OLIGODENDROCYTES AND THE IMMUNE SYSTEM

Jack P. Antel and Theodora C. G. Ruijs

Department of Neurology and
McGill University
Montreal Neurological Institute
3801 University Street
Montreal, Quebec H3A 2B4

CNS DEMYELINATING DISEASES AND THE NERVOUS SYSTEM

I. Human Disorders

The existence of a common and frequently disabling human neurologic disease, multiple sclerosis (MS), which is characterized pathologically by inflammation (the hallmark of immune response) and demyelination within the central nervous system (CNS), has focussed interest on whether and how immune-mediated mechanisms can induce the observed tissue injury (Table 1). Inflammation and demyelination are also the hallmarks of acute disseminated encephalomyelitis (ADEM), a uniphasic disorder commonly encountered after immunization with nervous system-containing vaccines (e.g. Pasteur vaccine for rabies prevention) or specific exanthematous viral infections (measles) in which immune sensitization to myelin constituents can be demonstrated (Johnson et al., 1984). The more recently defined human disorder, HTLV-1-associated myelopathy (HAM) or tropical spastic paraparesis (TSP) is associated with oligodendrocyte (OGC)/myelin and axonal destruction and development of viral protein-directed cytotoxic lymphocytes; persistent virus is not yet detectible at the site of tissue injury within the CNS, invoking the postulate that immune-mediated mechanisms rather than direct viral mechanisms are involved (Moore et al., 1989). Similar considerations may apply to cases of CNS demyelination associated with HIV infection. Progressive multifocal leukoencephalopathy (PML), which most frequently occurs in immunocompromised individuals, provides a precedent for direct viral injury of OGC.

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II. Animal Models

Animal models have been developed to indicate that primary immune-mediated, direct viral-mediated, and viral-induced immune-mediated mechanisms can produce diseases characterized by myelin destruction within the CNS (see Table 1). Experimental allergic encephalomyelitis (EAE) can be induced in genetically-susceptible animals of defined age (e.g. 6-12 weeks in rodents) and sex (female) either by active immunization with whole CNS tissue, whole myelin or specific myelin proteins [myelin basic protein (MBP) or proteolipid protein (PLP)] or "encephalitogenic" peptide portion thereof) or by passive transfer of myelin-sensitized $CD4^+$ T-lymphocytes. With selected immunization schedules, a relapsing form of EAE can be induced in genetically-defined recipients. Although disease can be induced with peptide fragments as short as 9 amino acids, the extent of demyelination observed is augmented when whole spinal cord is utilized as immunogen; the latter may involve generation of both myelin antigen-sensitized T cells (a sine qua non for the disease) and myelin-directed antibodies. The extent of demyelination in EAE also increases with disease chronicity. Neuroimaging studies of cases of MS suggest that demyelination is also a late event compared to inflammation per se and may represent the correlate to irreversible neurologic deficit (Arnold et al., 1992). The precise immune effector mechanisms of target tissue injury in EAE leading to demyelination are not yet defined. Potential immune-mediated injury of CNS myelin or their cells of origins, oligodendrocytes (OGCs) are described subsequently. Numerous immuno-therapeutic approaches directed at specific "encephalitogenic" T cells (e.g. anti-T-cell receptor antibody, T-cell vaccination), T-cell trafficking (anti-cell surface adhesioin molecule, anti-major histocompatibility complex (MHC) class II antibody therapy) or non-specific suppression of the immune system are demonstrated to effectively inhibit development of acute or, in some instances, chronic relapsing EAE. In EAE, at least two myelin peptides (MBP and PLP) can serve as "encephalitogens" for T cells; of interest, MBP is not usually considered as being expressed on the myelin membrane surface. How putative OGC/myelin antigens can be presented to the immune system is described below. Although antibody alone cannot be used to transfer EAE, specific antibodies (particularly ones directed at glycolipids - e.g. myelin-OGC glycoprotein (MOG) and galactocerebroside) have been demonstrated to act in concert with the "encephalitogenic" T cells to augment the extent of CNS demyelination in EAE and in MS (Lassman et al., 1988). In MS, autoantibodies associated with OGC rather than myelin constituents of white matter are described (Wolfgram and Duquette, 1976).

