Mechanisms of tissue injury in multiple sclerosis: opportunities for neuroprotective therapy

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Summary. Development of neuroprotective therapies for multiple sclerosis is dependent on defining the precise mechanisms whereby immune effector cells and molecules are able to induce relatively selective injury of oligodendrocytes (OLs) and their myelin membranes. The selectivity of this injury could be conferred either by the properties of the effectors or the targets. The former would involve antigen specific recognition by either antibody or T cell receptor of the adaptive immune system. OLs are also susceptible to non antigen restricted injury mediated by components of the innate immune system including macrophages/microglia and NK cells. Target related selectivity could reflect the expression of death inducing surface receptors (such as Fas or TNFR-1) required for interaction with effector mediators and subsequent intracellular signaling pathways, including the caspase cascade. Development of therapeutic delivery systems, which would reach the site of disease activity within the CNS, will permit the administration of inhibitors either of the cell death pathway or of effector target interaction and opens new avenues to neuroprotection approach.

Introduction

In this report, we attempt to address the issues of the mechanisms of tissue injury which underlie the development of multiple sclerosis and how the extent of such injury may be reduced by neuroprotective therapies. We will consider these questions in terms of the following: a) how the detailed analysis of tissues derived from MS patients and serial neuroimaging studies establish the immunopathogenic basis of MS; b) the evidence that autoimmune mechanisms can mediate CNS demyelinating disease; c) the potential immune effector cells present in MS lesions d) the mechanisms whereby immune effector can induce the observed tissue injury e) and potential means whereby therapies directed at events occurring within the CNS can be of therapeutic value.

Pathogenesis of MS

Multiple sclerosis has been recognized as a clinical pathologic entity since the mid-1800s. The early pathologic studies described the multifocal regions of

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demyelination within the central nervous system (CNS) with the relative but not absolute sparing of axons coursing through sites of demyelination. These early studies recognized the presence of inflammatory cells within lesion sites raising the hypothesis that continues to the present that MS is an immune mediated disorder which may be initiated or sustained by virus infection. The advent of magnetic resonance (MR) based imaging technologies in the 1980s has provided a means to evaluate the dynamic evolution of the MS disease process in large numbers of MS patients. Studies using conventional MRI, based on displacement of water molecules, indicate that the frequency of new MRI defined lesion formation far exceeds the frequency of clinical relapses in patients in the relapsing-remitting phase of disease, the most common early disease phenotype. Many of these lesions are associated with disruption of the blood brain barrier as evidenced by gadolinium enhancement. Imaging-pathologic correlative studies indicate that these lesions are characterized by lymphocyte infiltration. Many of these lesions will disappear within 4–6 weeks indicating that inflammation needs not always be associated with irreversible tissue injury.

The chronic active MS lesion is characterized by presence of activated macrophages and microglia at the lesion edge with a relative paucity of lymphocytes. MRI studies indicate that the volume of persistent lesions does increase over time even in apparent absence of new influx of exogenous inflammatory cells suggesting that resident cells of the CNS can contribute to the tissue injury. At least 50% of MS cases eventually (10–15 years) evolve into a more progressive disease form with or without continued intermixed relapses.

Molecular and immunopathologic examination of active MS lesions have focused on the fate of the OL and whether one can distinguish whether the cell itself or its myelin membrane is the primary target of the disease (Lassmann et al., 1998). There is increasing recognition that there is a heterogeneity of pathologies ranging from lesions with significant OL loss, lesions with dying back of distal myelin membranes suggesting cell body dysfunction, and lesions with myelin loss with relative OL preservation (Lucchinetti et al., 1996). Antibody deposition is a characteristic of the lesions associated with apparent primary myelin injury. Controversy exists as to the extent by which OL death occurs in situ via apoptosis or lysis. This may in part reflect the limitations of tissue sampling. To be resolved is whether all the lesions in any one MS case have one distinct pathologic sub-type. Chronic MS lesions feature loss of both myelin and OLs.

