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The Pathobiology of the Oligodendrocyte

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A recent surge in interest in the role of the oligodendrocyte in the etiopathogenesis of, and recovery from, demyelinating diseases such as multiple sclerosis has stimulated extensive investigation into the structure and function of the cell in health and disease. Although first regarded as a relatively metabolically inactive cell with little capacity for vigorous reaction to injury, the oligodendrocyte is proving to be exceedingly varied, with a complex developmental background, a wide range of protein and lipid products made in large quantities, and a cell transport system requiring passage over considerable distances. In this respect, it has many similarities to the neuron, and one of the objectives of this paper will be to note recent observations concerning oligodendrocyte behavior and pathology which were previously described only in neurons. In addition, the range of pathologic alterations in the cell following various insults is proving to be far wider than the simple all-or-none reaction to unknown factors in multiple sclerosis and similar diseases. Similarly, whereas the cell was previously considered to have little or no regenerative potential, the current literature is replete with examples, not only of spontaneous regeneration, but also of attempts to enhance repair by a variety of stratagems, designed not only to study basic biological behavior, but to eventually help in the alleviation of human disease.

The purpose of this review is to highlight the important aspects of the pathobiology of the oligodendrocyte. No attempt will be made to provide a complete catalogue of all aspects of this fascinating cell, and where necessary, the reader will be referred to other sources for more complete details. This review will also not provide a comprehensive outline of the etiology and the pathology of the demyelinating diseases, other than to note, where relevant, their obvious relationship to the oligodendrocyte. For example, there will be no separate section on damage or repair in multiple sclerosis; aspects of multiple sclerosis will be discussed in the various sections dealing with mechanisms of cell damage and repair. Rather, an outline will be given of some of the newer observations being made on a wide range of oligodendrocyte behavior, and it is hoped that these will provide some insight into some of the questions that still intrigue clinicians and researchers in the field of myelin and the demyelinative disorders. For more details of those conditions, the reader is referred to previously published reviews (1, 2).

Because so many of the reactions of the oligodendrocytes to disease resemble those that occur during normal development, a full discussion of normal genesis, structure and function is essential for the understanding of the behavior during injury and regeneration. Those aspects which are common will be discussed in the earlier sections, and referred to later.

DEVELOPMENTAL BIOLOGY

The formation and maintenance of myelin is the major function of the oligodendrocyte. It is possible that the perineuronal satellite oligodendrocytes may serve to regulate the microenvironment around neurons, and there is emerging evidence (see below) that oligodendrocytes have a role in axon growth and maintenance. This requires an enormous metabolic load, as the unique lipid-rich myelin membrane, with its relatively simple specific protein content, is an extension of the cell processes, often formed at a great distance from the perikaryon. Each oligodendrocyte can myelinate up to 50 internodes, although this number is far lower in the case of large axons requiring thick sheaths. It has been estimated morphologically that an oligodendrocyte with a perikaryal surface area of 100 to 300 μm2 supports a surface area of myelin up to 2 million μm2 (3). During myelination, an oligodendrocyte can make up to 3 times its weight of myelin membrane per day (4). To do this requires not only the requisite protein productions, but specific transport mechanisms to deliver these lipids and proteins to the periphery.

Oligodendrocytes develop in the subventricular zones from the neuroectoderm, and migrate and differentiate into mature, probably post-mitotic myelin-producing cells. It is significant that a much larger number of cells than needed are produced, and up to 50% of the cells undergo apoptosis, thus reducing the number during development (5). This will be discussed in detail below. The origin of these cells has been the subject of some of the most intense investigation in the oligodendrocyte field. Although classical studies using ultrastructural analysis and autoradiography made great strides in establishing the features of differentiating cells, it was with the demonstration of sequential expression of stage-specific cell markers, revealed by immunochemistry in culture (6), and to a lesser degree in vivo, that more rigorous attempts to define oligodendrocyte lineage have been made. These

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have been presented in greater detail elsewhere (3, 7, 8), and a summary of the findings in oligodendrocyte differentiation will be given here.

Although precursor cells expressing vimentin and NCAM are recognized both in vivo and in vitro, the earliest committed precursors are cells that express the gangliosides GD3 and A2B5. These cells, which are classically bipolar, were first isolated from the rat optic nerve, and in a highly significant experiment, were shown to be bipotential and capable of differentiating into either oligodendrocytes or type 2 astrocytes depending on the tissue culture conditions (9). They were named the O2A progenitor, and have remained the prototypical cell of this type. In culture they are highly proliferative and migratory. Under the influence of serum, medium conditioned by astrocytes, or factors such as PDGF or FGF (10, 11), IGF (12), and TGF-B, these cells progressively lose the ganglioside staining. At the same time they begin sequentially to express the SCIP transcription factor, and stain with the O4 antibody against the pre-oligodendrocyte antibody (POA) (6), and the Ranscht antibody, GalC and O1 against galactocerebroside. Certain factors can inhibit development, and retinoic acid (RA) has been found to inhibit partially both differentiation and proliferation of precursor cells (13). As a final stage in maturation, the cells express the myelin proteins, the earliest of which to appear is CNP, followed by PLP, MBP and MOG. Although this represents a general trend, the exact onset of these antigens is still not fixed and it is still unclear whether the names given to the various stages, such as pro-oligodendrocyte, pre-GalC, and immature oligodendrocyte, denote real differnces. Nevertheless, it is probable that cells progressing along this lineage lose their migratory and proliferative capabilities, although again, the exact point at which this happens is not clear. Most authors have reported the cessation of proliferation before the aquisition of galC, but others (14) have found division at this stage and even later (15, 16). In pathological conditions, the situation may not be exactly the same, and here division of more differentiated cells may provide a pool of cells (see below under remyelination). The most well-described proliferative factor during development is PDGF (17), but FGF (10), NT-3, and IGF-1 (18) may have similar effects. The growth factor-induced proliferation, as well as related fos-induction, may be mediated through protein kinases. New mitogens, such as oligodendrocyte trophic factor (OTF), are continually being described. For some time it was felt that breakdown products of myelin, present during developmental remodeling and demyelination, might also be mitogenic, but recent studies have failed to confirm this (19). Axonal contact (20), mediated either through membrane molecules or through electrical activity (21), may lead to oligodendroglial proliferation.

