra- and extraparenchymal conditions.

2.2 Absence of Conventional Lymphatic Drainage

While the blood-brain barrier was thought to restrict the efferent movement of peripheral cells into the CNS, the absence of a conventional lymphatic system within the CNS was believed to interfere with the afferent arm of immunity, alerting the peripheral immune response to antigens within the CNS. However, despite the absence of typical lymphatic channels in the brain, a connection between the CNS and the lymphatics was demonstrated when a large fraction of radioiodinated albumin injected into the brain was isolated from cervical lymphatics (YAMADA et al. 1991). These and other results (CSERR et al. 1992; WELLER et al. 1996) show that cerebral extracellular fluids drain into the blood from the CNS along cranial nerves, primarily the olfactory nerve, and along spinal nerve root ganglia. Thus the outflow of cerebrospinal fluid (CSF) along cranial nerves and spinal nerve roots is the conduit

for cross-talk between the brain parenchyma, the brain vasculature, and the lymphatic system (YOFFEY and COURTICE 1970; CSERR and KNOPF 1992).

2.3 Influence of an Immunosuppressive Environment

The paucity of MHC expression on resident brain cells, the lack of lymphatic drainage, and the presence of the highly selective blood-brain barrier collectively suggest that immunological nonresponsiveness within the CNS is due to an absence of lymphocyte access to the brain or an absence of recognition of infected cells. However, the isolation and characterization of brain-enriched molecules with potent immunosuppressive qualities suggests that the brain may also actively restrict immune responses.

One group of molecules with recently described immunomodulatory effects on neurotropic viral infections are the sialic acid-containing glycosphingolipids known as gangliosides. Gangliosides are ubiquitous cell membrane components that can also be shed into the extracellular environment. Their multifunctional roles in cell metabolism include regulation of cell-cell interaction, differentiation, signal transduction, and growth regulation (reviewed in BERGELSON 1995).

The influence of soluble gangliosides on the immune response has been studied for over a quarter of a century. In general, gangliosides have been shown to have potent and apparently generalized immunosuppressive qualities, including the suppression of CTL, T helper cell, and NK cell proliferation (reviewed in BERGELSON 1995). Interestingly, one form of ganglioside (GM3) can activate cells, but these lymphocytes have a suppressor phenotype (DYATLOVITSKAYA et al. 1991). While the precise mechanisms used by gangliosides to downregulate the immune response are not fully understood, their effects appear to be diverse, influencing cell surface expression by insertion into the lipid bilayer and altering soluble mediator function by binding to such proteins as interleukin-2 (IL-2), interferon gamma (IFN-γ), and tumor necrosis factor (TNF) (DYATLOVITSKAYA and BERGELSON 1987).

Gangliosides are enriched within the CNS (WIEGANDT 1971; IRANI et al. 1996), especially within neurons. Recently, gangliosides were shown to selectively block the in vitro production of Th1-associated cytokines, such as IL-2 and IFN-γ, perhaps by blocking NF-κB activation (IRANI et al. 1996). Furthermore, gangliosides inhibited T cell proliferation by preventing their entry into the cell cycle (IRANI et al. 1996). The immunosuppressive role of gangliosides in the resolution of CNS viral infections was substantiated in mice when intraparenchymal T cells, recruited to the brain parenchyma in response to the neuronal infection caused by Sindbis virus, were arrested in the cell cycle, despite the presence of activation markers (IRANI et al. 1997). Thus, while activated cells routinely patrol the CNS (HICKEY et al. 1991), molecules within the CNS microenvironment may suppress or impair the effector mechanisms of intraparenchymal lymphocytes.

2.4 Restricted Replication of Viruses

Because of these unique aspects of the CNS environment, the degree of immunological surveillance and the vigor of the immune response is significantly less in the brain than in peripheral organs. Taking these facts into consideration, it is not unexpected that neurons have evolved unique capacities to prevent their destruction by viral infections. Indeed, probably because CNS neurons are a harbor from the immune response, a large number of DNA and RNA viruses are neurotropic.

