Cell-Mediated Immunity in Virus Infections of the Central Nervous System

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Virus infections of the central nervous system (CNS) are generally a consequence of systemic exposure, being preceded by replication in other sites throughout the body and thus sensitization of the immune system. The obvious exception is a virus such as the rabies virus, which may progress to the brain by retrograde flow along neuronal processes and consequently avoid immunologic surveillance. The peculiar features of virus-specific cell-mediated immunity (CMI) in the CNS are thus largely a function of the nature of the target organ. The existence of tight endothelial junctions and intimately associated astrocyte processes, the lack (though this is debated) of conventional lymphatic drainage, the low levels of expression of major histocompatibility complex (MHC) glycoproteins,[1] and the complex physiologic and anatomic interactions that are required for normal function all contribute to particular difficulties in the effective operation of host response and lead to unique aspects of pathologic damage.

There can be no doubt, given (for instance) the continuing debate concerning the possible role of viruses in triggering multiple sclerosis (MS)[2-4] and the existence of the somewhat enigmatic dementia associated with human immunodeficiency virus (HIV) infection,[5] that developing a better understanding of the cellular immune response and inflammation in virus infections of the CNS is a priority. The consequences of lymphocyte invasion, the pathologic changes induced by secondarily recruited monocytes and macrophages, and so forth must be addressed using the best available technology. This brief review does not attempt to cover all the relevant literature, but aims simply to highlight some areas of contemporary interest.

NATURE OF T-CELL RECOGNITION

The basic principle of CMI is that effector T lymphocytes are targeted onto the surface of cells expressing an abnormal phenotype defined by modification of class I or class II MHC glycoproteins,[6,7] the phenomenon known as MHC restriction.[8] Class I MHC-restricted T cells are CD8+, and those that recognize class II molecules are CD4+.[9,10] There is increasing evidence that the CD8 and CD4 molecules play an accessory role in binding together the T cell and target, or stimulator, cell.[11-14] The

rules governing the coordinate expression of CD4 or CD8 and the particular T-cell receptor (TC_r) genes are not yet understood.

The fact of MHC restriction reflects that the T-cell repertoire is in some way constrained to see variants of self MHC molecules as a consequence of events occurring during thymocyte ontogeny.[15] The role of the thymus in determining self tolerance, on the one hand, and the capacity to mount an immune response, on the other, is currently a subject of intense experimentation by molecular and cellular immunologists.[16-18] This work has been greatly facilitated by the identification of the TC_r genes and by the realization that there are distinct families of these genes. In general, however, it is not yet clear if a particular spectrum of TC_r gene expression is associated with restriction of T-cell effector function to one or another MHC molecule or if the TC_r repertoire is essentially random and depends simply on binding affinities. There are experiments to support both viewpoints.[19-22] It is possible that the MHC-restricted TC_r may have a low affinity for the relevant, unmodified MHC glycoprotein, while being able to bind strongly to one or another allogeneic MHC molecule.[23,24] What is apparent is that the TC_r repertoire is not derived by somatic mutation following a high affinity binding event in the thymus[25,26] along the lines originally suggested by Jerne.[27]

The current paradigm accepted by most investigators is that the two chains of the T-cell receptor combine to form a single, immunoglobulin-like binding site that recognizes a modified MHC molecule.[26-28] Whether an individual T-cell clone is restricted to class I or class II MHC phenotype seems to depend on interactions involving CD8 or CD4 both during ontogeny and in the context of the primary immune response.[10-14] The nature of the entity recognized by the TC_r is now becoming much clearer.[28-30]