Likewise, O4-positive cells are thought to have ceased to migrate (22). In culture, a certain degree of plasticity takes place, and dedifferentiation in response to factors such as PDGF and phorbol esters can occur (3). Although this developmental scheme can take place in isolated oligodendrocytes, it is clear both in vivo and in vitro that neuronal influences play an important role in oligodendrocyte differentiation as well as proliferation (21). The well-established temporal pattern of myelination known to the neuropathologist denotes an orderly, tract-specific process that has to be influenced by the axon; in tissue culture, both soluble factors released by the axon, and contact-mediated axonal influences are important regulators of differentiation and myelinogenesis (20, 21, 23).

Of major importance has been the question of whether these stages exist in vivo, or whether they represent tissue culture phenomena. Immunochemical staining and retroviral lineage tracing experiments have clearly shown that there are cells with similar staining characteristics to those described in culture, suggesting that a similar developmental process probably exists in the intact brain. O2A-like cells, staining with GD3 and A2B5, have been demonstrated in the subventricular zone, especially ventrally, from where they have migrated laterally and dorsally (24, 25). It should be pointed out, however, that some GD3-positive cells seen in vivo have been shown to be of microglial origin, rather than belonging to the oligodendroglial lineage. Retroviral lineage tracing, however, has generally shown that most precursors give rise to a single cell type (26), although some exceptions do occur. This may relate, however, to the stage of development (8), as it would appear that neonatal subventricular cells form both oligodendrocytes and astrocytes, whereas juvenile cells form only the former. In addition, it appears that the local CNS environment may play an important role in allowing for the preferential differentiation into either oligodendrocytes or astrocytes (8). This local influence, which has been demonstrated following transplantation of O2A precursors into the intact brain (27), may also be stage-specific, varying with the degree of development. However, it is fair to say that although cells with staining features of O2A cells are found in vivo, there is as of yet no clear evidence that they differentiate bipotentially into astrocytes, and indeed it is not certain that the type 2 astrocyte actually exists in vivo (24, 26). What is known is that O2A progenitor cells grown in culture and then transplanted in vivo can differentiate into both oligodendrocytes and astrocytes, perhaps under local influences (27, 28). This occurs with both normal cells, as well as with cells immortalized by viruses and oncogenes (29), the latter being of tremendous potential as a source of large quantities of transplanted remyelinating cells in demyelinating diseases.

In addition to fetal and neonatal optic nerve, progenitor cells have also been isolated from adult optic nerve, and

in this situation, their mobility and proliferative potential is seen to differ from the developing situation (30, 31).

The developmental patterns described above have been shown to be present in cultures derived from human cortex and white matter (31, 32), and some of the same cells have been demonstrated in human tissue sections. These cells are capable of differentiation and proliferation in culture. Of particular interest has been the demonstration of the precursor cell in adult human tissue, providing a potential source of remeylinating cells (31).

Myelin

The central nervous system myelin membrane is the main product of the oligodendrocyte, and its role in axonal physiology is well known. It is not the object of this paper to review the chemical and physical structure in detail (see reviews, 3, 4, 33), but some important details are important for the understanding of the function of oligodendrocytes. The myelin sheath, although derived from and continuous with the oligodendrocyte membrane, differs from it chemically; its lipid content is 70% of the dry weight compared to the 40% found in the oligodendrocyte, implying that a gradient of lipids and proteins exists from the perikaryon to the periphery. The major lipid component is phospholipid, about 40%, but cholesterol and glycolipids, of which the most promimnent is galactocerebroside, account for 26% and 32%, respectively.

The protein structure components of the myelin sheath are relatively small in number, but their absence can cause severe inherited orders of myelination (reviewed in 34). The major component is proteolipid protein (PLP), a 30Kda protein, which together with its alternatively spliced isoform, DM20, accounts for about 50% of the protein. This protein spans the membrane in 4 domains. Genetic mutations of this protein cause the Jimpy and Rumpshaker dysmyelinating mice, and Pelizaeus-Merzbacher disease in humans.

Myelin basic protein (MBP) comprises about 35% of myelin protein. It exists in multiple isoforms, ranging from 14–23Kda. Recently, a new gene that uses MBP exons has been described, called the Golli-MBP transcription unit, which appears earlier in development than MBP (35), and which may play a role in oligodendrocyte development. Genetic alterations of MBP produce the Shiverer mutation in mice.

2′-3′-cyclic nucleotide 3′-phosphotidase (CNP), an enzyme of 46 and 50Kda isoforms, is present mainly in oligodendrocytes and their processes, but is difficult to locate in compact myelin. Further myelin membrane proteins are the myelin-associated glycoprotein (MAG), and the myelin oligodenrocyte glycoprotein (MOG), both occurring at very low levels, although their immunoglobulin-like domains suggest an important role in cell adhesion. With the advent of new molecular techniques such as differential screening, no doubt many further proteins specific for oligodendrocytes will emerge.

Despite much work in vivo and in tissue culture, specific roles for these proteins in the formation and maintenance of myelin have not been proven. Some hints have been provided, which are summarized here (see also 33). The use of transgenic and knockout mice will certainly become of increasing importance in helping to understand the specific roles of these proteins, although some of these early studies have shown not only a redundancy in the system, but a situation more complex than originally thought; a MAG knockout mouse was able to generate a surprising amount of myelin, although this was subtly altered (36). The early appearance and cytoplasmic localization of CNP has suggested that it is a GTP-binding protein important in differentiation by binding ATP. DM20 also appears at an early stage and has been suggested to be involved in myelinogenesis, whereas the adhesive properties of MAG and its localization against the axon suggest a role in the initial axon/myelin contact. PLP, through its adhesive extracellular domains (37), and MBP, or at least some of its adhesive isoforms (38), are thought to be involved in myelin compaction, while MOG, in spite of its immunoglobulin domains, appears only late, and may have a role in myelin maintenance. All these proteins may form complexes with glycolipids, to form specific domains either in the membranes of the oligodendrocyte process or the myelin sheath itself, and indeed antibodies directed against galactolipids may block differentiation (3). All these lipids and proteins may be highly immunogenic and important in the pathogenesis of immune-mediated demyelinating diseases.

