PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2005) **360**, 1775–1795
doi:10.1098/rstb.2005.1700
Published online 16 August 2005

Glial grafting for demyelinating disease

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Remyelination of demyelinated central nervous system (CNS) axons is considered as a potential treatment for multiple sclerosis, and it has been achieved in experimental models of demyelination by transplantation of pro-myelinating cells. However, the experiments undertaken have not addressed the need for tissue-type matching in order to achieve graft-mediated remyelination since they were performed in conditions in which the chance for graft rejection was minimized. This article focuses on the factors determining survival of allogeneic oligodendrocyte lineage cells and their contribution to the remyelination of demyelinating CNS lesions. The immune status of the CNS as well as the suitability of different models of demyelination for graft rejection studies are discussed, and ways of enhancing allogeneic oligodendrocyte-mediated remyelination are presented. Finally, the effects of glial graft rejection on host remyelination are described, highlighting the potential benefits of the acute CNS inflammatory response for myelin repair.

Keywords: multiple sclerosis; central nervous system; oligodendrocytes; oligodendrocyte precursors; graft rejection; remyelination

1. INTRODUCTION

There are a number of human conditions characterized by loss of central nervous system (CNS) myelin, the commonest of which is multiple sclerosis (MS). In this disease an immune attack targets myelin sheaths and the cells that produce them, the oligodendrocytes, which results in focal areas of demyelination (plaques) scattered throughout the white matter of the CNS (Lassmann 2004). Although remyelination of the demyelinated axons can occur, this process becomes defective as the disease progresses resulting in remyelination failure (Lassmann 2004). Demyelination of axons affects axonal conduction, and is associated with axonal loss, which results in progressive neurological handicap (Weinshenker 1998). Because there is an association between remyelination failure and disease progression, enhancement of remyelination has become a therapeutic goal in MS research. In principle, remyelination of axons could be achieved by enhancing endogenous remyelination or by transplantation of exogenous myelin-forming cells. Attempts to enhance endogenous remyelination by infusion of trophic factors or cytokines have rendered variable results, and this could be due to the fact that trophic factors have pleiotrophic effects on the brain (reviewed in Imitola *et al.* 2003), or that the animal models used do not represent examples of remyelination failure. Transplant-mediated remyelination, however, has been achieved in a variety of animal models of demyelination and shown to result in functional recovery (reviewed in Baron-Van Evercooren & Blakemore 2004).

This article reviews our current understanding of glial cell transplantation in demyelinating diseases and addresses, based on the limited amount of specific information available, to what extent tissue matching will be an essential requirement for successful cell therapy.

2. CELL PREPARATIONS THAT COULD BE USED FOR TRANSPLANT-MEDIATED REMYELINATION OF DEMYELINATING LESIONS

Remyelination of the demyelinating lesion can be achieved by transplanting several different cell preparations. These include cells derived from the immature and the adult CNS, Schwann cells, olfactory ensheathing cells and cells from non-neural tissues. Even though the ability of cells to remyelinate demyelinating CNS axons has been established in animal models of demyelination, the suitability of some of these cell types for potential clinical therapies must be questioned. While Schwann cells remyelinate certain types of demyelinated lesions efficiently, with this process leading to the improvement of axonal conduction (Honmou *et al.* 1996), these cells are unable to coexist with astrocytes (reviewed in Baron-Van Evercooren & Blakemore 2004). This implies that these cells would not be suitable candidates for transplantation into MS lesions since these are characterized by astrocyte hypertrophy. Olfactory ensheathing cells integrate into astrocytic environments *in vitro* better than Schwann cells (Lakatos *et al.* 2000), and they generate less astrocytic response upon transplantation into normal white matter (Lakatos *et al.* 2003), suggesting they would be better candidates for transplantation into astrocyte containing demyelinated areas of the CNS. However, the exact

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One contribution of 16 to a Theme Issue 'Immunoregulation: harnessing T cell biology for therapeutic benefit'.

behaviour of the olfactory ensheathing cells within an environment consisting of demyelinated axons accompanied by astrocytes still remains to be established (Franklin 2002*a*).

Transplantation of pluripotent cells from outside the CNS has been attempted in order to achieve remyelination, with several reports indicating that bone marrow could be used to achieve remyelination of the demyelinated CNS axons (reviewed in Baron-Van Evercooren & Blakemore 2004). Also, it has been suggested that umbilical cord blood-derived stem cells can differentiate into oligodendrocytes *in vitro* (Buzanska *et al*. 2002). However, this differentiation has not been observed upon transplantation into a newborn rodent brain (Zigova *et al*. 2002). The ability of haematopoietic stem cells to generate neural cells has been questioned (Wagers *et al*. 2002), thus the use of these cells for potential remyelinating therapies must be considered with caution.

Oligodendrocyte lineage cells have been successfully used to achieve remyelination of the demyelinated CNS lesions (reviewed in Baron-Van Evercooren & Blakemore 2004). These studies show that the myelination potential of a cell preparation following engraftment is dependent on its degree of enrichment for immature cells. Therefore, foetal cells are more effective in myelin formation than neonatal or adult ones after engraftment into neonatal CNS, and oligodendrocyte precursors are more efficient than pre-oligodendrocytes or mature oligodendrocytes. However, it also appears that commitment of the multipotent neural precursors to oligodendrocyte lineage prior to transplantation is required to achieve significant remyelination following engraftment into glial-free areas of acute demyelination (Smith & Blakemore 2000). Furthermore, these cells will not generate oligodendrocyte progenitor cells (OPCs) when transplanted into normal adult tissue since the integration of such cells is inhibited by the presence of endogenous OPCs (Hinks *et al*. 2001).

Since the ability of oligodendrocyte precursor cells to perform remyelination in the astrocytic environment has been well established (reviewed in Baron-Van Evercooren & Blakemore 2004), these cells represent the best candidates for transplant-mediated remyelination in the clinic. The best sources of human OPCs are likely to be embryonic and foetal tissues (reviewed in Franklin 2002*a*) and, because achieving exact haplotype matching between donors and hosts is extremely difficult due to polymorphisms of human genes (Drukker & Benvenisty 2004), it is important to evaluate to what extent allogeneic OPCs require tissue matching in order to achieve remyelination of the demyelinating CNS lesions. The following sections will review the data available on the regulation of adaptive immunity within the CNS first and then the major histocompatibility (MHC) expression of CNS glia, as this represents the main determinant of graft rejection. From this analysis it will become apparent that glial cell engraftment for treatment of demyelinating CNS disorders may represent a 'double privilege' scenario since not only is the site of engraftment characterized by an exquisite regulation of immune responses but also the tissue to be engrafted may be of low immunogenicity since it is characterized by low constitutive MHC expression.

3. REGULATION OF ADAPTIVE IMMUNE RESPONSES WITHIN THE CNS

When considering immune responses to the grafts placed in the brain or spinal cord, one has to take into account that the CNS is considered as one of the immune privileged sites of the body, the others being the eye (anterior chamber, subretinal space, vitreous cavity, cornea), testes and placenta (Barker & Billingham 1977). Immune responses in all these sites are different from those occurring in other parts of the body.

(a) *Immune privilege of the CNS: historical perspective*

The notion of the immune privilege of the CNS was raised by the studies on survival of transplanted tissue and tumour cells in the brain conducted in the first half of the twentieth century (reviewed in Barker & Billingham 1977). These experiments showed that allogeneic cells survived longer in the brain parenchyma than in extracerebral sites. At that time the interpretation of these experiments was that the brain was isolated from the immune system, first because of the absence of classically defined lymphatics within the brain, and secondly because of the presence of blood–brain barrier (BBB) consisting of endothelial cells sealed together by tight junctions leading to the idea that both afferent and efferent arms of the immune response to intracerebral antigens were absent. In 1923, it was suggested that the brain tissue was an 'uncongenial environment for the lymphoid cells' (Murphy & Sturm 1923). However, these authors made the important observation that regional differences existed within the brain microenvironment in that transplanted cellular antigen survived longer when placed in the cerebral cortex (prolonged survival) than in the ventricles (rejection). This observation changed the view on the immune privilege of the CNS indicating it was not absolute. The experiments of Medawar (1948) suggested that the BBB was not sufficient to prevent the entry of immune cells into the CNS. He observed that skin allografts placed in the brain failed to induce the usual rejection response observed in peripheral sites, but these grafts would undergo rejection if the recipient had rejected a previous skin graft. His interpretation was that brain grafts could be rejected if a recipient contained a source of circulating lymphocytes activated by immunization of the recipient with a peripheral skin graft and that placing antigens in the brain only failed to stimulate naive lymphocytes. Experiments by Scheinberg *et al*. (1966) showed that allogeneic skin grafts placed in the brain accelerated the rejection of comparable grafts in the periphery indicating that presence of antigens in the brain could also stimulate the afferent arm of the immune response.

The concept of the immune privilege nowadays has been redefined away from a belief in 'ignorance' of CNS antigens by the immune system towards an understanding that the maintenance of the immune

privileged status of the CNS is an active process and that the immune responses to antigens in the brain are specifically regulated (Cserr & Knopf 1992). So, both afferent (lymphatic drainage) and efferent (access of effector cells) arms of the immune system operate within the CNS, but they function differently than in the peripheral tissues (Harling-Berg *et al.* 1999). Raju & Grogan (1977) defined a paradox of immune privilege by stating that the immune system is aware of and responds to antigens on foreign grafts, but it is unable to mount a destructive immune attack (reviewed in Streilein & Taylor 1997).