The animal models characterized by CNS demyelination developing weeks-months after initial viral infection exemplify the induction of immune-mediated mechanisms capable of producing demyelination after initial viral infection. The precise mechanism of the tissue injury in these models is again not yet defined. Immunosuppression will alleviate these chronic demyelinating disorders but, in some cases, may result in acute lytic infection (Wang et al., 1990). One postulated mechanism for development of immune-effector responses involves the "molecular mimicry" concept in which putative shared antigenicity between constituents of viral proteins and CNS myelin would result in viral-directed immune mediators being able to recognize the CNS constituent. Myelin-reactive T cells were reported to exist in rats with chronic JHM infection (Wege et al., 1984). T cells sensitized to a homologous region of mouse hepatitis virus have been used to induce EAE

(Fujinami et al., 1985). No significant sequence homologies are known between measles virus and human CNS myelin, although both measles and myelin-reactive T cells can be recovered from individuals with MS and controls; such cells are not shown to be cross-reactive (Pette et al., 1992).

Table 1. Examples of human and animal CNS demyelinating diseases involving immune- and/or viral-mediated mechanisms.

Human Disorder	Mechanism	Animal Model	Response to Immunotherapy
Acute Disseminated Encephalomyelitis	**primary immune-mediated**	EAE - acute	+
		- relapsing	+
Progressive multifocal leukoencephalopathy	**direct viral-mediated**	acute JHM corona-virus infection	-
HTLV-1-associated myelopathy (HAM)		Theiler murine encephalomyelitis (TMEV)	+
or			
Tropical spastic paraparesis (TSP)	**viral-induced immune-mediated**	chronic JHM-coronavirus encephalomyelitis	+
HIV myelopathy		Herpes simplex-induced OGC-myelin injury	+

Studies of demyelination following herpes simplex inoculation via the trigeminal nerve further implicate host factors in determining susceptibility or resistance to viral-induced immune-mediated injury. Kastrukoff et al. (1987) have demonstrated, using *in vitro* techniques, that intrinsic properties of oligodendrocytes (i.e., those derived from different mouse strains) can contribute to the extent of viral-induced immune-mediated injury. In this regard, genetic susceptibility to both EAE and MS is shown to involve multiple genes, in addition to those directly involved in determining immune repertoire and regulation of expression of the MHC antigens, the critical recognition molecules for antigen-specific T cells on endothelial or glial cells within the CNS.

IMMUNE-MEDIATED EFFECTS ON OGCs/MYELIN - *IN-VITRO* STUDIES

I. Cell-Mediated

The existence and nature of specific immune-effector mechanisms which can induce negative (injury) or positive (survival and growth) effects on the OGC and/or its myelin membrane have mainly been explored to date *in vitro*. *In situ* data has, to date, largely involved unsuccessful attempts to demonstrate expression of specific molecules on OGC important for interaction with immune effectors, particularly T cells. Major histocompatibility complex (MHC) molecules, although observed *in situ* on astrocytes and microglia in humans and rodents in the CNS in inflammatory conditions, are not yet documented on OGCs. The difficulty of examining OGCs *in*

Table 2. Cell-mediated immune injury of OGC/myelin.

T CELL	- OGC lysis by rodent MBP-reactive, MHC-restricted cell lines.
α/β	- $CD8^+$ - class I MHC antigen restricted
	- non-MHC, non-antigen restricted.
	- $CD4^+$ - class II MHC antigen restricted lysis of astrocytes.
	- non-MHC, non-antigen restricted.
γ/δ	- ? heat shock proteins are recognition molecules.
MACROPHAGE-MICROGLIA	
	- phagocytosis - myelin stripping.
	- Fc receptors - antibody-dependent cell cytotoxicity.
	- via complement receptor - binding to antibody on target cell.

vivo limits observations regarding adhesion molecules or cytokine receptors. Attempts to express MHC molecules selectively in OGCs using transgenic animal approaches have resulted in OGC destruction unassociated with inflammation (Turnley et al., 1991).