Process Formation and Axon/Cell Adhesion

Myelination, and by the same token, remyelination, requires that the complex array of proteins and lipids in myelin be available for interaction with the axon at the right time and in the right place. This is achieved by the formation of cell processes, almost the hallmark of the mature oligodendrocyte in situ or in primary cultures (3, 4, 39), in secondary cultures after processes have been sheared during the removal of cells (39), and in remyelination (40). The formation of processes is no doubt related to the particular cytoskeleton of the oligodendrocyte, and to the enormously high content of microtubules seen in these cells. Oligodendrocytes do not contain intermediate filaments, but do have actin microfilaments which comigrate with the microtubules. It has been shown that the microtubule-associated protein, MAP1B, is first expressed in pre-oligodendrocytes, and subsequently associates and distributes with microtubules during the formation of processes and the development of the mature oligodendrocyte phenotype. Process formation is also critically mediated by protein kinase C (PKC). Phorbol esters which stimulate PKC have been shown to

dramatically enhance process formation and myelin basic protein synthesis, whereas inhibitors of PKC block fiber outgrowth (39).

Not only is the cytoskeleton important for process formation, but it also plays a key role in the translocation and transport of myelin proteins to the periphery (3). mRNA for MBP, PLP, and CNP all seem to be produced on ribosomes in the perikaryon, and then are transported to the process tips during myelination at different speeds by the cytoskeleton for incorporation into the membrane. It is possible that MBP mRNA is transported prior to translation, which is done in situ at the periphery. This appears to be signal specific, as globin mRNA, a marker protein coinjected with MBP mRNA, is not transported to the processes with MBP mRNA. It is also important to note that the tips of cell processes contain active mitochondria with a high respiratory quotient, indicating an important role in early axonal contact and myelination (41). The molecular events underlying these processes remain the subject of further study, and are of great importance for the study of remyelination.

Time-lapse video microscopy has demonstrated some of the events occurring at the time of oligodendrocyte axon contact (42). Oligodendrocytes send out processes that locate axons prior to myelination. The processes produce lamellopodia, which after tentative contacts and retractions anchor to the axon by means of stout filopodia, which ruffle and roll around the axon within about 90 minutes (min). These experiments suggested that adjacent filopodia anchoring the axon become new lamellopodia, which then fuse laterally, but this remains a speculation. After several turns of the oligodendrocyte process, the cytoplasm is extruded, and myelin compaction takes place. The adherence of the oligodendrocyte process to the axon is an essential prerequisite for both myelination and remyelination (40, 43). Much of the work on factors involved in this adherence has been done in tissue culture, although the immunochemical finding of MAG, a putative adhesion molecule (see above), early in remyelination suggests that the same holds true in vivo as well. The importance of cell adhesion in initiating myelination has been shown using sheep oligodendrocytes in culture, where adhesion of the cells to the polylysine substratum induces phosphorylation of MBP, and oligodendrocytes selectively adhere to neurons in culture (43). There are numerous factors which are candidates for mediating axon/oligodendrocyte adhesion. These include NCAM, whose highly polysialylated form PSA-NCAM is expressed after demyelination with lysolecithin (44), neuron-gliaCAM, and MAG. The adhesive property of the latter molecule has been attributed either to the N-glycan bearing the HNK-1 carbohydrate epitope, or else to being a ligand of an endogenous lectin present in myelin, the cerebellar soluble lectin (CSL). In addition, gangliosides have been shown to be neuronal ligands for MAG. Other oligodendrocyte-localized adhesion molecules include intercellular-CAM and a newly described glial adhesion molecule (for references see 2). Recently, the expression of integrins on the surface of oligodendrocytes has been demonstrated (45), and has been shown to be developmentally regulated. It is not clear whether these molecules, the major extracellular matrix receptors for laminin, fibronectin and vitronectin, are required for actual axon/oligodendrocyte contact; the latter study (45) would suggest not, and it is possible that their main role is to anchor oligodendrocytes to astrocytes, which are the main secretors of these ECM molecules, or to aid in cell migration.

Migration

In both myelination and in remyelination, is is essential that the cells gain access to the unmyelinated axons, and the processes in each of these are probably analogous (46). In fact, during development it is obvious that all cells have to travel considerable distances from their site of genesis. What factors act as cellular attractants for these migrating cells is at present unknown; it is entirely possible that any of the factors discussed above as cell adhesion molecules may have this role, and during remyelination many cytokines and other products present during myelin breakdown may also play a part. PDGF has been specifically mentioned as a chemoattractant in culture (18). In the developing animal, autoradiographic evidence has shown that cells migrate out from the subventricular zones, and recently experiments with retrovirus labeling have confirmed and extended these studies (8). Much of the information regarding oligodendrocyte migration comes again from tissue culture studies (see above in development), where comparative assessments of the motility and migratory capacities of mature and precursor cells have been made, and where many of the adhesion factors, integrins, and extracellular matrix molecules have demonstrated (see above). In this regard it would seem that cells up to the stage of expressing O4 retain the ability to migrate (22). These processes have not been well-demonstrated in vivo, which is of course more difficult. Indeed, it has long been thought that oligodendrocytes lacked the capacity to migrate in the central nervous system, and even Schwann cells, traditionally thought to have far greater migratory capacity, when injected into an unmyelinated area, remained localized around the needle tract through which they had been inserted, and remained immobile (47).

The extensive use of transplantation of cells to enhance remyelination has changed the concepts of oligodendrocyte migration. This can occur using both adult and fetal tissues, using dissociated cells as well as tissue grafts. Different authors have shown migration of cells through the white matter (48), as well as along the meninges, along blood vessels, and through the CSF pathways. In

vivo, the migrated cells have been traced by retrovirus labeling or by labeling with the lacZ gene, expressing beta galactosidase. The age of the recipient is of importance in these studies, although in general it would appear that the most extensive migration occurs when fetal tissue is transplanted into fetal recipients, although successful migration has been described in adult animals receiving mature tissue (personal observations). Migration of cells occurs not only in undamaged normal tissue, but both glial and Schwann cells may migrate from the site of injection into an area of cord demyelinated by lysolecithin, or through areas of unmyelinated axons in genetic myelin mutant models (49). It is possible that the unmyelinated or demyelinated axons act as a specific attractant for these cells. Following migration, cells differentiate and mature (50), and myelination or remyelination then occurs (50, 51). In naturally occurring migration, endogenous Schwann cells may be prevented from entering the CNS by the glial limitans, and therefore the role of the astrocyte in enhancing preferential migration of the oligodendrocyte at the expense of the Schwann cell becomes of great importance (51).