(b) *Afferent immune system and the CNS*

In theory, antigens present within the brain could reach the immune system by two means. First, soluble antigens may exit the brain with draining cerebrospinal fluid (CSF) and interstitial fluid (ISF). Second, professional antigen presenting cells (APCs) could carry antigens from the brain into the regional lymph nodes where antigen presentation and initiation of the immune response would occur.

(i) *Drainage of fluids and soluble antigens*

The routes of soluble brain antigen drainage have now been clearly established (reviewed in Harling-Berg *et al.* 1999). For a long time, it has been recognized that substances introduced into the CNS efflux from the subarachnoid space into blood and cervical lymph (reviewed in Cserr & Knopf 1997). However, in laboratory animals, drainage into the lymph appears to be more significant immunologically (reviewed in Cserr & Knopf 1992, 1997). A great contribution to the understanding of CNS drainage was provided by a development of a rodent model with normal BBB permeability in the Helen Cserr and Paul Knopf laboratory (reviewed in Harling-Berg *et al.* 1999). In this model, antigen is microinfused into CSF or brain tissue through a cannula implanted one week before. The one week delay ensures restoration of normal BBB function. This model avoids the disadvantage of other studies which involved the disruption of the BBB by acute insertions of the needles and cannulas and also injections of large volumes of antigen which could immediately efflux into blood and lymph. Using this approach it has become clear that from the subarachnoid space, ISF and CSF can either drain into dural sinous blood, via arachnoid villi, or escape to extracranial tissue spaces, via prolongations of the subarachnoid space along certain cranial nerves (Kida *et al.* 1995; Cserr & Knopf 1997). These include olfactory, optic, trigeminal and acoustic nerves (reviewed in Cserr & Knopf 1997). The primary outflow pathway in common laboratory animals is along the arachnoid sheaths of the olfactory nerves, through the cribriform plate, to the nasal submucosa (reviewed in Harling-Berg *et al.* 1999). There is also good evidence that CSF transport in humans occurs through the cribriform plate into the nasal submucosa (Weller *et al.* 1992; Lowhagen *et al.* 1994; Caversaccio *et al.* 1996). Outflow from the spinal subarachnoid space also appears to involve drainage both into venous blood, via arachnoid granulations (Cserr & Knopf 1997), and into tissue spaces outside the spinal column, by passage along spinal nerve root ganglia (Kida *et al.* 1995).

Substances draining from the cranial subarachnoid space via the olfactory pathway can be cleared from nasal submucosa by passage into terminal lymphatics or into blood capillaries. Thus, proteins and other large molecular weight substances preferentially enter lymphatics and can be recovered in high concentrations, relative to blood plasma, from deep cervical lymph nodes and from the jugular lymph trunks (Bradbury & Westrop 1983; reviewed in Cserr & Knopf 1997). Smaller molecular weight substances (5000 Da or less), however, only appear in low concentrations in the lymph, being lost to the blood due to a greater ability to penetrate the permeable vascular endothelium in the nasal submucosa and lymph nodes (Bradbury & Westrop 1983).

(ii) *Immune responses resulting from the drainage of soluble brain antigens*

Administration of antigens into the brain, as well as into another immune privileged site—the anterior chamber of the eye, elicits a stereotypic immune response (Streilein & Taylor 1997). Therefore in these sites, delayed type hypersensitivity (DTH) responses are suppressed (Streilein 1987; Harling-Berg *et al.* 1989) and humoral immunity is stimulated (Harling-Berg *et al.* 1989; Wilbanks & Streilein 1990). Moreover, antibody responses will not be as inflammatory since concentrations of complement components are very low in normal rat CSF (Cserr *et al.* 1992) and antibody isotypes in mice immunized in the anterior chamber of the eye with soluble proteins were found to be biased to complement non-fixing IgG subclasses (Wilbanks & Streilein 1990). Development of cytotoxic CD8+ T lymphocyte (CTL) responses to infused brain antigens appears to be impaired as well. Gordon *et al.* (1997) assessed CTL development in cervical lymph nodes and the spleens of mice in response to microinfusion of mastocytoma cells into the caudate nucleus. This revealed that even though antigen-specific preCTL commitment in these lymph nodes occurred, direct CTL activity was absent. This failure to progress into direct CTL is consistent with Th2 (humoral) biased responses to antigens introduced into the brain.

(iii) *CNS-antigen presenting cells*

Although the experiments from Helen Cserr's laboratory established that soluble antigens from the CNS drain into the cervical lymph nodes of rodents, it is not yet clear whether the CNS contains cells capable of capturing antigens and carrying them into the cervical lymph nodes where they could prime naive T cells. Several cell types have been suggested to function as CNS APCs, and those include dendritic cells (DC), microglia and other CNS macrophages and astrocytes.

Dendritic cells. It appears that in the CNS, DCs are restricted to certain compartments. Thus, DCs are absent in normal rodent brain parenchyma (Karman *et al.* 2004) but present in the leptomeninges (Vass *et al.* 1986; Matyszak & Perry 1996; McMenamin 1999) and choroid plexus (Matyszak & Perry 1996). Human CSF also contains DC, and their number increases in MS (Pashenkov *et al.* 2001, 2002). This is consistent with

studies showing lack of immune reactivity to pathogens introduced into the brain parenchyma and vigorous responses to the pathogens in the CSF (Poltorak & Freed 1997). Even though normal brain parenchyma does not contain DCs, their presence is induced in pathological situations such as *Toxoplasma gondii* infection (Fischer *et al.* 2000), experimental allergic encephalomyelitis (EAE; Fischer & Reichmann 2001) and traumatic brain injury (Serafini *et al.* 2000). However, it is not completely clear whether the appearance of cells with DC features in the parenchyma during these CNS pathologies is due to a differentiation of an endogenous DC precursor or to the recruitment from the periphery. Work by Fischer & Reichmann (2001) suggests that microglia may be the DC precursor since upon stimulation *in vitro* with granulocyte–monocyte colony stimulating factor (GM-CSF) they start expressing CD11c (pan DC surface marker). It is also possible that blood-borne or meningeal DCs are recruited via the perivascular spaces (Fischer & Reichmann 2001). For example, in EAE, DCs invade the CNS as precursors together with other blood-derived cells (Serafini *et al.* 2000; Suter *et al.* 2000). It appears that DCs can migrate from the CNS into the draining lymph nodes since, when injected intracerebrally, they accumulate in the deep cervical lymph nodes (Karman *et al.* 2004).

An important question that needs clarification is the function of the DCs that appear in the CNS during inflammation. While CNS-DCs isolated from *Toxoplasma gondii* infected mouse brain functioned as potent APCs (Fischer *et al.* 2000), those isolated from EAE mice were unable to prime naive T cells and, moreover, they inhibited T cell priming by bone marrow-derived DCs (Suter *et al.* 2003). It therefore appears that certain DC subsets have a major role in maintaining peripheral tolerance to CNS antigens, which is not surprising since DCs have been shown to participate in the maintenance of self-tolerance by constant presentation of self-antigens (Heath & Carbone 2001; Steinman & Nussenzweig 2002). Therefore, further studies need to be conducted to clarify the role of DCs in the CNS.

Resident CNS APCs. Besides DCs, other cells have been suggested to function as APCs in the CNS. Microglia and perivascular macrophages are the primary candidates, especially the latter, since their role is indispensable for the development of EAE (Hickey & Kimura 1988). Perivascular cells enter the CNS continuously (Hickey & Kimura 1988; Hickey *et al.* 1992), take residence beyond the endothelial basement membrane (Lassmann *et al.* 1991; Hickey *et al.* 1992), ingest and accumulate materials via scavenger receptor mediated endocytosis (Kida *et al.* 1993; Mato *et al.* 1996) and become progressively granular as a result of this. From transplantation studies, there is some evidence that perivascular cells actually leave the CNS and return to the immune system tissues presumably carrying the materials found in the CNS (Broadwell *et al.* 1994). This could, therefore, be a way of priming the immune system against graft molecules and lead to graft rejection. However, it is not clear whether microglial cells and/or macrophages migrate out of the CNS in significant numbers and present antigens in secondary lymphoid organs. Also, microglial cells are significantly less efficient in priming naive T cells than professional APCs such as DCs (Aloisi *et al.* 1999). However, it is possible that these cells can develop into DCs under certain proinflammatory conditions. For example, as mentioned above, activated microglia have the potential of developing into DCs *in vitro* in response to GM-CSF, the major Th1-derived cytokine that stimulates DC development from bone marrow precursors and monocytes (Fischer *et al.* 2000; Hamilton *et al.* 2000).

Astrocytes. These are another cell type whose role in stimulation of T cells has been suggested (Fontana *et al.* 1984), but these cells probably do not have a major direct role in antigen presentation in the CNS (Karman *et al.* 2004). The upregulation of MHC II molecules by interferon-γ (IFN) seen *in vitro* (Cornet *et al.* 2001) is hard to reproduce *in vivo* (Aloisi *et al.* 2000), and they cannot induce a primary T cell response since they cannot migrate out of the CNS. It is also not clear whether these cells have a role in reactivation of T cells entering the CNS (reviewed in Karman *et al.* 2004).

(c) *Efferent arm of the immune system and the CNS: immune cell entry*

For a long time it was considered that the BBB prevented the entry of leukocytes into the CNS. However, it has become evident that a wide variety of white blood cells traffic through the CNS. Some leukocyte cell types traffic in the healthy CNS, as a part of normal physiology, while the entry of other cell types is a sign of pathology since they only appear in search of a specific antigen or in response to tissue damage (Hickey 1999).