Many of these viruses convert to a persistent phenotype upon infection of neurons. In such cases, viral nucleic acids and proteins are readily detected in the absence of direct cell death or induction of the immune response, and often in the absence of production of extracellular infectious progeny. While the detailed mechanisms by which viruses establish and maintain long-term persistent infections are largely unknown, both viral genes and host cellular genes have been shown to cooperatively promote viral persistence.

In the persistent infection caused by the murine virus lymphocytic choriomeningitis virus (LCMV), expression of nucleoprotein (NP) antigen and short (S) RNA (RODRIGUEZ et al. 1983; FAZAKERLEY et al. 1991) is readily detected in the CNS, but electron microscopy examination has failed to reveal mature viral particles budding from infected neurons within the brain parenchyma, and almost no extracellular infectious virus can be detected. This was also observed in recent studies using the PC12 neuronal cell culture system (DE LA TORRE et al. 1993), in which differentiation of the chromaffin-like PC12 cells to a neuronal phenotype was accompanied by a 3- to 4-log decrease in the production of infectious virus.

How neurons block the production of infectious virus despite viral gene expression remains unresolved and is relevant to the human infections caused by measles, rabies, influenza, poliovirus, and mumps and the animal infections caused by Borna disease virus (BDV), the coronaviruses, and the arenaviruses, among others. Since each of these viruses can establish productive infections in other differentiated cells, the dramatic reduction in infectious virus production in neurons suggests that the neuronal environment is in some way inhospitable for viral propagation.

While the cellular mechanisms that restrict infectious virus production in neurons are not known, a number of observations indicate that the intracellular level of cyclic nucleotides in neurons may be involved. For example, the shift from acute to persistent measles virus infection in mouse neuroblastoma cultures depends on functions that affect endogenous cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) levels (MILLER and CARRIGAN 1982). In human fibroblasts, the addition of cAMP-enhancing compounds significantly decreased the yield of herpes simplex virus (HSV) I (STANWICK et al. 1977).

Neural cells contain abundant cAMP, with levels being closely correlated with terminal differentiation, cessation of cell division, and induction of neuron-specific functions (GREENGARD 1978). Since cAMP activates cAMP-dependent kinases and cAMP-gated ion channels and can also interact with other second messenger signals, it is reasonable to speculate that changes in the intracellular levels of cAMP or its downstream effectors may be able to alter the efficiency of viral replication in CNS neurons.

How might decreased viral synthesis affect antigen presentation and resultant immune recognition in the CNS? Reduced viral production, especially of extracellular virus, would limit the availability of viral proteins to enter the circulation via the lymphatics and activate or recruit the peripheral immune response. Furthermore, reduced levels of replication also impact on the number of endogenously synthesized viral peptides which can serve as epitopes for class I MHC recognition. Finally, many viral infections have been shown to enhance the transcription of the antigen-presenting machinery; perhaps slowly replicating viruses would not trigger this activation, resulting in a lower level of class I MHC-presented molecules on infected neurons.

3 MHC Expression in Neurons

3.1 Expression of Class I MHC in Peripheral Nervous System Neurons

The hypothesis that has been proposed as to why CNS neurons do not express class I MHC molecules relates to the consequences of CTL-mediated lysis of these nondividing cells: depletion of a critical and nonrenewable population would have dramatic effects on the host. Therefore, if MHC expression and terminal differentiation are linked, one might expect that peripheral neurons, which can regenerate, might express class I MHC under certain circumstances. Indeed, both in vivo and in vitro studies have confirmed that peripheral neurons can express class I MHC RNA. However, whether these cells can present endogenously generated peptides on the cell surface, and whether peripheral nervous system (PNS) neurons serve as targets for CTL recognition and attack, remains unresolved.

In an effort to assay whether brain-derived cells could serve as targets for alloantigen-specific CTL lysis, primary PNS neurons from the superior cervical ganglia were cultured and used as targets in standard chromium release assays (KEANE et al. 1992). PNS neurons were lysed both by allospecific CTL and by purified granules obtained from lymphokine-activated killer (LAK) cells, whereas CNS neurons remained refractory to CTL killing, even after 3 weeks in culture. Interestingly, no expression of MHC class I was detected on the surface of the PNS neurons by immunohistochemistry; however, the authors argue that the degree of killing seen in these cultures (as much as 60% at effector to target ratios of 40:1) and the purity of their CTL population strongly suggests that neuronal death is mediated through interaction of T cells with target cells via T cell receptor-class I MHC association.