Recent pathologic and MR based analyses have re-emphasized the initial observations of Charcot that there is an element of axonal injury and loss in MS and this may account for a greater than previously expected portion of the neurologic disability which develops in these patients. The MR based technique, termed MR spectroscopy (MRS), which is dependent on suppression of the usual water signal detected on conventional MRI, permits detection of N-acetyl aspartate (NAA) which in the adult human brain is restricted to neurons. NAA values are reduced in acute MRI defined lesions associated with clinical deficits; these value recover, at least partially, in concert with clinical

recovery (De Stefano et al., 1998). Recent immunocytochemical studies of active MS lesions document a high frequency of transected axons in such lesions (Trapp et al., 1998). Other axons show features consistent with demyelination including expression of non phosphorylated forms of neurofilaments, a change which can result in loss of axonal volume, and a wide re-distribution of sodium channels. Measures of brain volume also show evidence of axonal loss based on presence of significant atrophy in MS. These observations suggest that axonal injury in MS reflects both direct axonal injury or loss of oligodendrocyte (OL)/myelin trophic support; conversely recovery could reflect reversal of either of these processes. Remyelination has been well documented in MS pathologic specimens.

Autoimmune mediated disease of the CNS

The observations made in the late 1880s that a number of individuals injected with the Pasteur anti-rabies vaccine, prepared using phenol treated rabies infected spinal cord, subsequently developed a paralytic illness now referred to as post-vaccination or acute disseminated encephalomyelitis (ADEM), established that the CNS could be the target of an auto-immune response. This illness, characterized by inflammation and demyelination within the CNS, typically, in contrast to MS, follows a uniphasic course. ADEM can be reproduced in animals by systemic injection of either whole neural tissue or purified myelin antigens or with T cells sensitized to such antigens; this model is now most commonly referred to as experimental autoimmune encephalomyelitis (EAE). EAE models have been developed which reflect each of the pathologies described in MS, as have models which follow a relapsing or progressive disease course. In these chronic disease models one can demonstrate that over time there is expansion of the neural antigens recognized beyond the initiating one, a process referred to as determinant spreading (Miller et al., 1997). This process could reflect either antigen presentation within the CNS or transport of antigen to regional lymph nodes. The Theiler murine encephalomyelitis virus and JHEM coronavirus models provide evidence that chronic immune mediated demyelinating CNS disease can also develop secondarily to initial direct CNS virus infection dependent on development of immune sensitization to neural antigens. Although in all these experimental models, autoreactive CD4 T cells are a requirement for initiating or sustaining the disease process, an array of additional cellular and humoral immune mediators, derived from infiltrating and/or resident cells may be the actual effectors of the tissue injury.

Immune effector mechanisms in MS

In this section we will consider the array of potential immune effector cells and molecules found in the active lesion sites and how the properties of these effectors or of the target tissues with which they interact determine the pathologic features of MS described above. We will specifically focus on how these

processes result in the relatively selective injury of oligodendrocytes and their myelin membranes, which characterizes MS.

The immune system can be divided into two components, namely the adaptive immune system and the innate immune system. The former is characterized by cells whose receptors undergo rearrangements as the cells undergo maturation; this confers fine antigen specificity to these cells although their responses are delayed relative to the innate immune system responses. α/β CD4 and CD8 T cells and B cells are constituents of the adaptive immune system. Either T cells or antibodies could recognize an OL/myelin specific antigen. The former recognize an antigen-MHC complex