(i) *T cells*

Nowadays, it is evident that T cell trafficking is a part of normal surveillance of the CNS (reviewed in Hickey 1999). It is has been generally considered that activation of T cells is required for their entry into the CNS. Thus, rat T cells specific for ovalbumin can be found in neural parenchyma if, and only if, the cells are infused into the animal in the activated, lymphoblast state (reviewed in Hickey 1999) and passive EAE can be induced only if CNS antigen specific cells are stimulated prior to administration (Holda *et al.* 1980; Panitch 1980; reviewed in Hickey 1999). The entry of activated T cells (when transferred) is independent from MHC compatibility with the host, specific T cell phenotype, and the presence in the CNS of the antigen that the T cells are specific for (Hickey *et al.* 1991). Therefore, trafficking of activated T cells into the CNS is thought to be a non-specific phenomenon. However, recently, a number of studies (Seabrook *et al.* 1998, 1999) have brought the activation requirement for the T cell entry into the CNS into question since they have reported that resting lymphocytes entered the CSF and probably the CNS of normal animals. Also, Brabb *et al.* (2000) detected naive, myelin basic protein (MBP)-specific T cells in the nervous system of CNS autoantigen-specific T cell receptor (TCR) transgenic mice, and these cells appeared to be rendered unresponsive by the CNS environment.

Even though T cell entry into the CNS occurs constantly, their concentration in the CNS is much lower than in those organs without a well-developed blood–tissue barrier (Yeager *et al*. 2000). Within the CNS itself, there appears to be distinct differences in the numbers of T cells found at different levels of neural parenchyma with the highest number of T cells within the lumbar spinal cord (Hickey 1999). Moreover, the ability of T cells to gain entry at different levels of CNS seems to be related to the vulnerability of these levels to the inflammation associated with the EAE (Berger *et al*. 1997). While T cell entry appears to be a non-specific phenomenon, the retention of the T cells in the CNS is specific. Only those cells that find their antigen in the context of MHC molecules in the CNS are retained (reviewed in Hickey 1999). It is not completely clear what happens with the T cells that have entered the CNS. Many die by apoptosis (Schmied *et al*. 1993; Bauer *et al*. 1998), which is especially the case for T cells that enter the parenchyma (Bauer *et al*. 1998). Those placed in the CSF disappear rapidly, but this happens probably because of cell migration, not cell death (Hickey 1999).

An unexpected role for the T cells, and one that may be important when considering the consequences of CNS graft rejection, is their ability to foster CNS repair (reviewed in Hohlfeld *et al*. 2000; Schwartz & Cohen 2000; Bieber *et al*. 2003). It has been shown that this neuroprotective effect of T cells is dependent on T cell subtype and activation state, so that Th2 cells are more protective than Th1 cells (Wolf *et al*. 2002).

(ii) *Natural killer cells*

Not much is known about the entry, function or fate of natural killer (NK) cells in the healthy CNS or as inflammation develops. It is known that these cells represent up to 10% of the total infiltrates during EAE (Zhang *et al*. 1997; Matsumoto *et al*. 1998). These studies, however, suggested that these cells may play a moderating or suppressive role since their depletion renders acute EAE more severe. NK cells become detectable in the spinal cord a day after the first T cells arrive during rat EAE, and their presence is detected only when T cells entering CNS have located their antigen and the CNS is destined to develop inflammation (Hickey 1999).

(iii) *B cells*

In the healthy CNS, the IgG ratio between the serum and the CSF is approximately 1000 : 1 (Cserr & Knopf 1997). Yet in some diseases, high concentrations of IgG are present in the CSF. This indicates that plasma cells must have a way of entering the CNS and becoming established there. In the system used by Cserr laboratory mentioned above it was found that if the antigen to which the animal had been sensitized was introduced into the brain, plasma cells and B cells specific for that antigen appeared, but extensive inflammation did not occur (Harling-Berg *et al*. 1989). If an antigen to which the animal had not been sensitized was introduced, minimal changes were observed. It therefore appears that, just like in the case of T cells, the entry of B cells is not specific, but their retention in the CNS is.

(iv) *Monocyte–macrophage family members*

This group of cells includes microglial cells (Sedgwick & Hickey 1997), perivascular (microglia, macrophage) cells (Sedgwick & Hickey 1997), meningeal macrophages, choroid plexus macrophages (Matyszak *et al*. 1992), epiplexus cells and the standard phagocytic macrophages or 'Gitter cells' in the areas of parenchymal damage. Parenchymal microglia takes up residence in the CNS during foetal life (Perry 1994), while other monocytic elements are turning over throughout life (Hickey *et al*. 1992; Perry 1994; Sedgwick & Hickey 1997). Meningeal macrophages are replaced quickly and continuously from the bone marrow; perivascular cells and choroid plexus macrophages also turn over, but less rapidly (Hickey *et al*. 1992). It appears that the activation of the monocyte family is important to enhance transendothelial migration, but it is not essential (Persidsky *et al*. 1997).

(d) *Local factors in the CNS suppress the development of inflammation*

The environment in the immune privileged sites such as the brain and the anterior chamber of the eye is anti-inflammatory. It has been established that aqueous humour of the eye suppresses T cell proliferation and modulates the activity of the macrophages (reviewed in Streilein & Taylor 1997). Also, exposure of peritoneal exudate cells to the aqueous humour of the eye or to CSF *in vitro* conferred the ability to these cells to present antigens in a 'deviant' manner which is known to lead to the induction of tolerance (Wilbanks & Streilein 1992). The capacity for this induction of deviant immunity was mediated, at least in part, by transforming growth factor (TGF)β. Gordon *et al*. (1998) showed that normal rat CSF suppresses the *in vitro* development of mastocytoma-specific CTL activity in restimulated splenocytes from Balb/c mice, a strain unable to reject this tumour from the brain. This suppression was dependent on TGFβ, as reversal of suppression was achieved with a specific neutralizing antibody. Other CNS molecules capable of modulating immune activity include interleukins (IL-1, IL-6) and neuropeptides (substance P (SP) and vasointestinal peptide (VIP); reviewed in Benveniste 1992; Bellinger *et al*. 1997). Therefore, factors present within the CNS environment also contribute to the suppression of destructive immune responses.

4. STUDIES ON NEURAL GRAFT SURVIVAL

Most studies undertaken so far investigating the survival of allografts into the brain have focused on the survival of neurons as a means to replace those lost in neurodegenerative diseases. While prolonged survival in the brain compared to most other sites has been frequently observed, a number of studies have reported rejection (Mason *et al*. 1986; Nicholas *et al*. 1987; Date *et al*. 1988; Poltorak & Freed 1989). This rejection appears to be unpredictably variable, considerably delayed or incomplete (Head & Griffin 1985; Mason *et al*. 1986). Therefore, experiments have been undertaken in order to investigate mechanisms of

rejection, factors influencing rejection, or possible ways to overcome it. Bartlett *et al.* (1990) investigated whether removal of MHC I-expressing cells could enhance the survival of neural precursors in the brain. They first determined that neural precursors isolated from E12 brains of mice do not express MHC class I molecules. Then, they compared the survival of E12 neuroepithelial whole cell preparation to the survival of the preparation of MHC I negative cells isolated from E12 neuroepithelium. Whereas E12 whole epithelium underwent rejection in allogeneic hosts, the MHC I negative preparation showed good survival even six months post-transplantation.

Survival of neural allografts appears to be influenced by the site of implantation. Sloan *et al.* (1990) compared the survival of solid tissue cortex placed either into the third ventricle, lateral ventricle or caudate putamen (parenchyma). Grafts placed into the parenchyma showed much better survival than those placed in the ventricles. Also, this survival was dependent on donor–host strain combination. For example, whereas PVG grafts into the AO rats were rejected and this was accompanied by a heavy immune infiltration, AO grafts into PVG rats showed very little cell infiltration and generally good survival. Another study also showed donor–host strain combination influence on the survival of neural allogeneic tissue. Isono *et al.* (1993) showed that in some donor–host strain combinations, certain grafts survive even if the hosts were sensitized with donor skin twice. For example, Lewis ($RT1^l$) grafts survived well in Brown Norway $RT1^n$, PVG $RT1^c$, AO $RT1^u$ and PVG $RT1^u$ even after sensitization, whereas Brown Norway grafts did not. Therefore, the authors implied that the haplotype of the graft could influence rejection. They also noticed that susceptibility of the host strain to EAE appeared to be related to their susceptibility to rejection. Thus, EAE susceptible strains (Lewis $RT1^l$ and F344 $RT1^l$) showed more vigorous rejection responses than other, EAE non-susceptible strains. These observations suggest that survival of neural grafts is influenced by the haplotype of the donor, host MHC allele and susceptibility to autoimmune disease. Another factor determining graft survival appears to be the immunoresponsive gene. This is an MHC linked gene that controls alloimmune responses to class I antigens in rats and makes strains low responders against some MHC haplotypes or high responders against others.

While all these studies address the survival of allogeneic neural tissue, very few studies have specifically studied the survival of allogeneic oligodendrocytes. A study by Li & Duncan (1998) examined the responsiveness of *myelin deficient* (md) rats to allogeneic glial cells. In these studies, cells were prepared from ACI (RT1a) spinal cords of 6–7 days old rats and transplanted into the spinal cords of 7 or 8 days old md pups. These allogeneic grafts were rejected by two weeks post-transplantation. In this experiment, about 20% of the cells were microglial cells, and the recipients were neonatal pups. Extensive studies of allogeneic oligodendrocyte survival in normal adult animals have not been performed.