In vivo, the ability of peripheral neurons to express MHC and to be lysed by CTL is less clear. Following a peripheral nerve lesion of rat facial and sciatic motor nerves, an induction of class I MHC antigens was detected. The MHC expression was transient for some lesions (e.g., MHC expression decreased 21 days after nerve crush, coincident with nerve regeneration), although MHC antigen remained detectable in nonregenerating lesions, such as a cut nerve (MAEHLEN et al. 1988, 1989; O'MALLEY and MACLEISH 1993). While there is no evidence that upregulated MHC expression

results in the CTL-mediated lysis of these neurons, MAEHLEN et al. (1989) speculate that MHC expression may serve to signal cell death by a nonimmunological process, a hypothesis supported by later studies with "electrically silent" CNS neurons (NEUMANN et al. 1995).

Increased neuronal MHC expression could be due to the local inflammation, edema, and neighboring cell dysregulation that is induced by physical damage to neurons. Therefore, the effect of a neuron-specific, relatively noninflammatory lesion, such as a viral infection, may help to understand the link between intra- and extraneuronal stimuli. In an in vivo model of acute HSV infection of peripheral nerves (SIMMONS 1989; SIMMONS and TSCHARKE 1992), class I MHC protein expression in the PNS was upregulated transiently in satellite and Schwann cells (both of glial origin), but not in neurons within the HSV-infected spinal ganglia (PEREIRA et al. 1994). The authors report that, while no protein was expressed on HSV-infected neurons, class I MHC mRNA was detected, leading to two possible interpretations. In one case, these mRNAs correspond to classical MHC molecules, implying that the block in cell surface protein expression is post-transcriptionally controlled. This block could restrict translation of the MHC RNA themselves or could abrogate the function of accessory antigen-presenting molecules required to bring the MHC antigens to the cell surface. Alternatively, these transcripts could correspond to nonclassical MHC antigens, which would not be detected as proteins with the antibodies used in this study.

Thus the variability in PNS neuron MHC, RNA and protein expression is governed in part by the system (in vivo or in vitro) and the stimulating event (viral infection, nerve crush, axotomy). In general, however, PNS neurons appear to respond to deleterious stimuli; the role of upregulated MHC "classical or nonclassical" on the neuronal surface remains to be elucidated.

3.2 Expression of Class I MHC in CNS Neurons In Vitro

Most studies that evaluate neuronal antigen-presenting capacity in vitro find that MHC expression is low or absent in quiescent neurons, but can be induced by such stimuli as cytokines, viral infections, or direct injury. These reports collectively argue that the normal, low to negligible expression of class I molecules reflects a regulatory control rather than a genetic lesion in CNS neurons (LAMPSON and FISHER 1984). Two reports, however, suggest that MHC proteins are either constitutively absent or constitutively expressed on cells of neuronal origin. One study used the neuroblastoma line N2A to convincingly demonstrate constitutive expression of MHC, equaling that of a control ependymoblastoma line (TING et al. 1987). These cells were also efficient targets for CTL, but not for NK cells, implying that "classical" TCR-MHC interactions between CTL and these neuroblastoma cells occurred. These results differed from previous reports using the same cell line that were not able to detect MHC expression (LAMPSON et al. 1983; WONG et al. 1984; MAIN et al. 1985). TING et al. (1987) noted that detection strategies that depend on antibodies are exquisitely sensitive to antibody concentration and suggested that this may explain the discrepancy between their findings and previous reports.

In 1993, MASSA et al. (1993) evaluated the ability of IFN-γ to induce MHC expression in primary embryonic neuronal cultures and found that, while IFN-γ could activate MHC transcription in cultured astrocytes, primary CNS neurons remained refractory to the cytokine stimulus. As discussed later in this chapter, the block was attributed to an absence of induction of a nuclear factor which binds to the IFN-responsive consensus sequence.