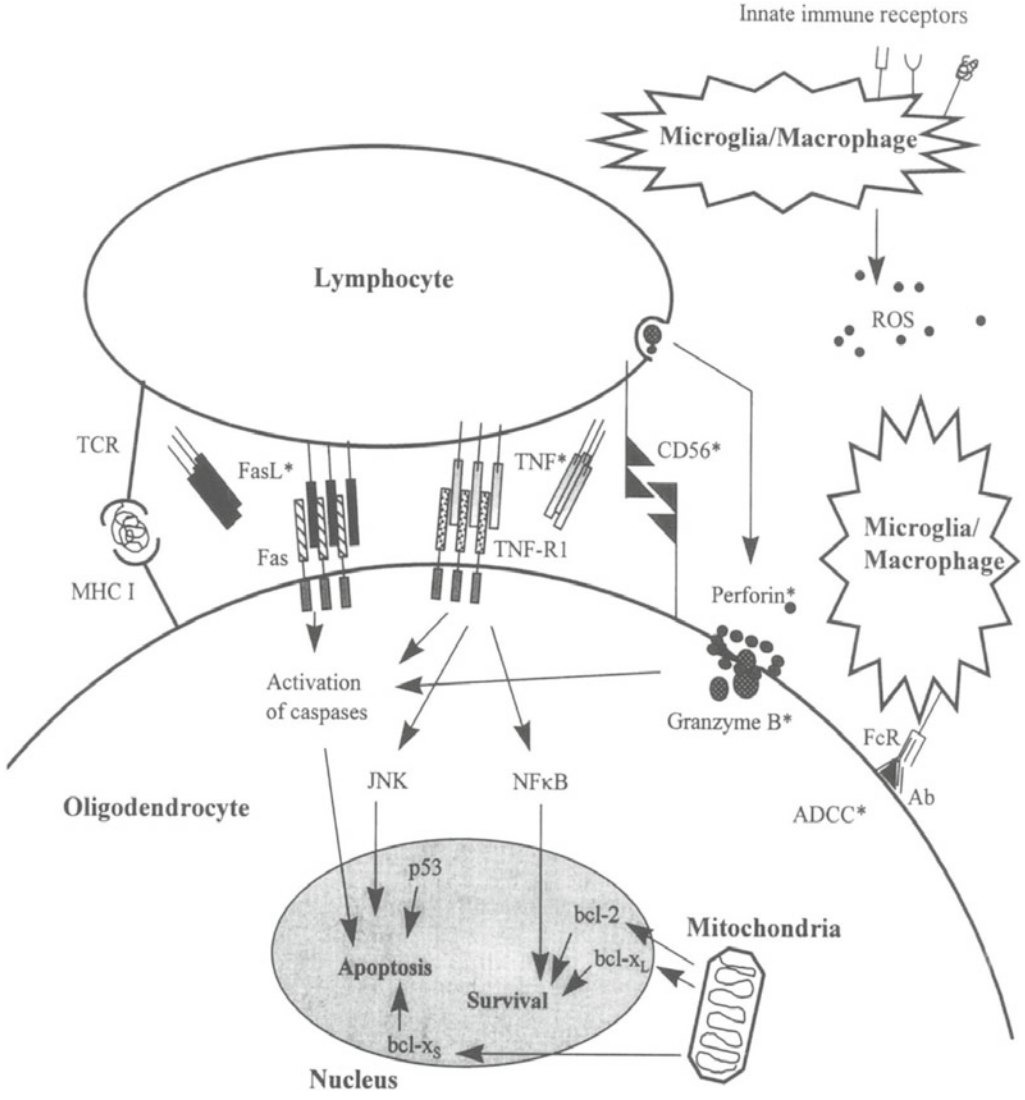

Fig. 1. Schematic representation of some of the potential immune effector cells and mediators which can induce oligodendrocyte injury and the intracellular signaling pathways involved in the injury response. The pathways which also involve NK cells are represented with a *

whereas the latter recognize their target directly. The cells of the innate immune system have fixed receptors which when engaged result in rapid responses. Cells included in this system and which are found in MS lesions include macrophages/microglia, NK cells, and γ/δ T cells. As will be discussed cells and molecules derived from both systems can effect oligodendrocyte directed injury.