Both class I and class II MHC-restricted T cells are apparently recognizing MHC glycoproteins that have been modified by the binding of foreign peptides.[28-30] Whether or not a viral molecule is expressed in an intact form on cell surface may thus be largely irrelevant in CMI; the fact is that the T-cell response to many viruses seems to be directed mainly at essentially "internal" components, such as the matrix and nucleoprotein in influenza.[31,32] The key event is therefore whether a particular viral peptide can bind with sufficient affinity to the relevant MHC glycoprotein. This presumably reflects that viral proteins are being degraded at sites where they encounter MHC molecules. The main characteristic of class II MHC association, which is a function of the less frequent class II MHC-positive cells that tend to have well-recognized antigen-processing functions, may be that such events occur in specialized organelles such as lysosomes. Class I MHC expression is associated with many more cell types, including fibroblasts and tumor cells, and may reflect more generalized degradative processes that are occurring in the cytoplasm. Questions concerning the nature of antigen processing and the recirculation of MHC molecules from the plasma membrane[33,34] are now of great interest. The system must operate in such a way that it does not become saturated with "self" peptides derived from, for instance, "minor" histocompatibility antigens.[35]

The current paradigm concerning the interaction between the peptide and the MHC glycoprotein is, for instance, that the class II molecule has a single peptide binding site of broad specificity[28] which may involve as few as four residues of the peptide. Analysis with a 14-residue peptide of ovalbumin and the class II molecule Ia^d showed that substitution of single amino acids in the peptide changed the binding affinity for Ia^d comparatively rarely, whereas recognition by the TC_r was much less permissive. The model proposed by this group[28] is that T-cell recognition involves the formation of a trimolecular complex in which the peptide is sandwiched in a β-sheet of extended conformation between Ia and the T-cell receptor.

Sense has thus finally been made of the old proposal that the T-cell receptor

recognizes either a virus-induced modification of the H-2 molecule or some complex of virus and H-2,[8,36] with viral peptide being substituted for virus.[28,30] The realization that antigenicity for T cells is a function of peptides associating with MHC glycoprotein also has particular implications when considering CNS pathology. Many candidate neurotransmitters are peptides[37] in the size range that could potentially bind to MHC molecules. This may be part of the reason that at least in the normal physiologic state the CNS is substantially MHC negative.[1]

The low level of MHC expression on normal brain cells can be reversed *in vitro,* at least for a proportion of cells, by culture in the presence of various agents (Table 1). The most potent in this regard is gamma interferon (IFN-γ), which is produced by activated T cells[44] and causes much higher and more rapid induction of both class I and class II MHC glycoprotein expression than does any other promoter tested.[42] The effect is also seen *in vivo* when IFN-γ is injected intracerebrally into normal mouse brain.[37] That we did not observe enhanced class II MHC expression on resident CNS cells in mice with acute lymphocytic choriomeningitis[45] may simply reflect that the disease process proceeded too rapidly.

Work from ter Meulen's laboratory has established that exposure to the inactivated JHM coronavirus is sufficient to induce astrocytes to become class II MHC positive.[42] Astrocytes secrete an interleukin-1-like molecule[46] and can act as both antigen-processing cells and stimulators for T-lymphocyte proliferation.[47] Furthermore, the capacity of JHM virus to cause class II MHC glycoprotein expression on astrocytes is rat-strain dependent and correlates directly with the development of virus-related demyelinating disease in genetically different rats.[48] The general argument that viruses may trigger autoimmune disease by causing aberrant class II expression on potential stimulator cells[49] thus applies clearly to the brain.

MHC PHENOTYPE AND VIRUS-INDUCED NEUROLOGIC DISEASE

The question of correlation of MHC phenotype with induction of virus-related immunopathology in the CNS has been addressed for two experimental systems:[48] the acute, fatal disease resulting from intracerebral injection of adult mice with LCM virus,[50] and the chronic demyelination associated with persistent infection of the CNS by Theiler's murine encephalomyelitis virus (TMEV).[51-54] The TMEV model is particularly fascinating, as the relationship between MHC gene expression and effector T-cell function would seem, on superficial analysis, to be inappropriate: susceptibility to demyelination is associated with class I MHC phenotype, whereas the T cell responsible for demyelination may be CD4+ and thus class II MHC restricted.[55,56]