These disparate findings may not be as controversial as they initially appear; TING et al. (1987) correctly notes the inherent problem of comparing a neuroblastoma cell line with neurons in vivo: it is possible that only dividing neurons, e.g., neuroblastoma, normally express class I MHC, linking MHC expression with mitotic activity and differentiation status. Consistent with this hypothesis, the primary neurons used by MASSA et al. (1993) were evaluated 8 days post-culture, a time when these cells are no longer mitotically active.

Cell division may not be the only parameter that determines the inducibility of MHC genes in neurons. Recently, NEUMANN et al. (1995, 1997) used the combined approaches of electrophysiology and reverse transcriptase-polymerase chain reaction (RT-PCR) to monitor MHC transcription in the presence or absence of cytokine stimulation. While other reports had evaluated the effect of cytokine addition to neurons in culture (LAMPSON and FISHER 1984; JOLY et al. 1991; DREW et al. 1993), the added approach of patch clamping allowed correlations to be drawn between MHC expression and functional activity in individual neurons. In this recent report, transcription of MHC class I genes was very rare in neurons with spontaneous action potentials, as determined by whole-cell patch clamping of cultured hippocampal neurons. However, in electrically silent neurons, defined as those without spontaneous action potentials, class I MHC transcription was detected; class I MHC protein expression could be seen only in electrically silent neurons treated with IFN-γ. Thus neurons may possess the basic requirements to interact with $CD8^+$ cells, but expression of these molecules is tightly regulated. In cases of overt neural damage and loss of neural activity, MHC upregulation might serve to target a damaged cell population. In a follow-up study, NEUMANN et al (1997) confirmed that basal neuronal expression of MHC class I genes differed significantly from gene expression in other cells; while transcription of the heavy chain appeared intact in most primary neurons, there was a failure to transcribe either β_2-microglobulin (the light chain of the class I MHC heterodimer) or the transporter proteins TAP-1 and -2 (NEUMANN et al. 1997). Consequently, unstimulated primary neurons failed to express MHC on the cell surface. The addition of IFN-γ induced β_2-microglobulin and TAP transcription in some, but not all cultured neurons. To understand how the expressing and nonexpressing cells differed, reagents which suppressed electrical activity were added to the cultures and MHC surface expression was monitored. Suppression of the neuronal electric activity by the addition of a sodium channel blocker led to induction of class I expression on virtually all neurons, reinforcing the connection between MHC expression and a lack of electrical activity reported earlier (NEUMANN et al. 1995).

In one of the first papers to appear on the issue of neuronal MHC expression (WONG et al. 1984), IFN-γ was found to induce a dramatic expression of H-2 antigens on virtually all oligodendrocytes, astrocytes, and microglia, while MHC expression was found on at least "some" neurons. While later reports noted the potential problem of

non-neuronal contamination in these studies (BARTLETT et al. 1989), the disparity in MHC expression observed in the original report, when viewed in the context of recent findings, may reflect replicative or functional differences between neuron populations.

Viral infection has also been shown to increase MHC expression on cultured neuronal cell lines. In one study, persistent infection of neuroblastoma cells (C1300) with measles virus (Edmonston strain) was shown to induce MHC protein expression on the cell surface (GOPAS et al. 1992). The motivation for this work was not to correlate the relevance of their neuroblastoma line to in vivo neuronal MHC expression, but rather to parallel neuroblastomas of children. The authors argue that viral infection of malignant neuroblasts may serve both to upregulate MHC expression and to provide unique immunogenic (viral) peptides for CTL recognition and lysis (GOPAS et al. 1992). In support of this hypothesis, persistently infected C1300 cells not only expressed higher levels of cell surface MHC, but also could be recognized and lysed by measles virus-elicited CTL.