In spite of the ever-increasing knowledge of the steps leading to successful myelination and remyelination outlined above, it is clear that there is still some axonal/oligodendroglial signaling mechanism that initiates the final stage of myelination. That the steps of differentiation, proliferation, process extension, and axon wrapping may all operate independently has been shown in experiments where sheep oligodendrocytes added to rat dorsal root ganglion neurons proceded through all the steps short of myelin compaction; the same cells transplanted into Shiverer mice brains could form compact myelin (43). This confirms that the intact CNS was obviously providing some factor or stimulus lacking in the culture system and also may account for the local environment effect on differentiation noted above (8).

The Influence of Oligodendrocytes on Axonal Growth and Regeneration

Recent experiments have demonstrated that one of the important factors inhibiting the regeneration of mammalian neurons and axons is the presence of oligodendrocytes and myelin. This was initially shown in culture, where axons failed to grow on sections of mature white matter and their growth cones, when exposed to mature oligodendrocytes, collapsed. Antibodies raised against the growth-inhibiting factors isolated from CNS myelin, and labeled IN-1 andIN-2, can be administered both in vivo and in vitro, and have been shown to enhance axonal regeneration. These are directed against CNS antigens, and may be more specifically directed against MOG (52). Destruction of oligodendrocytes using specific oligotoxins may also enhance regeneration of axons (53). It is of interest that the oligodendrocytes of fish, in which axon regeneration occurs quite readily, support growth of both fish and mammals. To further complicate the picture, Xenopus, oligodendrocytes from optic nerve, which are known to regenerate, support axonal regrowth in the frog, whereas those from the adult spinal cord, which does not regenerate, do not (54), indicating a local change in control of oligodendrocyte function. This inhibition fulfils a very important role of preventing random regrowth of axon sprouts once pathways have been established and myelinated by stabilizing axon numbers, but carries with it the teleological downside of not permitting extensive regeneration after damage; in addition, oligodendrocytes play a role in the maturation of axons, as in the absence of these cells, axon diameter remains small, resembling those seen in the premyelinated neonate (55). Finally, it is known that some oligodendrocytes and their precursors express nerve growth factor (56), and may play a role in neurite maintenance.

MECHANISMS OF OLIGODENDROCYTE DAMAGE

Damage to the oligodendrocyte is seen in a large number of conditions, human and animal, both spontaneous and experimental. It is beyond the scope of this review to describe all these diseases and models, and recent reviews are available (2). Rather, this paper will attempt to discuss the pathogenetic mechanisms leading to injury and death of the oligodendrocyte, the pathways of which are common to many of these diseases. It is important to note that whereas the end result of oligodendrocyte dysfunction is demyelination, not all demyelinative diseases are caused by destruction of the cell, and may be secondary to damage to the myelin sheath.

The dysmyelinating diseases are those in which mutations of the myelin or other genes interfere with the normal formation of myelin, and often have other effects on cellular function, such as perturbation of the cell cycle in the PLP mutant, Jimpy. These diseases have been reviewed recently (34), and they will not be discussed here.

An etiological classification of diseases affecting the oligodendrocyte is given in Table 1. It will be noted that in some diseases, the mode of damage is unique; in cuprizone and ethidium bromide toxicity, the mitochondrial DNA forms abnormal dimers, leading to abnormal respiration and necrosis, and following radiation, the DNA of the cell is damaged, leading to apoptosis. In addition, some viruses cause oligodendrocyte damage by direct infection, such as the papova virus in PML, and the coronavirus, the mouse hepatitis virus. However, in a large number of other diseases, such as the immune-mediated diseases (e.g. multiple sclerosis and its experimental analogues), the damage is caused by numerous factors, many of which are final common pathways. These include those viral diseases in which a strong immunological reaction mediates the oligodendrocyte damage accompanying the infection, such as Theiler's virus,

TABLE 1
Diseases Affecting the Oligodendrocyte: An Etiological Classification

Primary disorders:
1. Genetic mutations of oligodendrocyte genes (e.g. Pelizaeus-Merzbacher disease in humans; jimpy or shiverer mouse mutants).
2. Immune-mediated oligodendrocyte diseases (may cause direct damage to the myelin sheath as well) (e.g. postinfectious encephalomyelitis, acute hemorrhagic leukencephalitis, multiple sclerosis in humans; EAE in animals).
3. Viral diseases:
   a) Viral diseases directly infecting the oligodendrocyte (e.g. progressive multifocal leukoencephalopathy, possibly HIV in humans; mouse hepatitis virus in animals).
   b) Viral disease damaging the oligodencrocyte via immune mechanisms (e.g. herpes simplex in humans; canine distemper virus, Theiler's virus in animals).
4. Acquired toxic/metabolic oligodendrocyte diseases:
   a) In humans (e.g. thyroid deficiency in development, radiation, anoxia/ischemia).
   b) In animals (e.g. cuprizone toxicity, ethidium bromide toxicity).

Secondary disorders:
Oligodendrocyte dysfunctions secondary to Wallerian degeneration and axonal dysfunction (e.g. trauma, ischemia, or toxicity).

distemper virus, Semliki Forest virus, and even some coronavirus infections. Further details of all these diseases are available in recent reviews (2, 57), but the common pathogenetic mechanisms will be reviewed below.

Molecules Affecting Oligodendrocyte Injury

The macrophage, through its interaction with the resident cells of the nervous system, is a potent producer of many of the molecules toxic to the oligodendrocyte, in vivo and in vitro (57–61), and blocking the action of these cells may abrogate the pathology. Many cytokines have been implicated in the cytotoxicity of oligodendrocytes, but gamma-interferon (G-IFN) and TNF appear to have roles that are very well established. G-IFN enhances EAE, and its blockade may attenuate disease (60). It is seen in EAE in memory T cells, and has been found in T cells in the cerebrospinal fluids of multiple sclerosis patients, as well as at the edge of lesions (58). It is not, however, found in nonimmune conditions such as Wallerian degeneration. It also may indirectly cause cell damage by enhancing adhesion of microglia to the oligodendrocyte.