5. STUDYING THE SURVIVAL OF GLIAL ALLOGRAFTS: IMPORTANCE OF THE MODEL

While a number of models exist in which it is possible to study the myelination potential of transplanted cells, choosing a model suitable for studying the immunogenicity of myelinogenic cells is governed by a number of constraints. This section analyses the models used to demonstrate transplant-mediated myelination and comments on their suitability for investigating the survival of allogeneic myelinogenic cells. The basic principle underlying all these models is that axons are available for the progeny of the transplanted cells to myelinate.

(a) *Developing nervous system*

Many studies have used the developing nervous system as a transplantation site for assessing the myelin-generating potential of introduced cells with cells injected into the CNS of E19 embryos or newborn rat and mouse pups. These recipients represent a highly permissive environment for myelination, as transplanted cells will be responding to developmental cues. They are thus ideal for assessing myelinating potential; however, because of tolerance induction to antigens introduced in early embryonic and neonatal life such recipients are unsuitable for evaluation of immune responses to grafted cells.

(b) *Myelin mutants*

The myelin mutants comprise a group of animals in which myelin sheath production is perturbed due to defects in myelin protein related genes. These are classified into different groups according to the myelin protein gene affected:

(i) MBP mutants (the *shiverer* mouse and Long Evans rat) lack MBP which leads to abnormal myelin sheath formation. However, oligodendrocyte processes associate with axons forming thin and uncompacted myelin which, after a few weeks of age, could interfere with transplant-mediated remyelination (Gout *et al.* 1988). The utility of this model lies in the fact that it is easy to identify MBP positive myelin sheaths when tissue from normal animals is transplanted into the mutants CNS; however, to achieve significant myelination in mature animals demyelinating lesions must be induced (reviewed in Baron-Van Evercooren & Blakemore 2004).

(ii) The phospholipid protein (PLP) mutants include the *myelin deficient* (md) rat, the *shaking* pup and the *jimpy* (jp) mouse. In these animals mutations or duplications of the PLP gene result in death of oligodendrocytes as they begin to myelinate the axons (reviewed in Ikenaka & Kagawa 1995). In the md rat (Boison & Stoffel 1989) and the jp mouse (Farkas-Bargeton *et al.* 1972) as well as the canine shaking pup (Duncan *et al.* 1987), a small number of oligodendrocytes survive and synthesize a small amount of myelin. Theoretically the severity of the myelin deficiency should allow for significant transplant-mediated myelination. However, the disadvantage of using these

animals is that they have a short life span (21–28 days) which makes evaluation of long term consequences of remyelination impossible. Even though the shaking pup has a slightly longer life span, nursing these animals is very time consuming and expensive.

(iii) Transgenic animals overexpressing the PLP gene represent another model for studying transplantation of pro-myelinating cells (Espinosa de los Monteros *et al.* 2001). Homozygous mice have severe hypomyelination and a short life span similar to jimpy mice while heterozygous mice have a long life span and could be used as adult graft recipients since normal myelination is followed by progressive demyelination starting at three to four months (Kagawa *et al.* 1994).

(iv) Myelin associated glycoprotein-FYN (non-receptor tyrosine kinase of the SRC family) knockout mice are hypomyelinated (Biffiger *et al.* 2000). These animals have a normal life span and have been used as transplant recipients, although the experiments so far undertaken have been performed in postnatal animals (Ader *et al.* 2001).

(v) Another myelin mutant animal is the *taiep* rat. In these animals, a microtubular defect in oligodendrocytes results in a disruption of the normal myelination process in certain areas of the CNS and failure of maintenance of the myelin sheath elsewhere, leading to progressive demyelination which results in a tissue pathology that is very reminiscent of the chronic areas of demyelination found in MS (Duncan *et al.* 1992). Recent studies in adult *taiep* rats have demonstrated two important limitations for achieving remyelination in a clinical context and for transplantation studies in mature myelin mutants. First, it was confirmed that depletion of endogenous OPCs is an essential requirement to achieve repopulation of tissue by transplanted OPCs (Foote & Blakemore 2005*b*) and secondly the studies highlighted the requirement for an acute inflammatory response to generate remyelinating oligodendrocytes from OPCs (Foote & Blakemore 2005*a*). The implications of these studies are that OPC transplantation into tissue containing endogenous OPCs will not result in widespread colonization of tissue, and even if OPCs can be established within areas of chronic demyelination this will not be followed by remyelination unless the cells are activated (e.g. by the induction of an acute inflammatory response).

Therefore, even though the myelin mutants represent useful models for studying the pro-myelinating potential of transplanted cells, they may not be the most suitable models for undertaking rejection studies. The first disadvantage is that most of these animals are short lived so that transplantation studies have to be performed in developing animals, which, as mentioned before, leads to the induction of tolerance. Secondly, studies of rejection require animals with normal immunoresponsiveness. Even though one study addressed the immune status of an md rat and found it to be normal (Li & Duncan 1998), this issue in other mutant/transgenic animals has not been investigated. Thirdly, any precise study of rejection responses to mismatched tissue requires the use of inbred animals, and the most attractive of these models, such as the *taiep* rat, is derived from an outbred strain of rats (Sprague–Dawley). Finally, one has to keep in mind that transplantation of normal myelinating cells into mutant animals will imply genetic mismatches between the donor and the host that in themselves could stimulate an immune response.

(c) *Retina*

The non-myelinated retina has also been used as a model for graft-mediated myelination; however, the extent of myelination that can be achieved in mature animals is limited (reviewed in Baron-Van Evercooren & Blakemore 2004).

(d) *Focal lesions induced in adult animals*

Focal areas of demyelination can be induced in adult animals by injection of chemicals (gliotoxins) into white matter areas that specifically kill glial cells without killing the axons (reviewed in Baron-Van Evercooren & Blakemore 2004). The most commonly used toxins are lysolecithin (lysophosphatidyl choline) and ethidium bromide (EB). Lysolecithin has a particular toxicity for myelin but spares some oligodendrocytes and possibly their progenitors within the area of demyelination. EB-induced lesions are larger than those induced by lysolecithin. This DNA intercalating agent kills both astrocytes and oligodendrocytes as well as oligodendrocyte precursors throughout the area of demyelination. This toxin has been used to produce demyelination in both the dorsal funiculus of the spinal cord and in the caudal cerebellar peduncle. Injection of antigalactocerebroside (anti-GalC) antibody and complement has also been used to produce demyelinating lesions in the CNS, and it appears that large volumes of this preparation are required to induce significant areas of demyelination. Toxin-induced lesions in adult animals represent suitable models for studies of graft rejection since transplant-mediated remyelination would be evaluated in adult animals with presumably normal immune responsiveness, and the use of inbred strains for these experiments would ensure appropriate manipulations of MHC mismatches. Since both EB and lysolecithin-induced lesions represent better-developed models than anti-GalC antibody and complement produced lesions, and EB lesions are larger than lysolecithin induced ones and of known toxicity for oligodendrocytes and OPCs, we have used this as the preferred toxin-induced model of demyelination to study rejection of glial grafts. As will be discussed later, such lesions have intrinsic remyelinating capacity and therefore a means of distinguishing graft-mediated remyelination from that undertaken by host cells must be employed.

(e) *Diffuse lesions*

Several models of demyelination exist in which demyelinating lesions are more diffusely distributed

throughout the CNS. These include EAE, demyelination induced by intrathecal injection of cholera toxins B-subunit conjugated to saporin, cuprizone intoxication, and various virus-induced demyelinating diseases (reviewed in Baron-Van Evercooren & Blakemore 2004).

These models have not been used as frequently as toxin injections and myelin mutants to assess the myelination potential of transplanted cells because they are characterized by complex pathophysiological features, widespread dispersion of the lesions and the fact that all of them show spontaneous remyelination. Possibly the most widely used of these models is EAE. In this model, demyelination is induced by sensitizing animals to myelin component by injecting CNS homogenate, purified myelin or myelin proteins in Freund's adjuvant. Monophasic type of EAE (acute EAE or passive transfer EAE) shows little demyelination while those with remission and exacerbation (chronic-relapsing EAE or antibody-dependent EAE) have widely dispersed lesions throughout the CNS with preference for the spinal cord. The few studies performed in this model report the potential of survival and migration of the transplanted cells and their ability to improve clinical recovery (Tourbah *et al.* 1997; Pluchino *et al.* 2003). While it will be necessary ultimately to evaluate allograft survival in a model of autoimmune demyelinating disease, EAE may be inappropriate for studies whose primary focus is the study of the immunogenicity of glial cell preparations. In addition, it has to be appreciated that several features of MS are not reflected in EAE. For example, while EAE is a CD4-mediated disease, CD8+ cells appear to predominate in MS lesions. Moreover, while IFN-γ administration or tumour necrosis factor (TNF) α blocking ameliorate EAE, these treatments exarcebate MS (reviewed in Lassmann & Ransohoff 2004).

6. TRACKING THE FATE OF TRANSPLANTED CELLS

Since normal glial populations of the CNS have an intrinsic replacement capacity, it is important that there is a means of clearly identifying myelin formed by the transplanted cells. Therefore, an important issue that must be addressed when carrying out allografting experiments is the identification of the transplanted cells in a manner that will not interfere with allograft survival, or raise the possibility of immune recognition of the marker being used, since this could complicate the interpretation of the results.