Much emphasis, to date, has been placed on the role of myelin specific CD4 T cells in the immunopathogenesis of MS. This cell type is the predominant T cell within the acute MS lesion. The CD4 T cell is the cell type that is required for the adoptive transfer of EAE. Myelin reactive CD4 T cells generated in vitro can possess cytotoxic potential (Antel et al., 1994). However, oligodendrocytes do not seem to express MHC class II molecules, a requirement for antigen restricted CD4 T cell mediated cytotoxicity. To date, CD4 myelin reactive T cells have not been shown to induce MHC restricted lysis of OLs in vitro. A subpopulation of such T cells are, however, capable of expressing additional surface molecules which will permit non-MHC restricted interaction with OLs (Antel et al., 1998). We found that MBP reactive T cell lines which expressed CD56 (neural cell adhesion molecule) were cytotoxic to human OLs in vitro: the OLs also express CD56 permitting the homotypic interaction with these T cells. The in vitro conditions which promote CD56 expression on T cells, namely cell activation by pro-inflammatory cytokines, would largely seem to be simulated by the in situ inflammatory milieu of the active MS lesion (Vergelli et al., 1996). Efficiency of killing by such T cells was dependent on additional adhesion molecules including ICAM, also expressed by OLs. Target cells lacking CD56 expression were not susceptible to such injury. Activated NK cells, which also express CD56 also induce non MHC restricted OL cytotoxicity.

α/β CD8 T cells are the classic cytotoxic lymphocytes. These cells recognize foreign peptides presented by MHC-class I molecules widely expressed within all tissues. As regards OL susceptibility to antigen restricted T cell injury, we have shown that OLs express MHC class I molecules in vitro and are susceptible to MHC restricted cytotoxicity mediated by MBP peptide specific CD8 T cells (Jurewicz et al., 1998). OLs are also highly susceptible to non MHC restricted injury mediated via γ/δ T cells; the candidate recognition molecule for these cells include members of the heat shock protein family, which are shown to be inducible on OLs.

Myelin specific antibodies have been demonstrated in the CSF and tissues of MS patient. Such antibodies, acting in concert with serum complement proteins which can bind to Abs, could induce direct OL injury. Secreted Abs produced by neuroantigen-specific B cells can link the adaptive immune system with the innate (non-specific) immune system. NK cells and macrophages/microglia have innate immune receptors (Fc-receptors) allowing them to recognize surface bound antibodies (opsonized cells) and to mediate antibody dependent cell cytotoxicity (ADCC). This will eventually lead to phagocytosis of cellular debris by the microglia/macrophages.

Molecular mediators of OL injury

The actual molecular mechanisms by which the effector cells described above interact with and induce injury of OLs continue to be defined. In the inflamed CNS, these effector cells produce an array of molecules capable of inducing cell death. These molecules can be considered in terms of those which exert their cytotoxicity via receptors expressed by the target cells and those which are receptor independent mediators. The former can be divided into surface bound and soluble ligands. Amongst the receptor independent molecules are reactive oxygen species (ROS) including nitric oxide and super-oxide anion (O^{2-}), excitotoxins (glutamate), and proteases; microglia, macrophages, and to some extent astrocytes are major sources of these molecules. The perforin/granzyme system is the apparent predominant mediator of the OL cytotoxicity induced by α/β and γ/δ T cells and NK cells (Zeine et al., 1998). Perforin is a pore forming protein that aggregates and punches holes in the membrane of the target cell. Granzyme B (grbB) then infiltrates the target cell through the perforin formed tunnels and induces apoptosis by feeding into the death receptor pathway as described below. In our in vitro studies, the panel of cytotoxic lymphoid cells evaluated predominantly used non receptor mediated mechanisms, presumably perforin/granzymes.

In contrast to the above, receptor-dependent injury is mediated by so called death receptors which are members of the tumor necrosis factor (TNF)-receptor superfamily (review in Thorneberry et al., 1998). Such receptors (R) would include CD95 (Fas) and TNF-R1. Fas and TNF-Rs are detected on OLs in situ in active MS lesions (D'Souza et al., 1996a; Dowling et al., 1996). The ligands (L) for these death receptors are amongst the growing family of TNF-related molecules. Fas-L and TNF molecules are expressed on the cell surface of cytotoxic lymphocytes, NK cells, macrophages, and possibly microglia, especially when these cells are activated as occurs in an inflammatory milieu. In the immature human CNS, astrocytes are also a potent source of this cytokine (Lafortune et al., 1996). Both TNF and Fas can be proteolytically cleaved from the cell surface by membrane associated metalloproteases, giving rise to a soluble form. Both the soluble and the membrane bound form of Fas-L induce trimerization of Fas on the target cell resulting in activation of a signaling cascade that in some cells culminates in cellular apoptosis. For Fas and TNF-RI, this involves recruitment of caspase 8 which in turn initiates proteolytic activation of other ICE family members (caspases) which ultimately leads to apoptosis. Activation of TNF-R1 signaling also activates other signaling pathways, including those involving NFκB transcriptional complexes and jun kinases. The NFκB pathway has now been identified to provide survival/anti-apoptotic signals.