A second example of virus-induced MHC expression in vitro evaluated the susceptibility of BDV-infected neurons to CTL recognition and lysis (PLANZ et al. 1993). In the wild, infection of horses and sheep with BDV often leads to an encephalomyelitis accompanied by severe neurological disorders (LUDWIG et al. 1988); the inflammation has led to the characterization of Borna disease as an immunopathological condition (NARAYAN et al. 1983). Both $CD4^+$ T helper cells and $CD8^+$ CTL are found in CNS lesions. Effector lymphocytes isolated from brains of persistently infected rats showed haplotype-specific cytotoxic activity when incubated with BDV-infected primary neurons, confirming that these neurons can be targets for virus-specific CTL (PLANZ et al. 1993).

3.3 Expression of Class I MHC in CNS Neurons In Vivo

While in vitro studies of neuroblastomas and cultured primary neurons have told us much about the constraints on neuronal MHC induction and the stimuli that can overcome such constraints, these systems may not reflect the MHC status of neurons in vivo. For example, immunosuppressive molecules normally present in the CNS, such as gangliosides, would not be expected to function in vitro. Furthermore, the process of culturing primary neurons often involves trypsinization and incubation with serum-containing medium; these conditions could provide essential "background" stimulation for MHC expression on cultured cells. Consequently, to establish whether certain pathological conditions are accompanied by enhanced MHC expression, an immunohistochemical study (LAMPSON and HICKEY 1986) correlated MHC expression in human brain biopsies evaluated as "histologically normal" with those containing a range of neuropathological lesions, including glial tumors. HLA expression could be found adjacent to vessel walls in all brains, regardless of their condition. However, MHC expression did not overlap with any parenchymal cell, including neurons, oligodendrocytes, microglia, or astrocytes (LAMPSON and HICKEY 1986). It should be noted that none of the specimens examined in this communication showed obvious lymphocytic inflammation. Given the suggestion that activated lymphocytes

and their products may induce neuronal MHC expression (FONTANA et al. 1984; HICKEY et al. 1985), the absence of MHC, even in glial tumor-bearing individuals, may not be surprising. However, as these authors point out, exposure to circulating lymphocytes alone is not sufficient to induce MHC; class I expression is also absent from parenchymal cells in areas of the brain that lack a blood-brain barrier (WHELAN and LAMPSON 1985).

A more extensive study of 40 human brain specimens by SOBEL and AMES (1988) was able to detect class I expression in parenchymal cells, but notably no expression was seen in neurons (as determined by their location in the brain, not by neuronal antibody double labeling). The expression patterns, similar to the conclusions drawn by Lampson and Hickey, did not change as a function of age, sex, duration between death and tissue preservation, systemic illnesses at the time of death, or CNS lesions. Importantly, the 40 brains surveyed included a number of neuropathologic diseases expected to induce immune cell infiltrates, including atherosclerosis, hydrocephalus, and metastatic carcinoma.

Neither of these studies, however, included patients with neuronal viral infections which had been shown in vitro to upregulate MHC expression. In subacute sclerosing panencephalitis (SSPE), the rare but lethal neurodegenerative disease of the CNS following acute measles virus infection, MHC induction on neurons was found in five out of six autopsy specimens (GOGATE et al. 1996). However, when MHC and neuronal markers were used to colocalize the signals in these sections, MHC expression was rarely found on infected neurons. The disparity between measles virus infection and MHC expression could be explained by two hypotheses. In one scenario, enhanced neuronal MHC expression may not be a direct consequence of viral infection, but may be controlled by exogenous factors induced by viral replication in the CNS. Alternatively, MHC-expressing, measles virus-infected neurons may serve as efficient targets for antiviral CTL, with infected cells cleared by the cytolytic immune response prior to acquisition of the brain tissue (GOGATE et al. 1996).

Because analysis of autopsy specimens does not afford the opportunity to evaluate the progression of the disease and the factors that govern MHC expression, animal model systems of neurotropic viral infections have proven informative. In experimental viral infections of mice and rats and in one case of spongiform encephalopathy caused by the scrapie agent, infection of neurons correlated with MHC induction (DUGUID and TRZEPACZ 1993; BILZER and STITZ 1994; PEARCE et al. 1994).