TNF, and its related cytokine, lymphotoxin (LT), has been shown to be toxic to oligodendrocytes in culture (62, 63), and is produced by autoreactive T cells, and by macrophages in EAE, EAN, and Wallerian degeneration. As discussed above, TNF causes oligodendrocyte cell death via apoptosis (63), rather than by cell lysis.

Important effectors of cell damage include oxygen radicals, which cause cell injury in a wide variety of situations. The are known to cause peroxidation of lipids, and their effect on membranes implies that they cause cell necrosis or lysis, rather than apoptosis. They may affect the oligodendrocyte, or the the myelin sheath, and have been found in the acute stages of EAE, but are absent in the chronic or quiescent lesions (64). Oxygen radicals are commonly elaborated by the macrophage in an oxidative burst, as a final pathway of many disorders. Scavengers of these radicals may diminish disease.

Another putative mediator of cell damage is nitric oxide, a product of activated macrophages (65), often stimulated by autoreactive T cells. Incubation of microglia with target oligodendrocytes increases nitric oxide production in a contact-dependent manner, and this can be prevented by antagonists of nitric oxide (65). Oligodendrocytes in culture are more vulnerable to morphological alterations than microglia or astrocytes. Finally, although there has been some controversy over this, nitric oxide is thought to kill cells by necrosis, rather than by apoptosis (66).

There has been some interest in the role of complement in causing cell injury. Rises in intracellular calcium leading to cell death have been shown in vitro following pore formation caused by the membrane attack complex also known as the terminal complement complex. This is produced by macrophages (58). The C9 component of complement has been detected in MS and EAE tissues, and may be consumed in the CSF of these patients. Further evidence for involvement of the complement system in oligodendrocyte damage is the demonstration of the terminal complex on oligodendrocytes prior to demyelination. It should be noted, however, that some studies have failed to show that human oligodendrocytes are susceptible to complement damage in culture (67).

It has been further suggested that oligodendrocytes may be particularly vulnerable to changes in intracellular calcium, induced by either complement, perforin, or calcium ionophores such as ionomycin (58, 68). The calcium ionophore A23187 (69) may induce an injury mimicking complement attack, which may be abrogated by calmodulin. Beside the direct attack on the oligodendrocyte, calcium ionophores may also directly attack the myelin, causing vesiculation, macrophage stripping and demyelination. The calcium influx induced by complement membrane attack on oligodendroglia does not necessarily always lead to cell lysis, which depends on the oscillatory or nonoscillatory nature of the influx.

Although eicosanoids have been demonstrated in multiple sclerosis tissue, and prostaglandins increase dramatically during disease exacerbation, the effect on oligodendrocytes is probably only secondary to the that on the macrophage. Again, however, eicosanoid inhibitors may

attenuate the disease by decreasing chemoattraction for macrophages.

Macrophages and other cells also release a variety of proteolytic enzymes, some of which have been found to be increased in demyelinating diseases (70). Calpain and other neutral proteases and phospholipases have been shown in culture to degrade myelin, and acid proteases such as cathepsin have been found in tissue and CSF in multiple sclerosis; their effect on the oligodendrocyte is less clear.

The role of T cells in causing oligodendrocyte destruction has been the subject of much investigation, and has been extensively covered in recent reviews (57). How these activated T cells, so prominent in immune-mediated demyelination, actually cause the lysis of oligodendrocytes is less clear. It is obvious that CD4 cells may express G-IFN, TNF, Interleukin-6 (IL-6) and other cytokines injurious to these cells as discussed above. However, CD4 cells may also lyse oligodendrocytes through a mechanism independent of TNF and other factors (71). This may be achieved by direct adhesion through the Fas-FasL death domain system, leading to apoptosis. In addition, there is strong evidence to suggest that gamma-delta-T cells, which recognize heat shock protein 65 on oligodendrocytes (72), are important oligotoxic effectors, perhaps operating through G-IFN. These cells have been found in the more chronic stages of multiple sclerosis, co-localized with oligodendrocytes expressing heat shock proteins.

B cells have long been known to play an important role in the pathogenesis of demyelinating disease, and their effect on myelin degeneration in vivo and in tissue culture has been described (see 2, 57). From the standpoint of the oligodendrocyte, it would seem that B cell lysis is effected through the complement mechanism discussed above.

Finally, an interesting role for heat shock proteins (HSP) in mediating oligodendrocyte disease has been well described in recent years. An excellent recent review in this journal, to which the reader is referred (73), details the biology and occurence of these proteins in multiple sclerosis. A summary of the specific role in oligodendrocyte injury will be presented here. HSPs, which can be both constitutive and inducible, act to stabilize the protein structure and cytoskeleton in both stressed and unstressed cells. They are grouped in different classes depending on molecular weight. Early sudies showed that oligodendrocytes, especially in acute lesions, expressed HSP-65 (72), and that these cells co-localized with gamma-delta-T cells. The HSP was also found in proliferating cells at the lesion edge, either suggesting a protective role for this protein, or alternatively raising the possibility that the newly dividing cells may be more susceptible to further attack. This induction of HSP in oligodendrocytes subjected to heat stress may be mediated by IL-1 (74). The induction of HSP72 by heat stress appears to be less prominent in oligodendrocytes than in neurons or astrocytes. HSP70 has been found in large quantities in multiple sclerosis, but its localization and role in pathogenesis are less clear. Finally, a small molecular weight HSP of 23Kd, identified as alpha-beta-crystallin, has been identified in multiple sclerosis brains and has been proposed as a candidate auto antigen (75) in demyelination.

It is obvious from the above discussion that multiple mechanisms leading to cell injury are possible, and indeed probably operate not only in different diseases, but in the same disease at the same time. It is, however, important to attempt to dissect these mechanisms out, as current and future strategies for therapy will develop out of blockade of these specific mechanisms.