We have used dissociated cultures of human adult CNS derived OLs to evaluate the susceptibility of these cells to injury mediated via TNF-R superfamily. We compared responses of OLs, with those of other primary and transformed neural and non neural cells in order to determine whether and how a target cell can determine selective susceptibility or resistance, to effector mechanisms which themselves are not antigen restricted. We considered

that selectivity could be conferred either by the presence of the receptor on the target cell surface or by the intracellular signaling cascades that it induced. As a source of human OLs, we have used surgical tissue resected from young adults undergoing temporal lobe resections as a surgical treatment for intractable epilepsy. Our method has been described in detail (Yong et al., 1997). For comparative studies we have utilized fetal human derived astrocytes and neurons, U251 human glioma cell line, and human lymphoid cell line.

We found Fas to be present on human OLs in culture albeit at very low levels compared to astrocytes and glioma cells. When the receptor was triggered by FasL or by an activating anti-Fas mAb, OLs seemed to undergo lysis (LDH release) rather than apoptosis (TUNEL staining) (D'Souza et al., 1996a). We are currently pursuing studies as regards a possible upregulation of that receptor by immune regulators, and whether such an upregulation might result in detection of activation of the apoptosis pathway (Pouly et al., 2000). Fetal astrocytes, despite their high levels of Fas expression, were resistant to Fas mediated killing (Becher et al., 1998).

We have observed that exposure of OLs to relatively high levels of TNFα (1,000 units per ml for 4 days) resulted in very little cytotoxicity as measured by LDH release but did result in a significant proportion of the cells showing evidence of DNA fragmentation as measured by TUNEL staining (D'Souza et al., 1995). Toxic effects of TNFα and β on OLs derived from different species and varying maturity are reported (reviewed in Raine et al., 1997). In our studies, fetal neurons and astrocytes were more resistant to TNFα induced injury than were the OLs, whereas Jurkat cells were more susceptible. One should however consider that most of the in vitro studies have been conducted in serum deprived medium.

TNF is now established in various cell systems to induce a number of intracellular signaling pathways including caspases, JNK and NFκB. We can document induction of all these pathways by TNF in human OLs. We further observed that JNK activation was also associated with up-regulation of p53 expression, which would eventually lead to apoptosis, as shown by studies in which OLs infected with a p53 containing recombinant adenovirus construct would undergo significantly increased apoptosis (Ladiwala et al., 1999). We have found that the human OLs constitutively expressed the p75 low affinity NGF receptor but not high affinity NGF receptors (Ladiwala et al., 1997). Signaling through this receptor by use of NGF has previously been reported to induce cell death in immature rodent derived OLs. We did not, however, observe any cell death in our human OLs upon exposure to NGF, nor JNK activation, even though NFκB was activated. These findings would support the postulate that signaling via caspases and JNK would represent an injury response whereas signaling via NFκB is likely to be protective.

Placing the results of our in vitro studies with TNFα in context of in situ studies of autoimmune CNS disease in which TNF expression is manipulated or its activity inhibited is difficult given that this cytokine participates in multiple immune regulatory, as well as immune effector processes. TNF when selectively overexpressed in the CNS at high levels induces demyelination (review in Probert et al., 1997); at modest levels it increases the severity and

chronicity of EAE (Taupin et al., 1997). However, TNF knock out animals are also more susceptible to development of EAE and the disease is more severe (Probert et al., 1997). Such data infer an immune regulatory role for this molecule.