Infection of mice with a highly neurotropic variant of mouse hepatitis virus (MHV) establishes a limited infection within the brain and has a low mortality rate (PEARCE et al. 1994). Clearance of virus from the brain is associated with infiltration of $CD8^+$ lymphocytes, suggesting that CTL-mediated lysis may contribute to viral clearance. Infection of astrocytes by MHV had previously been shown to induce MHC expression on these infected cells (GOMBOLD and WEISS 1992); in the study by Pearce and colleagues, upregulation of class I antigens in the nerve fiber layer was also seen, suggesting that some neurons may be capable of MHC antigen presentation. A similar correlation between CD8 T cell infiltration and MHC upregulation on neurons was found for BDV infection (BILZER and STITZ 1994). In this paper, treatment of BDV-infected rats with the anti-CD8 monoclonal antibody OX-8 inhibited the immunopathologic reaction and reduced MHC class I antigen expression on neurons.

Collectively, it has been demonstrated that all neurons, peripheral or central, in vitro or in vivo, can be induced to express class I MHC. The stimuli that result in induction may be viral (e.g., measles), cellular (e.g., $CD8^+$ T cells), or soluble (e.g., IFN-γ), although not all members of these groups can induce class I antigen expression. For example, persistent infection with the arenavirus LCMV does not induce neuronal class I MHC (MUCKE and OLDSTONE 1992). Discovering the aspects of viral infection that govern MHC regulation remains a major effort in virology and immunology research, and the list of viral proteins that interfere with the antigen-presenting pathway continues to grow (reviewed in SPRIGGS 1996). It is important to note that, despite evidence of MHC expression on neurons, no reports exist of neuronal lysis in vivo by CTL. Consequently, despite transitory changes in blood-brain barrier permeability, movement of CNS antigens to the periphery via a lymphoid route, and presentation of foreign peptides by class I antigens, the "immune privilege" of the mammalian CNS is apparently preserved.

4 Basis of Neuronal Block in MHC Expression

4.1 Reduced Activity of Transcription Factors

If neurons can be induced to present antigens via the class I pathway, but normally do not, what intracellular mechanisms are responsible for suppression and how does an inducing signal override this blockade?

Amplification of the N-*myc* gene is correlated with an increased growth rate and enhanced metastatic ability of human neuroblastomas (BERNARDS et al. 1986). While the issue remains controversial (FELTNER et al. 1989), there appears to be an inverse correlation between expression of the N-*myc* gene and MHC class I expression: as the neuroblastoma progresses, the level of N-*myc* increases while the level of class I antigens decreases. The effect of N-*myc* on MHC expression was reversible with IFN treatment. Interestingly, this association between N-*myc* expression and class I synthesis was true only for neuroblastoma; expression of N-*myc* in fibroblasts was not correlated with levels of class I antigen expression (BERNARDS et al. 1986).

It was later found that two distinct elements in the MHC promoter render the class I genes susceptible to *myc*-induced suppression (LENARDO et al. 1989). N-*myc* reduced the binding of a transcription factor specific for one of these elements, the MHC class I enhancer. Consequently, in the presence of high concentrations of intracellular *myc*, the activity of this enhancer is compromised and cellular transcription of MHC genes is constitutively low. The identity of this nuclear transcription factor was shown to be the p50 subunit of the inducible transcription factor NF-κB (VANT VEER et al. 1993). Introduction of the NF-κB p50 subunit into neuroblastoma cells restored expression of class I molecules.

Why do neuroblastomas express such high levels of N-*myc*? To test the idea that N-*myc* expression results from the inactivation or loss of some N-*myc* repressor, VERSTEEG et al. (1990) fused neuroblastomas overexpressing N-*myc* with lines that

do not express N-*myc*. N-*myc* expression was turned off in the resulting hybrids, confirming the hypothesis that the absence of the N-*myc* suppressor can account for the unusually high levels in neuroblastomas. As predicted, the level of class I MHC transcription in these hybrids was restored, verifying the causal link between N-*myc* and class I expression.