The effects on the OGC/myelin complex of cell-mediated immune effector cells are summarized in Table 2. Current data suggest that OGCs do not express MHC class II molecules *in vivo* or *in vitro*, but do express class I MHC molecules, at least *in vitro* (Lee and Raine, 1989; Grenier et al., 1989). OGCs do express a series of receptors required for other cell interactions, including adhesion molecules. Human OGCs, in apparent contrast to rodent OGCs, do not express receptors for complement (Scolding et al., 1989). Our data also suggest they do not express receptors for the Fc portion of the Ig molecule, although contrary reports exist (Ma et al., 1980). Ovine OGCs are reported to be able to present antigen or mitogen to human T cells (Cashman and Noronha, 1986).

The *in vitro* effects of cellular-immune effectors on OGCs have been explored

using rodent- and human-derived cells. These assay systems have used OGCs in enriched (neuron-free) tissue culture systems; OGCs in these systems do extend processes expressing myelin proteins (see Fig. 1). Thus, these systems, to date, permit evaluation of the positive or negative effects of immune mediators on OGC survival and/or process extension, but not on myelination per se, if the latter is defined as wrapping of membrane around axons. Kawai and Zweiman (1990) reported that

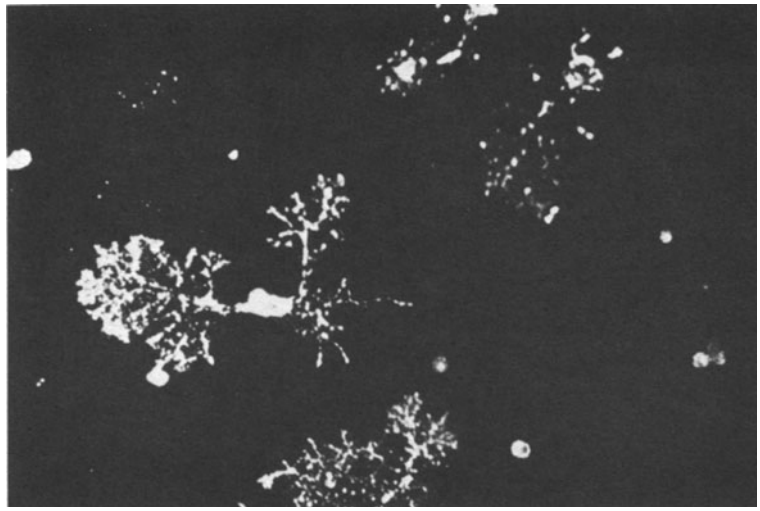

Figure 1. Dissociated 6-week old culture of human adult oligodendrocytes - immunostaining with galactocerebroside antibody.

myelin- reactive T-cell lines comprised largely of $CD4^+$ T cells reactive with MBP could induce lysis of OGCs, as measured by ^{51}chromium (Cr)-release cytotoxicity assays in an MHC-restricted manner, although the OGCs did not express MHC class II molecules. Antigen-presenting cells in the culture were a requirement. Whether the cytotoxicity involved recruitment of an intermediary cell present in the OGC culture, secretion of specific cytokines, or involved $CD8^+$ cells recognizing MHC class I molecules induced on the OGCs was not defined. Myelin-reactive $CD4^+$ T cells do acquire antigen-restricted cytotoxic capability when maintained *in vitro*, but MHC class II expression on targets is required for lysis to occur (Weber and Buurman, 1989; Fontana et al., 1984) (e.g. lysis of MHC class II-compatible rodent astrocytes or EB virus-transformed B cells by MBP-reactive T cells in the presence of exogenously added MBP.) Antigen-restricted MHC class II-restricted $CD4^+$ T-cell-mediated cytotoxicity is well demonstrated against viral infected cells - e.g. measles (Jacobson et al., 1984).