Identification of transplanted myelinating cells can be achieved in two ways: having reliable markers for transplanted cells or using demyelinating lesion models in which endogenous remyelination is suppressed.

(a) *Graft-specific markers*

Transplanted cells can be labelled *in vitro* prior to transplantation (exogenous labelling) using vital dyes such as fast blue (Espinosa de los Monteros *et al.* 1993) and bisbenzimide—Hoechst dyes 33 258 and 33 342 (Gansmuller *et al.* 1991) for subsequent identification using fluorescence microscopy. However, these exogenous labels as well as the use of mitotic S-phase markers such as tritiated thymidine or BrdU have the disadvantage of becoming diluted by repeated divisions of transplanted cells. In addition, the dyes can be taken up by host cells at the time of transplantation (Iwashita *et al.* 2000), and also there can be a direct transfer of label to host cells and macrophages when transplanted cells die.

Rather than using exogenous labels, genetic markers can be used in order to avoid problems of dilution and transfer associated with *in vitro* labelling. These markers can be introduced as genes for green fluorescent protein (GFP; Herrera *et al.* 2001; Windrem *et al.* 2002) or bacterial Lac-Z directly in the cells (Tontsch *et al.* 1994; Franklin *et al.* 1995), or the cells can be derived from transgenic animals (Friedrich & Lazzarini 1993; Lachapelle *et al.* 1994). Inherent genetic mismatches between the donor and the host can also be used. A useful paradigm is male to female transplantation utilizing repeat sequences on the Y chromosome as a marker (Harvey *et al.* 1992; O'Leary & Blakemore 1997).

The use of graft-specific markers that make use of genetic mismatches between the graft and the host could potentially induce an immune response to the engrafted cells. This has been shown for two of the most commonly used markers for transplanted tissues, Lac Z (Abina *et al.* 1996) and enhanced green fluorescent protein (eGFP; Stripecke *et al.* 1999; Rosenzweig *et al.* 2001; Andersson *et al.* 2003). Inherent markers such as the Y chromosome are probably more appropriate since, although H–Y is defined as an alloantigen (Scott *et al.* 1997; Simpson *et al.* 1997), studies involving engraftment of male glial cells into demyelinating lesions of the same strain of female rats resulted in survival of male cells one year post-engraftment (O'Leary & Blakemore 1997). Therefore, this transplantation paradigm appears to be suitable for the evaluation of long-term survival of allografts.

(b) *Suppression of endogenous remyelination*

Making demyelinating lesions and transplanting cells into the tissue in which the inherent remyelination capacity is abolished represents an alternative strategy to using markers for the identification of transplanted cells. This situation can be achieved by inducing a demyelinating lesion in tissue exposed to 40 Gy of X-irradiation (Blakemore & Patterson 1978). The exposure of rat spinal cord to this dose of X-irradiation inhibits remyelination by depleting the tissue of its endogenous OPC population (Hinks *et al.* 2001). The validity of this model has been established by a number of means. Firstly, there is a lack of remyelination in demyelinated lesions in animals killed up to two months post-irradiation (Blakemore *et al.* 2002). Secondly, remyelination is only seen following transplantation of pro-myelinating cells and the nature of remyelination is related to the type of cells introduced; so transplantation of an OPC preparation results mainly in oligodendrocyte remyelination of the lesion and transplantation of Schwann cells results only in Schwann cell remyelination. Thirdly, remyelination after xenografting is achieved only if animals are immunosuppressed (Smith & Blakemore 2000).

Fourthly, X-irradiation of 4 cm of the spinal cord centered on the lesion guarantees an absence of OPCs from the lesion even two months after lesion induction since it has been shown that endogenous OPCs repopulate OPC-depleted tissue at a rate of 0.6 mm per week in 2 month old animals (Chari *et al.* 2003). Thus, if the area of demyelination is situated in the centre of a 4 cm length of X-irradiated tissue, endogenous OPCs from non-irradiated tissue will not be able to reach the lesion site and contribute to remyelination for at least four months.

The main advantage of the X-irradiated gliotoxin model is that it allows for evaluation of the graft survival simply by examining the extent of remyelination within the lesion after engraftment. However, a possible disadvantage of this approach could be an induction of BBB dysfunction (Li *et al.* 2004). This effect results from a triggering of endothelial cell apoptosis, which results in a decrease in the endothelial cell density during the first week after irradiation. However, by 14 days post-irradiation, BBB integrity is restored (Li *et al.* 2004). Since in any transplant experiment involving focal induction of demyelination by gliotoxin injection the BBB will be disrupted twice (first, during lesion induction and second, during transplantation), induction of temporary BBB dysfunction during the first week to two weeks following X-irradiation may not be a compromising variable. Another effect of the dose of X-irradiation required to inhibit endogenous remyelination is the induction of white matter necrosis with longer survival times. Stewart *et al.* (1995) analysed the effect of exposure of rat cervical spinal cord to 25 Gy of X-irradiation and noticed major structural changes 107 days after exposure. In the previous studies in our laboratory using 40 Gy, signs of tissue necrosis were first seen three months post-irradiation, so the X-EB model appears suitable for addressing allograft survival for up to 60 days. A further side effect of X-irradiation with the doses required to inhibit remyelination is that it appears to enhance immune responses in the irradiated tissue. Thus, Love *et al.* (1987) found that local exposure of the spinal cord to 20 Gy of X-irradiation one month after EAE induction or 2–16 months after Theiler's virus inoculation resulted in local exacerbation of the disease that was not due to viral reactivation. This raises the possibility that cells may be more susceptible to rejection in irradiated tissue than in normal tissue and that studies using this model could overestimate the immunogenicity of allografts.

7. IMMUNOGENICITY OF GLIAL ALLOGRAFTS

MHC disparity is the leading cause of allograft rejection (Roitt 1994; Parham 2000), thus when considering grafting of CNS-derived progenitors as a replacement therapy, one has to consider their MHC expression both at the time of engraftment and when they have established themselves in the tissue.

(a) *Major histocompatibility complex molecules expression in the central nervous system*

Constitutively, cells in the CNS display no or very little expression of MHC antigens (Lampson & Hickey 1986; Vass & Lassmann 1990; Mucke & Oldstone 1992). The low expression of MHC antigens is believed to be an important factor contributing to the immune-privileged status of the CNS, since cells lacking MHC molecules are unable to interact with immunocompetent T cells (Lampson 1987). However, MHC I and MHC II antigen expression is upregulated within the CNS as a consequence of inflammation (Traugott *et al.* 1986) or infection (Olsson *et al.* 1987). The most potent inducer of MHC expression is IFN-γ, which is produced by activated T cells and NK cells (Linda *et al.* 1998). Different cell types within the CNS differ in a degree of constitutive MHC antigen expression as well as in their potential for MHC upregulation in response to cytokines.

(i) *Neural stem/progenitor cells*

Constitutively, neural stem/progenitor cells show either absence of MHC expression or very low levels MHC I expression (Bailey *et al.* 1994; McLaren *et al.* 2001; Drukker *et al.* 2002). This expression can be upregulated by IFN-γ treatment (McLaren *et al.* 2001; Drukker *et al.* 2002). When cells differentiate, MHC expression reflects the nature of their differentiation, so that the highest levels of MHC expression are observed when the cells differentiate into astrocytes (McLaren *et al.* 2001), while neuronal and oligodendrocyte populations maintain low levels of MHC I expression only.

(ii) *Microglia*

In culture, microglial cells express MHC I, but not MHC II. However, treatment with IFN-γ results in upregulation of MHC II antigens (Frei *et al.* 1987; Suzumura *et al.* 1987). The induction of MHC II antigen expression on microglia has also been shown *in vivo* after tissue engraftment into the brain (Finsen *et al.* 1991), on retinae grafted into the CNS (Perry & Lund 1989), and in ischaemic and kainic acid lesioned adult hippocampus (Finsen *et al.* 1993). The presence of MHC II antigens on microglia has also been shown in lesions of acute EAE (Vass *et al.* 1986), Alzheimer's disease (Dickson *et al.* 1993), MS (Gobin *et al.* 2001), as well as in a mouse model of Parkinson's disease (Kurkowska-Jastrzebska *et al.* 1999). It has been shown that MHC II expression on microglial cells increases with age in non-human primates (Sheffield & Berman 1998) and in the normal rat brain (Ogura *et al.* 1994), indicating an increase in microglial activity with age.

Interestingly, microglia in rat brain upregulated MHC II expression after high doses of lipopolysaccharide (LPS; Buttini *et al.* 1996) or IFN-γ (Steiniger & van der Meide 1988) were injected intravenously, which suggested that peripheral immune challenge can lead to microglial activation. Vass & Lassmann (1990) found regional differences in the numbers of MHC II expressing microglial cells. The highest density was found in the lumbar spinal cord and cerebellar white matter, while the lowest density was in the forebrain. The areas of highest constitutive microglial MHC II expression corresponded with the preferential sites of inflammatory infiltrates found in EAE (Levine 1970).