Excitotoxicity in Oligodendrocytes

In spite of the tremendous amount of work done on the subject of excitotoxic damage to neurons, especially during hypoxia, relatively little information is available concerning the possible role that this process plays in damage to the oligodendrocyte. However, it is well recognized that the oligodendrocyte is sensitive to hypoxia/ischemia, and undergoes necrosis to a degree secondary only to the neuron (76). In vitro studies have shown that the oligodendrocyte is almost as susceptible to glutamate as the neuron, in contrast to astrocytes, which are resistant (77), and that the toxicity of glutamate was mediated by free radical attack. This has been suggested as the mechanism of cell death of oligodendrocytes, not only in ischemia and infarcts, but also in conditions such as periventricular leukomalacia. Oligodendrocytes of the O2A lineage have been shown to express glutamate receptors of both the kainate and the AMPA subtypes (78), and like neurons, they can be damaged by exposure to kainate as well as glutamate. In addition, hypoxia may damage oligodendrocytes reversibly and functionally. Cells subjected to hypoxia show reversible inhibition of alpha-hydroxy fatty acid and myelin basic protein synthesis, and hypoxia appears to affect signal transduction by targeting GTP binding protein-mediated processes. Hypoxia also rapidly induces the synthesis of ferritin in cultured oligodendrocytes (79). This appears to follow an early and potentially toxic disruption of intracellular free iron homeostasis caused by the hypoxia, and possibly acts to protect cells from iron-catalyzed lipid peroxidation. The high ferritin content of normal oligodendrocytes is an indirect indication of the potential vulnerablility of these cells to hypoxic damage. Changes in intracellular calcium, as discussed above, are most likely one of the final common pathways leading to cell death in excitotoxicity caused by anoxia, as in many other forms of cell injury.

Maturing oligodendrocytes also express alpha1 adrenoreceptors of the 1A type (80). The potential significance of these in pathologic processes is unclear, and it may be

that they mediate the neurotransmitter released by the neurons as a function of development.

Apoptotis in Oligodendrocytes

Cell death can occur by necrosis, characterized by disruption of the plasma membrane, organelle and cytoplasmic swelling, random DNA cleavage and an associated inflammatory response, as well as by apoptosis (programmed cell death), where there is nuclear and cytoplasmic condensation and endonuclease-mediated DNA fragmentation into regular ladder-rung nucleosomic packages of 180 to 200 base pairs. Although the same etiological factor may at times cause both types of cell death (66), it is important to try to differentiate the two processes, as the strategies for treating them may be entirely different. Indeed, although nitric oxide has been shown to cause both types of cell death in other systems, in oligodendrocytes, its effect appears to be confined only to necrosis (66). In multiple sclerosis and similar diseases, where apoptosis is known to occur in oligodendrocytes (81, 82), oligodendrocyte cell death may be prevented or ameliorated by such strategies.

During normal oligodendroglial development, numbers of cells are controlled both by the number of mitotic cell cycles, a process susceptible to PDGF and FGF, as well as by programmed cell death (5, 83). It has been shown that in the developing rat optic nerve, about 50% of newly formed oligodendrocytes normally die. In vitro, these cells, when deprived of serum or trophic molecules, display the features of apoptosis. This programmed cell death can be largely prevented by the addition of molecules like PDGF (84), insulin-like growth factor (IGF), CNTF, leukemia inhibitory factor, and interleukin-6. CNTF has also been shown to protect oligodendrocytes from natural and TNF-induced apoptosis, but not from complement-induced necrosis (85). FGF has been shown by some to be capable of preventing apoptosis in vitro, although other studies have failed to confirm this.

The protooncogene BCL-2, through its encoded protein, enhances cell survival by blocking programmed cell death, and has recently been demonstrated in oligodendrocytes by immunostaining and immunoblotting (86), whereas the tumor suppressor gene p53, known to be a mediator of apoptotic cell death, can be demonstrated to be induced in oligodendrocytes undergoing apoptosis following exposure to dimeric interleukin-2 (87).

The question of the type of oligodendrocyte cell death seen in multiple sclerosis remains the source of some controversy. Some pathological studies have demonstrated the universal presence of apoptotic cells bearing myelin markers at nearly all stages of the disease (81, 82), and the presence of TNF, a known inducer of apoptosis, has been shown in EAE and MS lesions (58). On the other hand, it is most likely that in the MS lesion multiple factors play a role and both processes are operative. In a study designed to differentiate the two processes of cell death, membrane lysis, measured by chromium or lactate dehydrogenase release and caused by exposure to activated CD4 cells, indicated necrosis in cultured oligodendrocytes, whereas apoptosis, measured by the TUNEL technique, followed exposure to TNF (63, 71). Administration of CNTF protects against TNF-induced apoptosis (88). Necrosis of oligodendrocytes also follows exposure of the cells to gamma/delta lymphocytes. All of these mechanisms are thought to be candidates in the pathogenesis of the MS lesion.

Apoptosis of oligodendrocytes takes place in other situations as well. Following spinal cord trauma, apoptotic oligodendrocytes are seen, although Bcl-2 expression is not upregulated (89). In addition, apoptotic oligodendrocytes are seen in the spinal cords of rats infected with HTLV-1, and are thought to be at the basis of the demyelinative myelopathy seen in this situation (90). Finally, apoptosis has been seen in oligodendrocytes in Alzheimer disease (91).

Dying-back Gliopathy

A further mechanism of oligodendrocyte damage is the so-called distal or dying-back gliopathy (see 2). This was first described in the inner tongues of Schwann cells in the PNS of Syrian hamsters, and subsequently in the oligodendrocyte in Cuprizone toxicity. In this situation, the damage to the cell occurs slowly; the perikaryon and its essential processes appear preserved, while the cell is unable to maintain its functions at the periphery, where the inner cytoplasmic tongue degenerates first. This process is analogous to the well-described dying-back phenomenon seen in the distal axon, and again points out the similarities of cells that have extensive transport and cytoskeletal functions, and their vulnerability to injury. Ultrastructural studies of the early lesion in multiple sclerosis have shown similar patterns in the myelin, suggesting that this mechanism operates here as well, following injury to the oligodendrocyte (92).