Defects in MHC class I transcription are not the only obstacles to antigen presentation in neuronal cell lines. JOLY and OLDSTONE (1992) found that the OBL-21 cell line, previously shown to express low levels of the class I heavy chain (JOLY et al. 1991), also expressed negligible levels of the TAP-1 and -2 transporter proteins (previously called HAM molecules), which are responsible for shuttling proteolyzed peptide fragments into the endoplasmic reticulum. While the levels of N-*myc* in this cell line have not been established, nor is it known whether N-*myc* overexpression is the cause of decreased transcription of the TAP genes, it is generally appreciated that many antigen-presenting genes share common regulatory motifs, leaving open the possibility that N-*myc* can sequester nuclear transcription factors that regulate multiple genes involved in antigen presentation.

The above studies employed transformed cell lines as model systems, again raising the relevance of neuroblastoma lines to primary neurons or neurons in vivo. MASSA and colleagues (1993) evaluated the ability of class I antigens to be upregulated by IFN-γ in primary astrocytes, neurons, and oligodendrocytes. They found that expression in oligodendrocytes and astrocytes could be enhanced by the addition of the stimulatory cytokine, but that neurons remained refractory to the effects of IFN-γ. The differences in inducibility among these cell types was due to differences in the expression of nuclear factors specific for the NF-κB-like region I enhancer within the class I promoter. Thus, similar to neuroblastomas, the absence of class I MHC in neurons appears to be linked to a decreased abundance or activity of DNA-binding transcription factors. A critical difference between primary neurons and neuroblastomas is their response to cytokine stimulation. Addition of IFN-γ or TNF-α induces MHC expression in most neuroblastoma cells (DREW et al. 1993), whereas primary neurons are generally refractory to cytokine stimulation.

The expression of the IFN-β gene is regulated by enhancers similar to those found in the MHC genes (BURKE and OZATO 1989). One might therefore predict that the IFN-β gene would be similarly downregulated in primary neurons, although surprisingly the data indicate that it is highly expressed in neurons following treatment with IFN-β-inducing stimuli (WARD and MASSA 1995). The authors discuss the potential benefit of this differential regulation: neurons successfully escape MHC class I-restricted lysis by downmodulating MHC cell surface expression, while retaining the beneficial properties of IFN-β production, which include restriction of viral infection within a cell population (SCHIJNS et al. 1991).

Finally, while transcription factors do play a pivotal role in MHC regulation, *cis*-acting signals appear to also regulate cell type-specific MHC expression (MURPHY et al. 1996). Cell-specific expression of class I antigens is achieved in part by a series of negative and positive regulatory elements located within the extended class I MHC promoter (WEISSMAN and SINGER 1991). The constitutive ability of this promoter to function in a given cell type is dependent on the activity of the activating and silencing domains within the promoter. Thus it is possible that the class I promoter in neurons

is constitutively functional, but is actively repressed by upstream elements. MURPHY and coworkers (1996) demonstrated that the removal of four elements upstream of the transcriptional start site of the class I promoter of the non-MHC-expressing human neuroblastoma line CHP-126 restored constitutive MHC expression. Two of these "silencer" elements display differing functions in CHP-126 and HeLa cells. Silencer B, located between nucleotides 402 and 503 within the class I MHC promoter, suppresses MHC expression in neuroblastomas, but enhances expression in HeLa cells; likewise, silencer A, which suppresses expression in the CHP-126 cells, has no detectable function in HeLa cells.

The authors acknowledge that NF-κB plays a critical role in cytokine-induced expression. However, while NF-κB may be necessary for cytokine-induced expression, it is not necessary for constitutive expression, since an upstream enhancer is able to activate the promoter in the absence of NF-κB. Thus suppression of constitutive MHC synthesis appears to reside, at least in part, at the level of *cis*-acting elements within the MHC promoter, in addition to the altered function of transcriptional regulators (MURPHY et al. 1996).

4.2 Reduced Susceptibility to Cytotoxic T Lymphocyte-Mediated Lysis and the Potential Role of Cytokines

An underlying assumption of this chapter is that increased neuronal MHC expression confers a higher susceptibility to CTL-mediated recognition and lysis. Three studies suggest that this may not be an accurate assumption and that neurons may be refractory to CTL lysis at a level beyond TCR-MHC recognition.