The explanation for this may rest in the studies of Rodriguez and colleagues[53] with the H-2^{dm2} mutant. The dm2 mutation results in the expression of a molecule with the extracellular NH_2-terminal of H-$2D^d$ associated with the COOH-terminal extracellular portion of H-$2L^d$. This has presumably resulted from the unequal crossing over of the H-$2D^d$ and H-$2L^d$ genes, leaving the N exon and part of the C1 exon of H-$2D^d$ joined to the L^d gene beginning somewhere in the C1 exon.[57] Molecular experiments using "exon-shuffling" protocols[58,59] have shown that such rearrangements often result in the loss of MHC-restricted T-cell recognition directed at, in this case, H-$2D^d$ and H-$2L^d$.

Mice that express both H-$2D^d$ and H-$2L^d$, or have deleted H-$2L^d$ (H-2^{dm2} mutant),

TABLE 1. Induction of MHC Glycoprotein Expression on Brain Cells

Origin of Cells	Inducing Agent	Cell Type	MHC Class	Reference
Cultured cells from baby mice	IFN-γ[a]	Oligodendrocytes	I	Wong *et al.*, 1984,[38]
		Microglia	I	1985[1]
		Some neurons	I	
		Astrocytes	I & II	
Adult human brain, cultured for 10-135 days	Nil	Astrocytes	II(9-24%)	Kim *et al.*,
		Oligodendrocytes	II(4-16%)	1985[39]
First passage cells from newborn mice	IFN-γ or Con A Sn[b]	Astrocytes	I & II	Fontana
		Astrocytes	I & II	*et al.*, 1986[40]
11-14-day cultures from newborn mice	IFN-γ	Oligodendrocytes	I	Suzumura *et al.*,
	rec IL-2[b]	Oligodendrocytes	I	1986[41]
Primary cultures from newborn rat	IFN-γ	Astrocytes	II	Massa and
			18% in 4h	ter Meulen,
	Phorbol ester Ca^{2+} ionophore muramyl dipeptide inactivated JHM virus	Astrocytes	II	1987[42]
			10-19% in 5 days	
Sections of human brain	Abscess or tumor metastasis	Astrocytes and endothelium	II	Frank *et al.*,
			II	1986[43]

[a] Type 1 interferon (IFN) may also induce class I expression, but is much less potent than IFN-γ.
[b] Con A Sn = supernates from Con-A-stimulated lymphocyte cultures; rec IL-2 = human recombinant interleukin-2.

clear the virus and do not develop demyelination.[53] The interpretation is therefore that an H-2D^d-restricted, CD8+ T cell is responsible for clearance of TMEV from the CNS. If this does not occur, persistent viral antigen presentation of class II MHC positive cells (possibly astrocytes or microglia/macrophages) may target the virus-immune CD4+ cells that directly or indirectly cause the demyelination.

The implication is therefore that H-2^{dm1} is not an immune response (Ir) gene for CD8+ effectors in TMEV infection. That is to say, H-2^{dm1} is unable to associate with any TMEV peptide$^{28-30}$ to form a configuration that is recognized by an appropriate TC_r. Furthermore, considering the H-2 haplotypes that were used in this study, this would also be true for H-2K^b and H-2K^d. The existence of a very limited spectrum of class I MHC Ir genes has been established for another group of small RNA viruses, the alphaviruses.[60] It may not be a coincidence that the alphaviruses have also been used to develop mouse models for T-cell-mediated demyelination in the presence of viral persistence.[61]

Unfortunately, this simplistic model correlating virus clearance with class I and class II MHC phenotype to explain the TMEV demyelination model is not obviously applicable to the findings of Clatch, Melvold, Lipton, and colleagues.[51,54] This group, using SJL × B10 recombinant mouse strains, exploited the correlation between H-2D^s homozygosity and demyelination and found no clear correlation with virus clearance. One parameter that might be worth checking is the anatomic localization of the residual virus in the CNS: the virus titers are low, and it could be worthwhile to use immunohistochemistry to determine exactly where the infection is persisting.