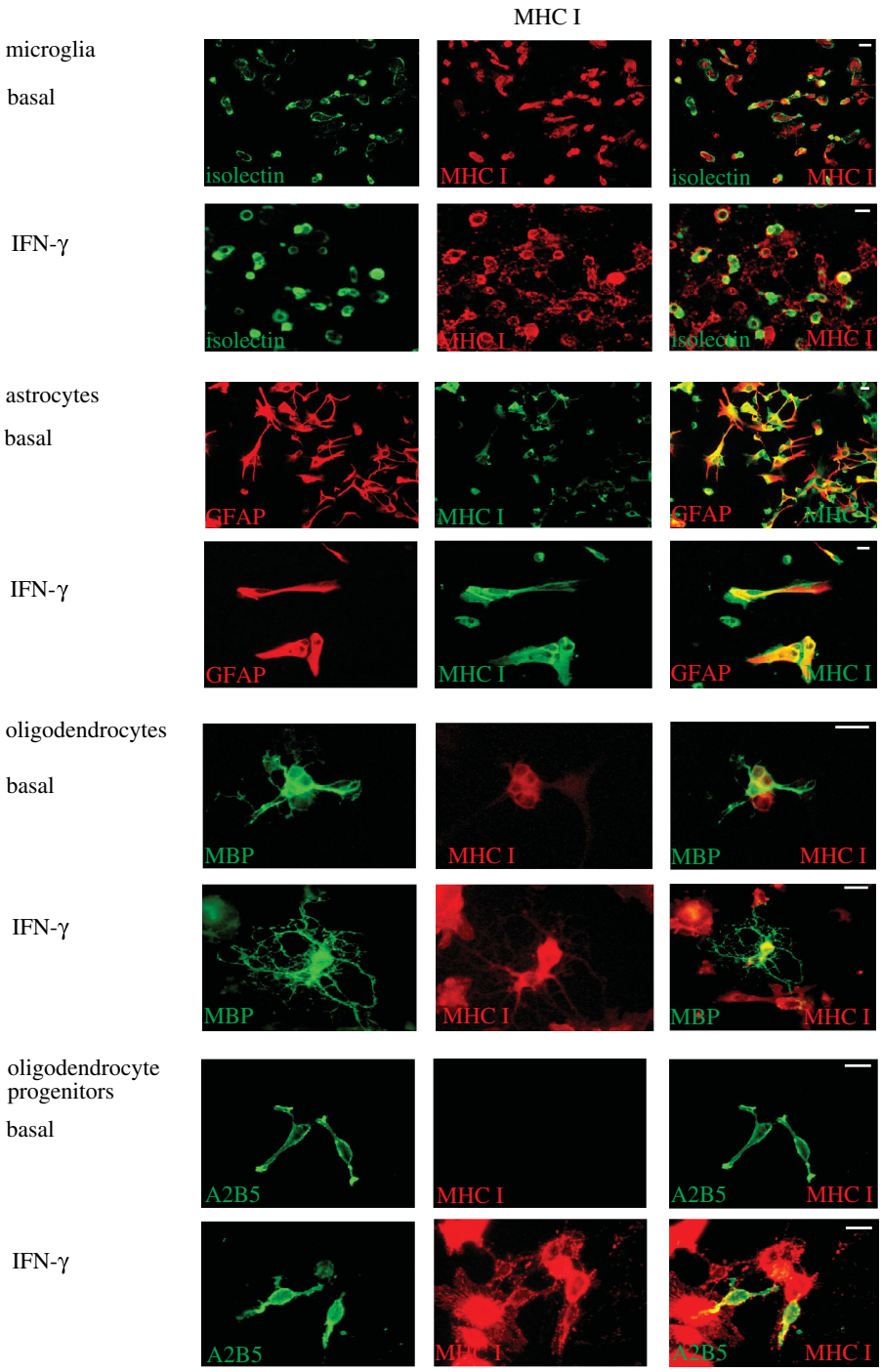

Figure 1. MHC I expression of neonatal rat mixed glial preparations after one to two weeks of culture. Constitutive expression was detected on microglia and astrocytes, and in low levels on oligodendrocytes. MHC I expression was detected on oligodendrocyte progenitors only after exposure to 10 ng ml^{-1} of IFN-γ overnight. Scale bars, 10 μm.

(iii) *Astrocytes*

In vitro, astrocytes constitutively express MHC I (Satoh *et al.* 1991; Massa *et al.* 1993). Fontana *et al.* (1984) detected MHC II expression on astrocytes and showed that these cells were able to present antigens to T cells in a specific manner restricted by the MHC. A later study (Frei *et al.* 1987), however, showed that astrocytes are completely immunonegative for MHC II in a normal state, and that it is a signal from T cells, probably mediated by IFN-γ, which induces MHC II expression. Other studies confirmed that treatment with IFN-γ (Wong *et al.* 1984; Lee *et al.* 1992; Satoh *et al.* 1995) as well as a combination of IFN-γ and TNF-α (Nikcevich *et al.* 1999) induce expression of MHC II on these cells *in vitro*. Massa and co-workers demonstrated that hyperinduction of MHC class II molecules on astrocytes *in vitro* was strain dependent (1987). Thus, astrocytes from Lewis rats (highly susceptible to EAE) expressed much higher levels of MHC II molecules than astrocytes derived from an EAE-resistant strain, Brown Norway rats, and the same was shown when a mouse EAE-susceptible strain, SJL, was compared to an EAE-resistant strain, BALB-c (Massa *et al.* 1987).

In vivo expression of MHC II antigens is a matter of controversy. Even though intrathecal application of IFN-γ induced MHC II expression on astrocytes, this immunostaining was weak and patchy, and it occurred

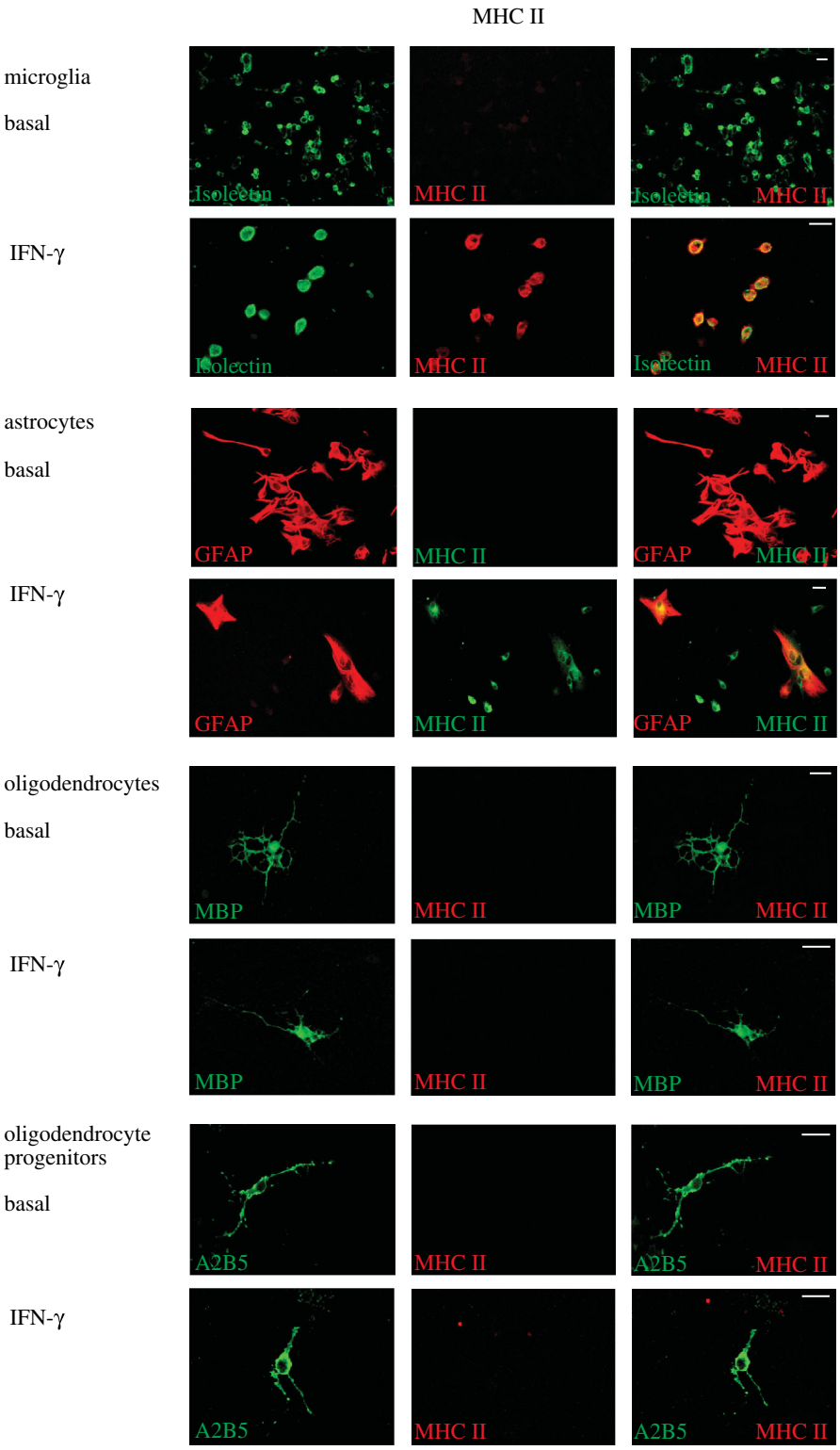

Figure 2. MHC II expression of neonatal rat mixed glial preparations after one to two weeks of culture. No constitutive expression was detected. Upon IFN-γ stimulation overnight, microglial cells readily upregulated MHC II expression and low levels of expression were detected on astrocytes. No MHC II expression was detected on oligodendrocytes or oligodendrocyte progenitors, even when cells were exposed to high concentrations of IFN-γ. Scale bars, 10 μm.