Protection

Multiple strategies related to protection of OLs or other neural cells to immune mediated injury can be envisioned. These could be directed either at the effector or target side of the injury response. Modifying the CNS environment to become less supportive of immune reactivity would decrease production of the array of immune effector molecules referred to previously. A challenge in context of CNS restricted disease is whether novel means to deliver molecules with anti-inflammatory effects to the CNS directly can be developed. Such possibilities include use of generation of CNS antigen specific T cells producing such molecules. The antigen specificity is a requirement for their recruitment and persistence in the CNS. Enhanced anti-inflammatory cytokine production by T cells is the proposed means of action of such MS therapies as oral tolerance, Copaxone, and altered peptide ligand immunization. Further studies are expected using CNS specific T cells or neurotropic viruses, engineered to overexpress such molecules, as a means to deliver these molecules to lesion sites within the CNS.

We have previously shown that astrocytes can protect OLs from some types of injury such as those dependent on reactive oxygen species (ROS), presumably by removing these mediators (Noble et al., 1994). Astrocytes are also the source of several growth factors which have been shown to promote OL survival as well as remyelination (CNTF, PDGF, FGF, IGF). On the other hand, astrocytes also produce NO, which might act as an injury mediator (Tran et al., 1997). Similarly, microglia are a source of molecules which can both effect injury, as outlined previously, or promote regeneration. Thus understanding and eventually manipulating the properties of the CNS environment is a therapeutic approach that will remain under study.

As regards protection based on therapies directed at the target cell, our in vitro OL injury assay systems illustrate that protection measures are dependent on the nature of the injury. We observed that CNTF, a molecule produced within the CNS mainly by astrocytes, could protect the human OLs from TNF induced injury but not from Fas mediated injury (D'Souza et al., 1996b). This molecule was not effective in protecting the OLs from either cytotoxic T cell mediated injury, which as mentioned likely reflect a perforin/granzyme mediated effect or from direct Fas mediated injury. CNTF and other neurotrophins have been already tested clinically in neurodegenerative diseases, specifically ALS. These studies illustrate the concerns regarding systemic side effects and the challenge of how to ensure delivery of such molecules into the disease site. Further caution is required with regard to cytokine manipulation therapy in view of results that whereas a systemically administered TNF-R:Ig molecule inhibits EAE, exacerbations of MS are increased. This also brings up the general dilemma as to when one proceeds

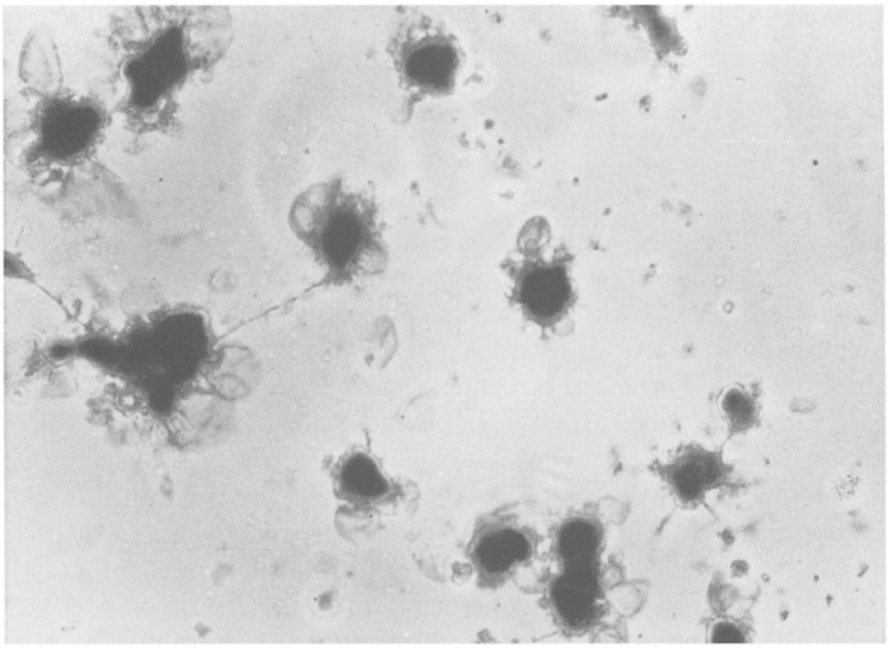

Fig. 2. Adenovirus-mediated gene transfer of lacZ into adult human OLs in culture; at 24 h post-infection — almost all cells show lacZ expression as determined by beta-galactosidase staining