The acute lymphocytic choriomeningitis (LCM) immunopathology model is superficially much more straightforward. The disease is induced by class I MHC-restricted, CD8+-activated T cells.[62,65] Earlier experiments indicated that H-2L^d is the sole class I Ir gene for lymphocytic choriomeningitis virus (LCMV) in the H-2^d haplotype.[66] This caused us to ask if the H-2^{dm2} mutant,[50] which has deleted H-2L^d,[67] would develop clinical LCM. The answer was in the affirmative, but with a slightly delayed time of onset. Further analysis revealed that in the absence of H-2L^d, H-2K^k emerged as an Ir gene for targeting LCMV-specific cytotoxic T lymphocytes (CTL). This dominance of a strong (H-2L^d) over a weak (H-2K^d) class I allele is poorly understood. It could be that they compete for the same viral peptide, with H-2L^d having a higher affinity. However, LCMV-infected targets expressing both H-2K^d and H-2L^d are lysed by H-2K^d-restricted LCMV-immune CTLs. Similar immunodominant effects have now been observed in several experimental systems[6,68] and must be considered when assessing correlations between class I MHC phenotype and disease susceptibility.

MHC EXPRESSION AND T-CELL TARGETING TO THE CNS

The LCMV model has been used to examine this question in a systematic way, at least for the induction of viral meningitis. The great advantage of this system is that the severity of inflammation can be determined accurately by counting cells in the CSF. It is thus possible to make a quantitative analysis of cellular extravasation into the site of disease. Early adoptive transfer experiments showed clearly that homology between donor and recipient for class I MHC phenotype was sufficient for the induction of severe meningitis, whereas matching for class II alleles had little effect.[69] More recent studies have confirmed that the key cell is the CD8+ immune

lymphocyte, whereas deletion of the CD4+ cells from cytotoxic effector populations, if anything, simply enriches the capacity to cause meningitis.[64]

We then asked if this effect reflected that the transferred lymphocytes were first multiplying in MHC-compatible lymphoid tissue and then localizing nonspecifically to the site of virus growth[70,71]; there is evidence from the experimental allergic encephalomyelitis (EAE) model that any activated lymphocyte can cross the brain endothelium.[72] The alternative was that the capacity of effector T cells to cause meningitis required that there be MHC-restricted T-cell target interactions at the site of pathology,[68] the brain, and the cells of the blood-brain barrier. The experiment was performed by making [(A×B)F1 → (B×C)F1] bone marrow radiation chimeras (A, B, and C are different MHC haplotypes), leaving them for 8-12 weeks, and then using these mice as immunosuppressed, virus-infected recipients for T-cell transfer.[70,71] The situation in such chimeras is that the bone-marrow-derived compartment, including monocyte/macrophages and lymphocytes, is almost entirely of donor origin.[73,74] With time, the same is also true for the microglia.[75] We found that adoptively transferred B virus-immune T cells induced severe pathology, whereas those of the C phenotype were slightly less effective. However, the A LCMV-immune lymphocytes caused little inflammation, though they developed strong, virus-specific CTL activity in recipient spleen and could be shown to be present in blood.

These findings indicate that there is a need for an appropriate, radiation-resistant, MHC-compatible cell in brain if T cells are to be recruited into, and cause severe inflammation at, the site of growth of LCMV. The likely target is the endothelium which can be shown to be infected with this virus.[76] Blood-borne monocytes (either adhering to endothelium or localized in the inflammatory site) are apparently unable to assume this antigen-presenting role, though the inflammatory process in acute LCM consists of approximately 50% monocyte/macrophages. Models proposing that monocytes attach nonspecifically to virally modified endothelium and then target the T cells, or that promotion of the inflammatory process is a consequence of interactions between blood-borne cells that have extravasated nonspecifically into the site of virus growth are not supported by these experiments.