later and with less intensity than in microglia (Vass & Lassmann 1990). When rat hippocampal primordia from MHC classes I and II incompatible donors were grafted into the hippocampus of adult rats, no MHC II upregulation on astrocytes was observed (Lawrence *et al.* 1990). The documentation of class II positive astrocytes in diseases such as EAE and MS has been conflicting (Hickey *et al.* 1985; Hofman *et al.* 1986; Matsumoto *et al.* 1989; Lee *et al.* 1990). Several studies found MHC II immunopositive astrocytes in brains of MS patients (Hofman *et al.* 1986; Traugott 1987; Zeinstra *et al.* 2000). In the normal white matter, no MHC II positive astrocytes were found (Traugott 1987; Zeinstra *et al.* 2000). However, in another study (Gobin *et al.* 2001), astrocytes in MS lesions were found to express transcription factors controlling

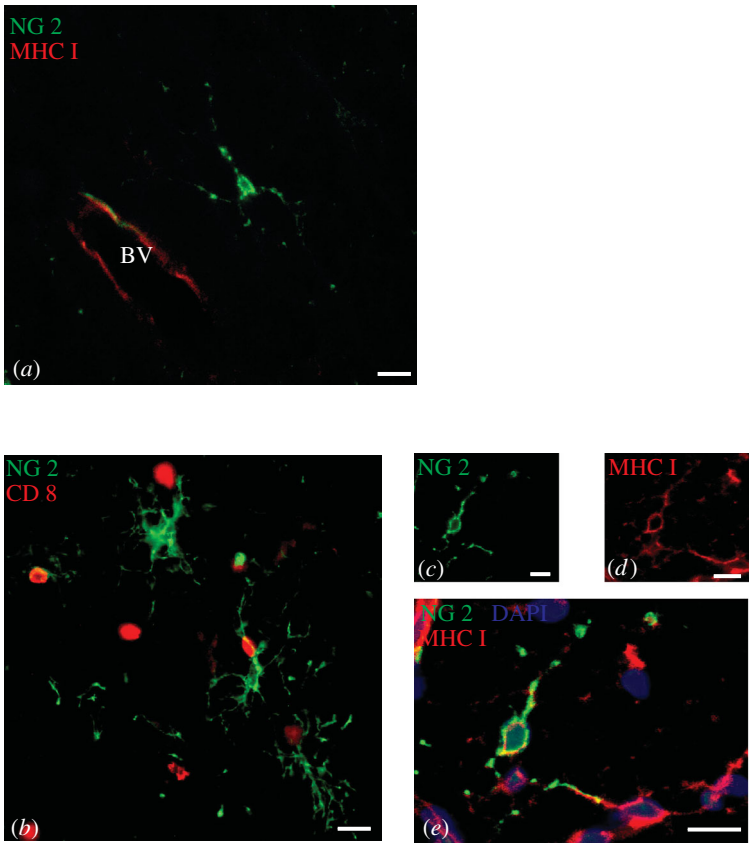

Figure 3. MHC I is expressed on transplanted oligodendrocyte progenitors (OPCs) only when inflammatory cells are present in the tissue. (*a*) Following 40 Gy of X-irradiation endogenous OPCs are killed. This allows transplanted OPCs (visualized using antibodies to the NG2 proteoglycan) to repopulate the depleted tissue. In the absence of inflammation they do not express detectable levels of MHC I molecules. MHC I expression is present on endothelial cells of blood vessels (BV). (*b–e*) Following immune cell infiltration (*b*) OPCs upregulate MHC I expression (*c–e*). Scale bars, 10 μm.

MHC expression weakly, which corresponded to weak MHC I and no MHC II immunoreactivity. In this study, MHC II expression was restricted to microglia.

(iv) *Oligodendrocytes*

Oligodendrocytes show low levels of surface MHC class I molecules in culture (Wong *et al.* 1984, 1985; Massa *et al.* 1993). Presence of MHC I expression on oligodendrocytes has been shown in the brains of patients with progressive multifocal leukoencephalopathy (Achim & Wiley 1992), as well as on oligodendrocytes isolated from corona virus-infected mouse brains (Suzumura *et al.* 1986*b*). Treatment of oligodendrocytes with IFN-γ (Wong *et al.* 1985; Satoh *et al.* 1991; Massa *et al.* 1993), TNF-α (Mauerhoff *et al.* 1988), or with interleukin (IL)-2 (Suzumura *et al.* 1986*a*) induced an increase in surface MHC I levels. Secretion of factors by T cells can also induce MHC I expression on cultured oligodendrocytes (Suzumura & Silberberg 1985). Oligodendrocytes do not express MHC class II molecules in culture (DuBois *et al.* 1985; Satoh *et al.* 1991) or *in vivo* (Grenier *et al.* 1989). MHC II mRNA production and immunoreactivity on oligodendrocytes has been induced by IFN-γ in rat optic nerve cultures, but this happened exclusively in the presence of the glucocorticoid dexamethasone (Bergsteindottir *et al.* 1992). IFN-γ on its own (Wong *et al.* 1984; Suzumura *et al.* 1986*b*; Satoh *et al.* 1991; Bergsteindottir *et al.* 1992) or in combination with TNF-α (Turnley *et al.* 1991) did not induce MHC II expression on oligodendrocytes.

(v) *Oligodendrocyte progenitors*

So far, there have been no studies examining MHC expression on OPCs. We have found that under basal conditions, rat neonatal OPCs grown within mixed glial cell cultures do not express detectable levels of MHC molecules (figures 1 and 2). Exposure of the cultures to IFN-γ resulted in detectable MHC I, but not MHC II expression. OPCs present within normal rat spinal cord did not express MHC molecules, neither did transplanted allogeneic OPCs when established in normal tissue. However, within areas of lymphocytic infiltration, MHC I expression was detected on transplanted OPCs (figure 3).

8. OPC ALLOGRAFTING FOR REMYELINATION

In experimental models (syngeneic situation), remyelination can be achieved by transplanting OPCs, either as components of mixed glial cell preparations that also contain a significant percentage of astrocytes, or as preparations purified for the cells of oligodendrocyte lineage. Since examination of MHC expression on glial cultures shows that cultured microglia and astrocytes can be induced to express MHC class II (figure 2), these cells can be identified as potential rejection inducers. One can therefore hypothesize that graft composition may be an important determinant

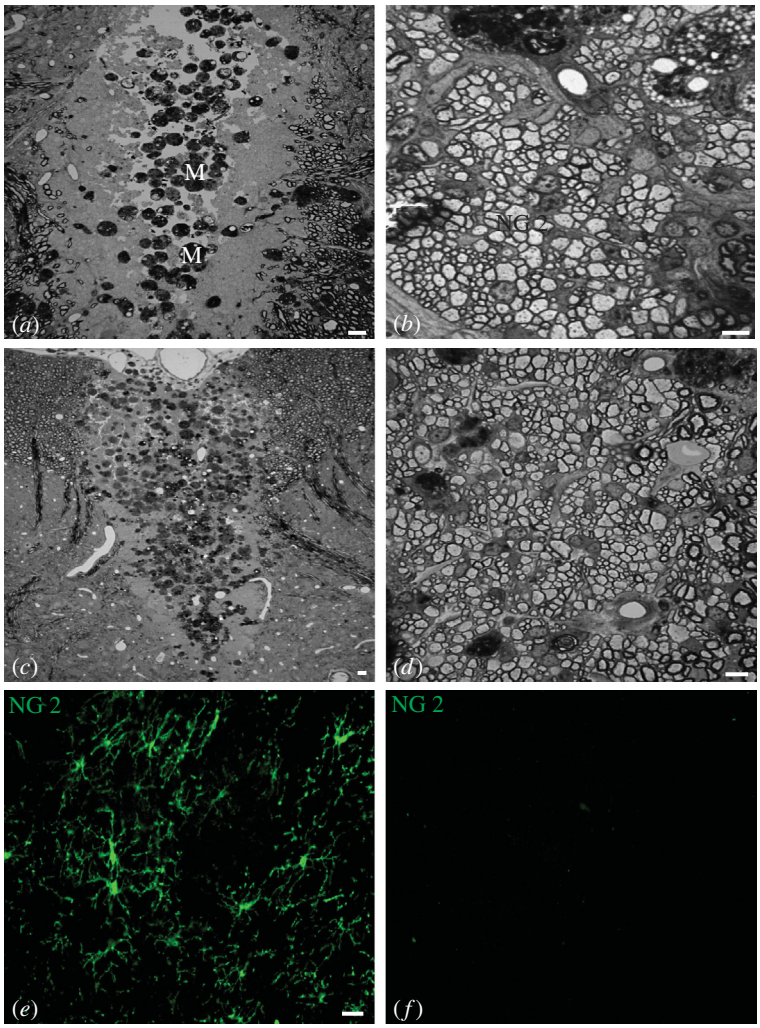

Figure 4. Allograft-mediated remyelination can be monitored by injecting cells into areas of demyelination induced by injection of 0.1% ethidium bromide (EB) into spinal cord white matter in which remyelination by host cells has been inhibited by exposing the spinal cord to 40 Gy of X-irradiation. Using this approach, it can be shown that survival of transplanted allogeneic oligodendrocyte lineage cells and the persistence of the remyelination they undertake is influenced both by donor–host haplotype combination and graft composition. (*a*) Shows the non-remyelinating nature of the area of demyelination four weeks after injection of EB. Demyelinated axons are clumped together and the only cells present are myelin debris filled macrophages (M). Normally myelinated tissue bounds the area of demyelination. (*b*) When such lesions are transplanted with syngeneic mixed glial cultures 3 days after their induction, the demyelinated axons are completely remyelinated by oligodendrocytes generated from the transplanted OPCs by four weeks. (*c*) Area of demyelination showing a diffuse distribution of myelin debris filled macrophages at four weeks in an allografted animal. By four weeks there was no evidence of oligodendrocyte remyelination in the area of demyelination in 100% of Lewis rats transplanted with PVG derived mixed glial cell cultures. (*d*) When Lewis rats are transplanted with PVG derived oligodendrocyte enriched preparations, 75% of the animals show oligodendrocyte remyelination eight weeks after engraftment. (*e*) In Lewis rats transplanted with PVG mixed glial cell cultures OPCs can be found in the tissue surrounding the area of demyelination using anti-NG2 antibodies two weeks post-engraftment (prior to evidence of a rejection response) but are absent eight weeks post-engraftment (*f*). Scale bars (*a*) and (*c*), 20 μm, (*b*), (*d*), (*e*) and (*f*), 10 μm. (*a–d*) Toluidine blue stained resin sections.

influencing allograft-mediated remyelination, with preparations containing astrocytes and microglia being more immunogenic when transplanted as allografts than preparations enriched for the cells of oligodendrocyte lineage.