REMYELINATION

Repair of damage or loss is a vital reaction of any tissue, and recent studies have confirmed the ability of CNS myelin to be regenerated in a significant and functional way. Although regeneration and remyelination occur much more vigorously in the peripheral nervous system, it is now well established that in the central nervous system, in both naturally occurring and experimental demyelinating diseases, remyelination takes place in humans as well as animal species. Much current work now centers around the attempts to manipulate and augment this remyelination. The general features of this remyelination have been well described, and the reader is referred elsewhere for complete details (2, 40). Because

there are no chemical differences between normally myelinated and remyelinated axons, the identification of a remyelinated axon depends on morphological features, namely a myelin sheath that is inappropriately thin, and an internodal length that is inappropriately short, for the size of the axon. Some confusion may arise with a partially demyelinated axon, but knowledge of the timing of the injury may help to differentiate between these two situations.

In general, once an axon has been demyelinated, and the offending agent withdrawn, the remyelinating cells will send out cytoplasmic processes around the axons within a week, and will proceed to wrap the axon in a spiral fashion, recapitulating the normal myelination process, with an inner and an outer cytoplasmic tongue. This leads to compaction of myelin, which may occur in clusters, indicating the ability of the remyelinating cell to remyelinate more than one internode, as in development. Remyelinated myelin never regains its normal thickness, and the normal linear relationship between axon size and sheath thickness is never regained. Experimental studies of development suggest that at the time of myelination the oligodendrocyte receives an axonal signal that determines the myelin thickness, and that if this occurs at a later time when the axons have reached their terminal size, this signal is never received again. All evidence suggests that remyelinated myelin, although thinner than normal, is ultrastructurally and chemically normal. Myelin proteins such as myelin basic protein and myelin-associated glycoprotein are present not only in the sheaths in a manner similar to that seen in normal development, but are also seen in the remyelinating oligodendrocyte prior to the onset of this process (2).

There are numerous factors necessary for successful remyelination to occur; most of these have been summarized previously (40). It is of great importance to realize that in general these are the same factors and steps seen in normal myelination, and the necessitity of an adequate number of cells, cell proliferation, migration and differentiation, process extension, axon wrapping, and myelin compaction have all been extensively discussed above in the section on development. The first is the supply of available remyelinating cells. In experiments on demyelination using chronic or recurrent Cuprizone toxicity, Theiler's Virus, and chronic experimental allergic encephalomyelitis, remyelination is enhanced and the remitting disease allows for the survival of larger numbers of oligodendrocytes. The source of the remyelinating cells has become the object of intense study. Most studies on central nervous system remyelination have demnonstrated that the oligodendrocyte is the cell primarily responsible for repair. Under certain conditions it appears that the perineuronal satellite oligodendrocyte may also be capable of remyelination. Although Schwann cells can carry out remyelination under certain circumstances in a variety of demyelinating conditions, this does not appear to be highly significant, and seems to occur mainly where the glial limitans has been breached and where astrocytes, especially type 1, do not form a barrier to interaction, and where O2A precursors are not present to compete with the Schwann cells for remyelination (93).

There is an accumulating body of evidence to suggest that the remyelinating oligodendrocyte is derived in most cases from a precursor cell. This is also intuitive, as the biology of oligodendrocyte lineage would suggest that this cell is the most versatile, mobile, and proliferative. As has been previously discussed extensively in the section on development, the most likely candidate is a precursor cell similar to the O2A cell. The evidence that precursor cells are present in the adult has been presented (see above). This is evident in the the Cuprizone model, where it was shown that proliferation occured prior to remyelination in cells identified by ultrastructural study as immature cells, which subsequently matured into oligodendrocytes (2). A further example is seen in the remyelination following the injection of antigalactocerebroside into the optic nerves of cats (94), where the presence of vimentin, as well as HNK-1 staining, marked the cells as immature. Some authors have used the staining of oligodendrocytes with HNK-1 to claim that newly divided immature cells are formed in acute multiple sclerosis, and would be responsible for remyelination (95). In the coronavirus model of demyelination (96), immunostaining for O4 antigen denoting an O2A-like cell revealed an increase in these cells following demyelination. In addition, experimental transplantation of O2A cells into demyelinated lesions results in extensive and successful remyelination (97).

There is, however, some evidence that remyelination may be carried out by surviving mature cells, and it is even possible that some of these cells may be capable of proliferation with a subsequent expansion of numbers. In multiple sclerosis, it has been shown that surviving cells, assumed to be relatively well differentiated because of staining with MOG, are present in lesions, often where the demyelination is early (81, 82). Other authors have noted the potential for the involvement of mature cells in remyelination (98). Proliferation of mature cells has also been noted by the uptake of tritiated thymidine in vivo following trauma, and lysolecithin (see 2). In vitro studies have provided a basis for this suggestion by demonstrating proliferative activity in mature cells (15). In another study (99), differentiated cells reacting with the RmAb and 007 antibodies were able to continue, dividing up to 4 or 5 times. The latter cells, which morphologically displayed a mature, extensive, cytoplasmic network, displayed migratory characteristics and an ability to regrow cell processes after injury. A possible role for growth factor expansion of the number of these mature cells has been suggested (16) following stimulation by FGF. It

should also be noted that oligodendrocytes may have a much greater tolerance for survival than previously believed. In experiments on Wallerian degeneration in the rat, oligodendrocytes surviving a metabolically quiescent phase for up to 2 years in the sectioned optic nerve were able to express myelin proteins and when re-exposed to axons (see 2).

It is therefore reasonable to assume that there will be different possible sources for regenerating oligodendrocytes, and that although precursor cells are more likely to be present to carry out this role, mature cells may also take part. The relative contributions of these will vary according to the type of injury, the mechanism of demyelination, the age of the animal and the lesion, and the presence of factors potentially stimulating the cells. More than one mechanism may be present in the same disease, or even in the same lesion.

The factors involved in cell migration, process extension, cell adhesion, axon wrapping, and myelin compaction do not appear to differ from those seen in development (see above).

Enhancement of Remyelination

Attempts to enhance remyelination as a possible therapy for demyelinating diseases have drawn heavily on the basic science experiments and the clinical observations described above. These strategies have centered around the factors required for remyelination, including the provision of adequate numbers of remyelinating cells, either by enhancement of numbers using growth factors, or by the transplantation of exogenous cells. In addition, remyelination has been enhanced by modulating the initial demyelinating disease, either specifically or nonspecifically.