CHARACTERISTICS OF VIRUS-INDUCED INFLAMMATORY EXUDATE

It is obvious that all categories of immune cells, and a variety of nonspecific effectors, can potentially localize to the site of a virus-induced pathologic process. The brain is no exception, with natural killer (NK) cells often making an early appearance,[77-79] CD8+ cytotoxic T cells and CD4+ lymphocytes being present for a time,[77,80,81] and B cells and plasma cells tending to remain *in situ* for considerable periods especially under conditions of viral persistence. I reviewed the then current situation in this area for the First International Congress of Neuroimmunology,[82] and there is little point in repeating the exercise here. However, more recently there have been two sets of systematic experimental studies with the Sindbis alphavirus, by Moench and Griffin,[80] and with LCMV,[81] in our laboratory.

Sindbis virus-induced meningitis is dominated by T cells,[80] particularly those of the CD8− phenotype (TABLE 2). The opposite situation applies in LCM,[81] where there are numerous monocyte/macrophages and the great majority of T cells are CD8+ CD4−. This finding is intriguing, as most people would consider that the

CD8− CD4+ population is the more potent at mediating monocyte recruitment to a site of pathology. However, it is also true that the CD8+ effectors are the cells that trigger delayed type hypersensitivity (DTH), as measured by footpad swelling, in the LCM model, and that this DTH correlates well with MHC-related patterns of disease susceptibility and CTL activity in CSF.[85,86] The CD8+ cells are also responsible for eliminating the virus from non-neural tissue,[87,88] though clearance from the brain may be incomplete.[89]

The reason for the very low levels of CD4+ T cells in CSF, but not blood, of mice with LCM may be that virus is growing in this T-cell subset[90] and they are thus being eliminated by CD8+ effectors. The dominance of T cells with the CD8+ phenotype applies in all models of LCM,[81] whether induced by direct inoculation of virus or by adoptive transfer of immune spleen cells into immunosuppressed, or unsuppressed, virus-infected recipients. It does not reflect that the majority of these CD8+ cells are LCMV specific. In fact, the preponderant CD8+ population seems to be nonspecifically recruited bystander cells that are not obviously involved directly in the disease process. Fatal LCM may, in fact, be triggered by relatively few specifically

TABLE 2. Characteristics of Meningitis at 6 or 7 Days after Inoculation of Virus

Cell Phenotype	Percentage of Positive Cells	
	LCM[81]	Sindbis[80]
Thy 1+	30	85
CD8+ CD4−	25	25
CD8− CD4+ (or Lytl+)	25[a]	40
Pgp,[b,c] or F/480+	60	0

[a] The level of CD4 staining was minimal, and both from this experiment and after nonspecific T-cell expansion *in vitro*, it was concluded that the CD4+ cells were not T lymphocytes.

[b] CD4+ T cells also predominate in the brain of people infected with Japanese encephalitis virus.[85]

[c] Monocyte/macrophages stain brightly for Pgp, whereas activated (but not resting) T cells are more weakly positive.[84]

sensitized, class I MHC-restricted CD8+ effectors that have localized to the site of virus growth in the CNS.

CONCLUSIONS

This brief review emphasizes the role of specifically sensitized T cells, and the sites of MHC glycoprotein expression, in the cell-mediated immune response to viruses growing in the CNS. Other aspects of this topic are addressed in greater detail elsewhere.[82,91] It should be recognized that virus-immune T cells, both CD4+ and CD8+, are likely to be specific for viral peptide in association with MHC molecules. Whether a particular viral protein is present in an intact form on cell surface may be largely irrelevant to T-cell surveillance, though the level of MHC glycoprotein expres-

sion may be crucial.[92] At least in the LCM model the induction of severe T-cell-mediated inflammatory process is totally MHC restricted, though many of the inflammatory cells are secondarily recruited as a consequence of the interaction of a few immune T cells with (perhaps) virus-infected endothelium. It therefore may not be too surprising that it is difficult to find evidence of clonality[93] in, for instance, CSF T cells from patients with multiple sclerosis.[94] Much of the inflammatory process in any acute viral disease of the CNS is likely to be "nonsense," though it would be intriguing to look at a much more chronic virus-induced inflammatory process (e.g., the TMEV model) from this aspect.

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