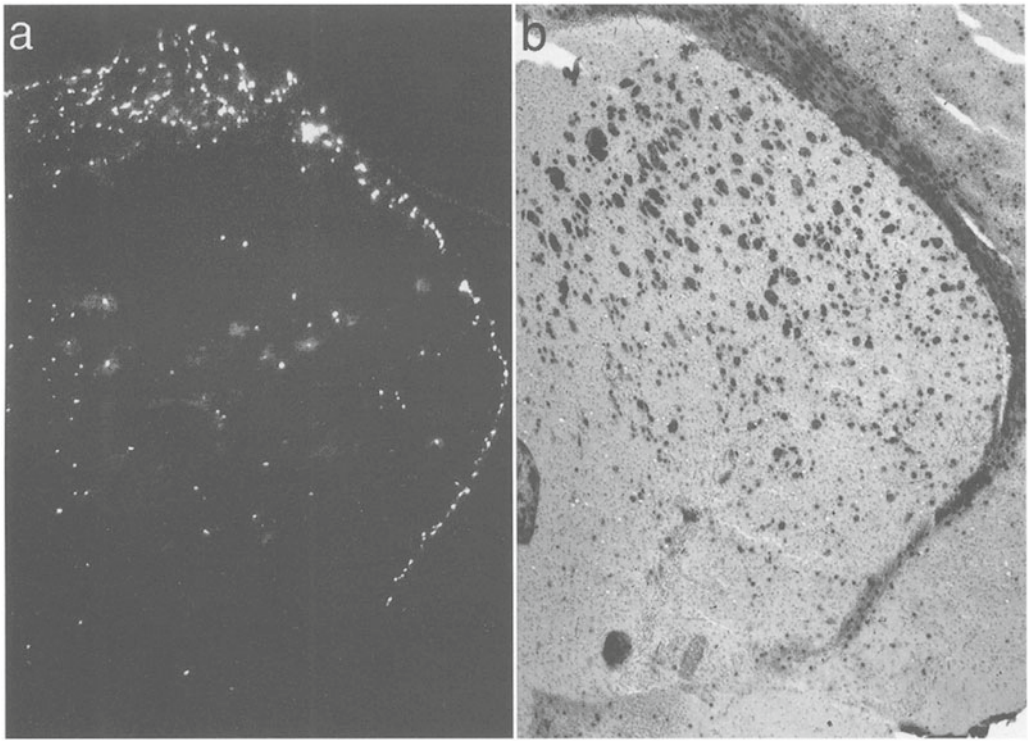

Fig. 3. Preferential infection of oligodendrocytes in the corpus callosum of MBP lacZ transgenic mice (gift from Alan Peterson) when AVCMV p53 is injected into the caudate. **a** Immunostaining for p53 expression in coronal sections of these transgenic mice shows localization of p53 to the **b** oligodendrocytes marked by MBP-regulated beta-galactosidase expression as seen on a serial section

from animal models to human clinical trials. A challenge is to design appropriate studies to evaluate mechanisms of actions of therapies in patients participating in such trials.

Protection related to the target side of the injury response could involve either manipulation of the signaling pathways involved in the injury response or induction of protective pathways. Anti-inflammatory molecules or specific new drugs might be used to modulate the activation or inhibition of signaling pathways. As regards intracellular signaling pathways, caspase inhibitors have already been shown to inhibit the severity of other neurological disorders, including ischemic injury (Cheng et al., 1998). Whether OL resistance to injury in vitro and in situ can be enhanced by induction or insertion of anti-apototic molecules (bcl-2, bcl-x_L) needs to be established. The advent of techniques such as viral vectors to insert genes into non dividing cells opens up the possibilities for developing this means of neuroprotection. Studies from our lab illustrate the use of recombinant adenovirus constructs as means to introduce genes in OL specifically, as shown in vitro (Fig. 2) and in situ (Fig. 3). Many of the injury effector mechanisms considered in this report are likely to play a role in various extent in other diseases of the CNS, thus the principles of neuroprotection learned from one disease or model are likely to have wide applicability.

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