The studies on the effects of growth factors on oligodendrocytes in development have stimulated the use of similar mitogens to enhance cell numbers and differentiation after demyelinating disease. Following the demonstration of enhancement of regeneration of oligodendrocytes with IGF (18), and the finding of receptors for IGF on oligodendrocytes during remyelination after Cuprizone toxicity (100), administration of Igf was shown to increase PLP production and quantity of remyelination in rats suffering from EAE (101), as well as to reduce clinical symptoms. The therapeutic use of PDGF and FGF has not been achieved in the in vivo situation, although numerous authors have predicted their usefulness in the future (18, 102), as well as the future potential for gene transfer therapy to introduce growth factor specifically into the brain where needed.

Immune modulation has been successfully shown to enhance remyelination in EAE. Treatment with MBP and antigalactoside resulted in diminishment of the immune reaction and subsequent enhancement of remyelination (103). These experiments led to the discovery that administration of immune IgG to animals demyelinated with Theiler's virus resulted in enhanced remyelination (102, 104). The activity of the IgG appears to reside in two monoclonal autoantibodies directed against multiple organs, but recognizing surface and cytoplasmic determinants on glial cells. Standard immunosuppression using cyclophosphamide also enhanced remyelination (104), and the use of antibodies against oligodendrocyte-destroying cytokines such as TNF-alpha may be a useful avenue in the future.

By far the largest literature on enhancement of remyelination arises out of the experiments on the transplantation of myelinating cells, the background of which has been covered above. Since the earliest demonstration that transplanted cells could migrate and remyelinate demyelinated lesions in the Shiverer mouse (105), investigations have been directed at determining the situations in which this may occur, the species restrictions, the optimal source of cells, and the optimal age. Although Schwann cells can be grafted and remyelinate, most studies have been on central glia (106). The myelination can occur both in normal (107) and in dysmyelinated animals such as the PLP mutant MD-rat and the shaking pup (47, 49), as well as in areas of induced demyelination (28, 51, 93). The proportion and type of cell can influence whether the remyelination will take place by transplanted oligodendrocytes or endogenous Schwann cells. Co-transplantation of type 1 astrocytes with the oligodendrocytes reconstitutes the glial limitans, and effectively inhibits Schwann cell remyelination. Although any cell along the differentiation spectrum from O2A to mature O1-positive can remyelinate, the most extensive remyelination is seen with the use of A2B5-positive O2A cells (28, 93), the numbers of which may be expanded with the use of growth factors. A defined or transformed O2A-like cell line, such as CG4, can successfully differentiate into oligodendrocytes and astrocytes and remyelinate lesions. Successful remyelination can occur following transplantation into adult animals. Although cross-species transplants are effective, the use of adult recipient animals leads to rejection, requiring the use of immunosuppression (93). This is complicated by the fact that human cells have been relatively unsuccessful as transplants (108). Finally, it should be pointed out that most of the transplants have been into relatively acutely demyelinated lesions, and the potential for transplantation into chronic lesions has not yet been fully explored.

DISORDERS OF THE OLIGODENDROCYTE CYTOSKELETON

As described above, the prominence of microtubules in the cytoplasm and processes of oligodendrocytes, and the complex trafficking of myelin constituents imply a major role for the cytoskeleton in the maintenance of cell

structure and transport. It is not surprising, therefore, that disorders of this system within the oligodendrocyte have started to become evident in the broad group of the degenerative diseases. In this regard, it is important to regard the oligodendrocyte as a cell with functions located at a distance from the perikaryon, in many ways very analogous to the neuron, and therefore susceptible to many of the same pathologic changes seen in that cell. A recent review in this journal (109) describes the full pathological details of all the conditions in which these aberrations occur. The recognition that, in multiple system atrophy (MSA), glial cells with the morphological features of oligodendrocytes contained "microtubular masses" which stained with the classical cytoskeletal reactions (110) was followed by the recognition that these are present in a variety of degenerative diseases, such as progressive supranuclear palsy (PSP) (111), corticobasal degeneration (CBD) (112), Alzheimer disease (113), Pick's disease, and argyrophilic grain dementia. As in the neurons affected by these conditions, the composition of the glial inclusions varies with these conditions. In CBD, the inclusions in oligodendrocytes identified with Leu-7 staining, which extend into the processes, are reactive with antibodies against epitopes along the entire tau molecule (112). No ultrastructural studies have been done on these inclusions to this date. In contrast, oligodendrocyte inclusions in MSA stain with antisera to tubulin, ubiquitin and alpha-beta-crystallin, while staining very weakly with anti-tau. Ultrastructurally, the filaments comprising the glial cell inclusions (GCI) in this disease are 20 to 30 nm in diameter.

The cytoskeletal inclusions seen in Alzheimer disease, labeled glial fibrillary tangles (113), have been seen in oligodendrocytes identified by their CNP and transferrin content. They contain tau and ubiquitin, and appear to be distinguishable from those seen in MSA both immunochemically and ultrastructurally; they contain straight filaments 16 nm in diameter.

The significance of these inclusions is not clear. Although they may be of some diagnostic value, they are of far greater importance in helping to understand some of the pathogenetic mechanisms in these degenerative conditions, as their presence suggests a widespread involvement of subcellular structures not restricted to the neuron. Secondarily, they may contribute to the white matter degeneration sometimes found in these conditions.

CONCLUSIONS

It is thus evident that the oligodendrocyte is susceptible to a wide variety of pathological insults, and therefore the range of its reactions is broad. The very specialized nature of its function in myelination requires a complex differentiation pattern, whereas the large metabolic load needing to be delivered at a site distant from the perikaryon requires a cell machinery in many ways analogous to the neuron. The cell is also exquisitely dependent on its surroundings and adjacent cellular elements for survival and function. It is therefore not surprising that modes of cell injury formerly thought to be confined to the neuron, such as excitotoxicity, dying-back, and apoptosis, as well as hypoxia and cytoskeletal abnormalities, have now been shown to occur in this cell as well. The potential for regeneration is likewise much greater than previously believed, while the role it plays in regulating axon growth and numbers has turned out to be fascinating during development, but more frustrating during axonal regeneration.

Nevertheless, the investigations cited above have helped build a clearer understanding of the cell and have opened the door to future investigations directed at therapy.

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