To examine this we compared the extent of transplant-mediated remyelination in the X-irradiated EB model of demyelination following transplantation of fully mismatched mixed glial cell cultures with that following transplantation of cultures depleted of astrocytes and therefore enriched for oligodendrocyte precursors. The transplantation of syngeneic mixed glial cultures led to excellent remyelination (figure 4*b*), while allografting of a similar preparation was followed by a rejection response against graft-derived oligodendrocytes (figure 4*c*) as well as transplanted oligodendrocyte precursors (figure 4*f*). This process started around one month and was completed by two months after transplantation. Rejection of mixed glial allografts varied between different donor–host strain combinations, so that PVG grafts into Lewis rats elicited rejection in all hosts at four weeks post-transplantation (figure 4*c*), but Lewis grafts into PVG showed survival in 50% of the hosts both at four and eight weeks post-transplantation. To test the effect of removing astrocytes on graft survival, we transplanted astrocyte-depleted, OPC-enriched, preparations using the PVG into Lewis combination that in the previous experiment

resulted in 100% rejection. We found that depleting astrocytes significantly improved graft survival in that allograft mediated remyelination was present in 75% of animals at two months post-transplantation (figure 4*d*). Furthermore, Lewis rats transplanted with PVG OPC-enriched allografts did not show DTH responses to either this preparation or PVG alloantigens (spleen cells), suggesting a lack of immune response against these grafts. Even though the surviving preparations were OPC-enriched, they were not pure in that about 20% of the cells were microglial cells. Since this cell type showed the highest levels of MHC expression of all the different glial cell types in our, as well as in other studies (reviewed under 'Major histocompatibility complex molecules expression in the CNS'), this would suggest that a certain threshold of MHC glial allograft expression determines whether rejection will be triggered or not. It may also be that the difference in the MHC expression is not the main cause of the difference in the survival between mixed glial cell preparations and OPC-enriched preparations. Since the main difference between these two populations is the astrocyte content, it is possible that the presence of this cell type may have introduced an important minor antigen which may have then elicited a rejection response. Alternatively, astrocytic presence may have also altered the cytokine environment of the lesion favouring a rejection response, since these cells secrete a variety of pro-inflammatory cytokines (reviewed in Benveniste 1992). It must also be remembered that the astrocytes present within transplanted preparations would have most likely been activated during the process of culture preparation for transplantation.

Therefore, the survival of glial allografts appears to be dependent on a balance of factors and is more likely to occur if preparations enriched for OPCs are transplanted (since these do not appear capable of sufficiently priming the host), even in the case of donor–host combinations that are likely to result in graft rejection. Long-term survival of transplanted allogeneic oligodendrocyte lineage cells remains to be investigated, but these experiments probably need to be performed in a different demyelinating lesion model because of the limitations of the X-irradiation EB lesion discussed in the model section of this review.

9. THE PARADOX

This review has focused on aspects of immune responses in the CNS pertinent to achieving graft-mediated remyelination in a demyelinating disease such as MS. However, it may not be crucial to prevent the rejection of engrafted tissue in order to achieve repair, and this is due to the complex pathophysiology of the disease. The following section will discuss how rejection of mismatched grafts may actually stimulate repair of demyelinating lesions since host pro-myelinating cells are present both within adjacent tissue and some areas of chronic demyelination in this disease.

MS lesions can be divided into two categories, those in which there is remyelination and those in which remyelination is absent or restricted to a narrow rim around the periphery of the demyelinated plaque (Lucchinetti *et al*. 2000). This indicates that the CNS has the ability to remyelinate demyelinating lesions in this disease, but this process fails for some reason. One explanation for remyelination failure with progression of the disease is that OPCs, in addition to oligodendrocytes, become targets of the immune attack in MS. This would result in demyelinating lesions depleted of OPCs. In such a situation, OPCs from neighbouring, non-affected tissue would have to be recruited into the areas of demyelination in order to achieve remyelination. Based on studies which examined the rate at which adult OPCs repopulate OPC-depleted tissue in the rat it has been proposed that, because of the slow rate at which this happens (0.2 mm per week in rats over 1 year of age; Chari *et al*. 2003; Foote & Blakemore 2005*b*), in large areas of OPC-depleted demyelination, it would take some considerable time for recruited OPCs to interact with demyelinated axons (Chari & Blakemore 2002). Many studies have shown a stimulatory effect of inflammation on remyelination (reviewed in Franklin 2002*b*; Bieber *et al*. 2003). Therefore, delayed interaction of OPCs with the demyelinated axons will mean these cells are not exposed to the conditions present in an acutely demyelinating lesion which would normally provide the stimuli required for their activation and generation of remyelinating oligodendrocytes. Support for this proposal comes from: (i) studies in which OPCs introduced into areas of chronic demyelination fail to remyelinate axons unless an acute inflammatory response is induced (Foote & Blakemore 2005*a*), (ii) experiments that show that the extent of remyelination is reduced when there is a temporal mismatch between demyelination and the interaction of OPCs with demyelinated axons (Blakemore *et al*. 2002) and (iii) the observation that OPCs and premyelinating oligodendrocytes can be found in some areas of chronic demyelination in MS without evidence of remyelination (Chang *et al*. 2000, 2002). Because of the importance of inflammation for remyelination one can suggest that rejection of engrafted cells may be beneficial for host OPC-mediated remyelination since it would provide the acute inflammatory stimulus required for efficient remyelination. Indeed, studies in which xenogeneic OPCs were transplanted into a demyelinating lesion offer support for this proposition (Blakemore *et al*. 1995). In these experiments, mouse OPCs were transplanted into a spontaneously repairing demyelinating lesion model induced in the rat spinal cord. Under immunosuppression the transplanted mouse OPCs remyelinated the demyelinated axons. With removal of immunosuppression, the remyelinating mouse cells were rejected, and this resulted in efficient remyelination by host cells. Similar results were obtained in our laboratory when transplanted allogeneic mixed glial preparations were rejected after transplantation into a spontaneously repairing demyelinating lesion model (unpublished data). Therefore, these experiments would suggest tissue matching in OPC transplantation for the treatment of a demyelinating disease such as MS may not be crucial since either survival or rejection of these cells could ultimately lead to remyelination.

10. GLIAL ALLOGRAFTING FOR CNS DEMYELINATING DISEASES: CONCLUSIONS AND QUESTIONS

This article has discussed the need for tissue matching of glial grafts in order to achieve remyelination of non-repairing demyelinating lesions such as those present in MS. The CNS is characterized by a highly specific regulation of immune responses, and the survival of allogeneic oligodendrocyte lineage cells appears to be enhanced in situations favouring graft rejection if preparations enriched for the cells of oligodendrocyte lineage are transplanted. The results of the studies described are, however only a first step when considering glial allografting for potential clinical applications. It has to be remembered that these experiments were performed in a toxin-induced rather than immune-mediated model of demyelination in which the system is not primed against cells of the oligodendrocyte lineage. The next step therefore must be to determine the fate of allogeneic OPCs transplanted into a model in which an immune reaction is ongoing or has occurred, such as EAE. What also needs to be determined is whether the survival of OPC allografts is due to the induction of active tolerance to these preparations. In another immune privileged site of the body, the eye, it has been well established that engraftment into the anterior chamber not only results in enhanced survival in comparison to the engraftment into the periphery, but also in a suppression of DTH responses to the engrafted antigens, a phenomenon named anterior chamber associated immune deviation (ACAID; reviewed by Streilein 2003). ACAID is mediated by the induction of regulatory T cells in the spleen (Streilein & Niederkorn 1981; reviewed by Streilein 2003). A similar phenomenon named brain associated immune deviation has been suggested in the case of ovalbumin placed in the striatum of mice with the regulatory cells responsible for DTH suppression present in the cervical lymph nodes rather than the spleen (Wenkel *et al*. 2000), which would be consistent with the differences in antigen drainage between the eye and the CNS. Further experiments would be required to establish whether allograft survival in the CNS requires an induction of regulatory T cells and possibly results into an immune deviation.

We would like to acknowledge Jennifer Gilson for technical assistance with animal surgery; also, we would like to thank Drs Su Metcalfe, Alana Thackray, Barbara Blacklaws, Cyril Barbezange and Geoff Butcher for discussions and advice concerning transplantation immunology. V.T. was supported by a Merck Sharp and Dohme fellowship and the Cambridge Overseas Trust.

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