In vitro studies have also shown that $CD4^+$ T cells, as well as $CD8^+$ T cells, can acquire non-antigen non-MHC-restricted cytotoxicity capability (Kabelitz et al., 1989; Patel et al., 1987). This capability, termed promiscuous killing, was demonstrated initially by expanding mitogen-activated $CD4^+$ T cells *in vitro* with IL-2 under limiting dilution conditions; such conditions would seem not dissimilar to those in the CNS under inflammatory conditions. The $CD4^+$ T-cell cytotoxicity is

best demonstrated *in vitro* utilizing cytotoxicity assays of prolonged duration compared to those used to demonstrate antigen-specific cytotoxicity or γ/δ T-cell-mediated cytotoxicity (Ruijs et al., in press). We have observed that $CD4^+$ T cells derived from mitogen (± IL-2) long-term activated cultures generated under limiting dilution conditions or under short-term bulk culture conditions can induce lysis of human adult OGC *in vitro* in an 18-hour, but not 5-hour, ^{51}Cr-release assay. The mechanism(s) of target killing by such cells is not precisely defined but is neither MHC- nor antigen-restricted. An increased number of molecules are now identified which are produced and released by T cells which can act upon target cells which are in close apposition. Smyth et al. (1992), examining $CD4^+$ T-cell-mediated cytotoxicity in an 18-hour ^{51}Cr-release assay, implicated TNF as a mediator of the effect. Our data confirm that susceptibility to lysis using short-term bulk phytohemagglutinin (PHA)-activated $CD4^+$ T cells correlates with tumor necrosis factor (TNF) sensitivity of the target cells. The susceptibility of human OGCs to bulk-activated $CD4^+$ T-cell-mediated lysis is intermediate between TNF-resistant and TNF-susceptible cell lines. Long-term $CD4^+$ T-cell lines can lyse TNF-resistant cell lines (Patel et al., 1987). $CD4^+$ T-cell-mediated lysis of human OGCs as described above could not be reproduced by cell culture supernatants or addition of TNF at concentrations beyond those produced by $CD4^+$ T cells to the media. Deleterious effects of TNF or T-cell supernatants on OGCs over a prolongued time period are discussed below. Our results support the concept of cytotoxicity involving both cell-cell interaction and soluble mediators released into the local environment.

$CD8^+$ T cells are considered the classic phenotypic cytotoxic cells whose activity is restricted both by antigen and MHC molecules (usually class I). We observed significant human OGC-directed specific cytotoxicity in a 5-hour ^{51}Cr-release assay using as effectors $CD8^+$ cells sensitized to the MHC class I antigens expressed by the OGCs (Ruijs et al., 1990a). Jewtoukoff et al. (1989) have described a $CD8^+$ T-cell clone expressing the α/β T-cell receptor (TcR) which was cytotoxic to both mouse and rat OGCs; MOG was identified as the putative target molecule. As mentioned, $CD8^+$ T cells can acquire "promiscuous" capability, which can result in OGC injury. These "promiscuous" $CD8^+$ T cells are $CD3^+$, α/β TcR positive, and thus differ phenotypically from natural killer (NK) cells. OGCs are reported to be resistant to NK cells (Satoh et al., 1990).