As described earlier in this chapter, cytokine treatment of neuroblastoma cell lines can upregulate cell surface MHC expression. However, despite increased levels of MHC, MAIN and colleagues (1988) found that neurons remained resistant to CTL-mediated lysis. The authors noted that "neuroblastoma lines [with increased MHC] did not form conjugates with primed T cells." Retrospectively, in light of the need for both MHC and costimulatory signals to result in target lysis, these neuroblastomas may be deficient in either the critical "second signal" or in cell surface adhesion molecule expression needed for binding of the CTL to its target.

The requirement for TCR-MHC recognition can be bypassed using purified granules obtained from CTL or LAK cells. Differing conclusions are drawn by two groups who assessed the susceptibility of primary neurons in culture to purified granules. One group (KEANE et al. 1992) reported that, while leucoagglutinin-treated primary CNS neurons triggered granzyme release from effector cells, neurons remained resistant to the cytolytic effects of these granules, unlike other cultured resident brain cells. They concluded that neurons possess protective mechanisms, beyond blocks in target cell-CTL interaction, that render them refractory to CTL-delivered cytotoxins. A more recent report (RENSING-EHL et al. 1996) readdressed this issue and found that both extracted granules and purified perforin could induce virtually complete lysis of neurons. The major differences between these studies were the age and type of neurons studied; in the paper by Keane and colleagues, whole embryonic brain was used as a source of neurons; in the report by Rensing-Ehl and

coworkers, cerebellar granule cells from day-7 neonates served as a source of neurons. These papers underscore the importance of the model system in interpreting these studies: differentiation status, host age, and neuronal subtype are all factors in immune susceptibility.

Finally, if neuronal MHC expression and CTL lysis are linked events, one might expect that reconstitution of class I MHC on the surface of neurons in vivo might render mouse CNS neurons more susceptible to CTL lysis following an inflammatory CNS lesion. However, when CNS neurons were driven to constitutively express a transgene-encoded MHC molecule (RALL et al. 1995) and were subsequently infected with the neurotropic virus LCMV, adoptive transfer of antiviral CTL resulted in recruitment of CTL to the brain parenchyma and more rapid clearance of virus from infected neurons, but no appreciable loss of neurons within infected brains. Thus MHC expression was sufficient to recruit CTL to the CNS parenchyma and to allow virus clearance, but clearance was not accompanied by cell loss (RALL et al. 1995).

How these CTL exert their virucidal, but not cytocidal, effects in vivo is not known. It is conceivable that factors released from neurons or glial cells, such as gangliosides, may interfere with CTL-neuronal interactions. Alternatively, intraparenchymal cytokine production may participate in the clearance of infectious virus from transgenic brains. Inhibition of viral synthesis in the absence of cell death has been documented in the CTL response to HBV infection of hepatocytes (GUIDOTTI et al. 1994) and in human immunodeficiency virus (HIV) (HSEUH et al. 1994). In these systems, inflammatory cytokines such as IFN-γ and TNF-α can downregulate viral expression without concomitant cell death. Studies are underway to test the ability of CTL-elaborated cytokines to resolve neurotropic viral infections in the absence of cell killing.

5 Conclusions

In summary, the paucity of neuronal expression of the class I MHC antigens seems to reflect a reluctance, rather than an inability, to synthesize these proteins. The burden borne by cells which can present foreign peptides in the context of class I MHC is that they will become targets of the cytolytic response. In most cases, such altruistic behavior benefits the host, since CTL lysis of virally infected cells will likely precede virus maturation, thereby limiting the spread of the infection. For some cells, however, such behavior would not benefit the host. In the case of placental trophoblasts that lie at the junction between the maternal and fetal systems, absence of MHC expression helps to protect the developing "graft" from maternal recognition. In the CNS, negligible MHC expression protects essential and nonrenewable neurons from immune-mediated cytolysis.

The potential of neurons to express class I MHC antigens, however, suggests that various stimuli, including cytokines, viral infections, and alterations in neuronal function may allow these cells to become targets for CTL recognition. The mechanisms by which these stimuli cause increased MHC expression and the ultimate

consequences of CTL-neuron interactions in the host remain issues of paramount importance to our understanding of immunologically mediated CNS diseases.

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