A lineage of T cells distinct from the above-described α/β TcR-expressing T cells is defined by expression of γ/δ chains of the TcR. These cells exhibit potent cytotoxicity directed against OGCs (Freedman et al., 1991a). The antigen on OGCs recognized by these cells remains speculative, with heat-shock proteins (HSPs) being candidate molecules; Tγ/δ cell recognition of HSPs expressed on microbial agents has been demonstrated (Haregewoin et al., 1989). The proportion of γ/δ T cells amongst inflammatory cells present in the CNS lesions of MS is increased (Selmaj et al., 1991a). *In vitro* co-culture of T cells with human OGCs results in a relatively greater expansion of Tγ/δ cells compared to some other cell lines (e.g. U937 monocyte tumor line) (Freedman et al., 1991b). OGCs, both *in vivo* and *in vitro*, express increased levels of HSPs, both constitutively and in response to heat stress, compared to astroglial cells (Selmaj et al., 1992; Freedman et al., in press). Although the above discussion on T-cell-mediated injury of OGC has specifically described cytotoxicity, one could postulate that lesser degrees of injury might also occur, which could manifest as impaired stability, production, or repair of the cell's membrane (i.e., myelin).

Macrophage/microglia-mediated injury of OGC/myelin could involve multiple mechanisms. These cells avidly phagocytose or ingest myelin debris which, in turn, could be processed and presented as antigens to infiltrating T cells (Frei et al., 1987; Williams et al., 1992). Macrophage/microglia stripping of myelin membranes has been demonstrated in MS lesions. The inflammatory mediators released by infiltrating T cells "activate" microglia/macrophages, and thus potentially contribute to their effector potentials. Macrophages/microglia can bind to cell targets via distinct receptors, some of which require intermediary molecules. Examples of these include antibody which binds via its specific receptor portion (Fab) to the target and via its Fc receptor to the macrophage/microglia cell; this process is termed antibody-dependent cell cytotoxicity (ADCC) and is a potential mechanism of immune-mediated OGC injury (Merrill and Zimmerman, 1991; Prineas and Graham, 1981; Scolding and Compston, 1991). Complement is an additional molecule which can link macrophage/microglia with a potential cell target (Bruck and Friede, 1990). As will be discussed, human and rodent OGCs may differ with respect to ability to bind complement.

II. Soluble Factor-Mediated

Soluble mediators produced by lymphocytes and monocytes were initially termed lymphokines (or interleukin) and monokines respectively, and shown to be important mediators of cell-cell communication within the immune system. These molecules are now shown both to interact with and be produced by cells within other body systems and are generally referred to as cytokines. With reference to the CNS, both the neuronal and glial elements respond to and produce specific cytokines; these molecules thus are important mediators of cell-cell interactions amongst endogenous CNS cells (e.g. neuron-glia, glia-glia) and immune-neural interactions under both physiologic and pathologic conditions. Cytokine interactions with neurotrophins and neurotransmitters indicate the complexities of modulatory influences which cytokines can have on the CNS. Thus, in assessing immune effects on OGCs, one need consider the overall effects of cytokines on the CNS environment which in turn would influence the biologic function of OGC/myelin in addition to the more specific or direct immune effects on OGC/myelin, as described below (Table 3).

Soluble molecules acting either alone or in concert with cell-mediated immune mechanisms, have been implicated as effectors of OGC/myelin injury. Crude supernatants derived from either T-cell macrophage or astrocyte cultures have been observed to induce OGC/myelin injury. Tuberculin-activated lymphocyte/monocyte cultures can induce demyelination when injected *in vivo* into the rabbit retina; this process is termed bystander demyelination and is shown to be mediated by soluble products, particularly those released by monocytes (Brosnan, 1988). Supernatants from cultures of T cells activated either by myelin-relevant or irrelevant antigens can induce both myelin injury in aggregated tissue culture and morphologic changes in OGCs grown in dissociated cell culture (Selmaj and Raine, 1988). These latter changes tend to be observed over days rather than hours, in contrast to the cell-mediated cytotoxicity effects. The major cytokines implicated as mediating the effects are TNF and lymphotoxin (LT). Specific effects on OGCs *in vitro* now observed using recombinant TNF and LT include retraction of process formation, altered expression of K^+ channels and cell death via the process of apoptosis (Selmaj

et al., 1991b; Soliven et al., 1988). As with effects of cytokines on other glial cells, one need consider that the net effect of inflammatory cell infiltration in the region of OGC/myelin most likely involves the combined positive and negative effects of multiple cytokines. Precedent for "positive" effects of cytokines on OGCs includes the observation by Merrill et al. ((1984) that T-cell-derived supernatant and recombinant IL-2 can promote proliferation of prenatal OGCs. To date, proliferation of adult OGCs has not been observed *in vitro*, but has been described *in vivo* in viral-induced demyelinating disease, and possibly in MS (Ludwin, 1987). The array of microglia/macrophage products which could induce OGC/myelin injury include proteases, free radicals, complement, and TNF (Griot et al., 1990).

The role of antibody in mediating OGC/myelin injury in autoimmune demyelinating disease requires clarification. In the human disease MS, enhanced levels of intrathecal Ig production is a disease hallmark. Although the bulk of this Ig is not myelin- or OGC-directed, "immunospot" assays do demonstrate secretion of antibody directed against a number of myelin antigens, including MBP, GalC, and MOG (Olsson et al., 1990). Sera of MS patients and EAE animals immunized with whole spinal cord contain anti-myelin antibodies, particularly against lipid moeities. In the EAE model, coincident systemic administration of anti-MOG antibody with sub-encephalitogenic numbers of myelin-reactive T cells results in a demyelinating form of EAE, not induced by either immune reagent alone (Schluesener et al., 1987). The array of potential immunogenic OGC and myelin proteins and their post-translational modifications remains to be defined.

Complement-mediated OGC injury, even in the absence of antibody, has been demonstrated using rodent OGCs (Scolding et al., 1989). Complement binding to OGCs results in generation of the membrane attack complex (MAC). This complex can induce vesicular disruption of the OGC membrane. Human serum is cytotoxic to rat OGCs, but not human OGCs (Ruijs et al., 1990b); the activity is destroyed by heating sera to 56° C. These data indicate that human OGCs may be less susceptible to complement-mediated injury than are rodent OGCs.

SUMMARY

The OGC/myelin complex would appear to be susceptible to an array of immune-mediated effector mechanisms which would be expected to be activated by an inflammatory response occurring within the CNS. Such responses could reflect responses primarily directed against a normal or altered OGC/myelin complex, responses against exogenous antigens present in the CNS or cross-reacting with CNS (molecular mimicry), or non-OGC/myelin-directed responses in which the latter are "bystander" targets. Most studies have focussed on the relatively readily observed effects of cell lysis or major morphologic changes. More subtle changes resulting either from primary injury to the OGC cell body or myelin membrane could be reduced production, maintenance, or repair of myelin. Precedent for cell injury resulting in reduced function without morphologic change is provided by evidence that viral infection can reduce function of pituitary (hormone production) cells without morphologic abnormalities - i.e., concept of selective loss of luxury functions (Oldstone et al., 1984). More precise definition of mechanisms of immune-mediated OGC/myelin injury should hold promise for more rational means to prevent or reverse its occurrence.

Table 3. Soluble immune system mediator injury of OGC/myelin.

Soluble mediators	**Effect on OGC-myelin**
T-cell-derived	
Direct	
- T-cell supernatant	promote OGC survival and proliferation
- TNF-α and β	induces demyelination *in vitro*, reduces OGC extension, alters K+ channel properties, mediates OGC cell death via apoptosis
Indirect	
- via microglia/macrophage	induce cytokine production
or astrocyte activation	or cell-mediated effector mechanisms
- induction of neurotrophins	
B-cell-derived (Ig, antibody)	
- anti-lipid, glycolipid	demyelination
- combined with T cell	demyelination
Complement	Ab-dependent or independent injury of OGC or myelin
Microglia/macrophage-derived	
- protease	
- free radicals	OGC/myelin injury
- protect with astrocytes	"bystander demyelination"
- TNF-α, complement	

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