Brain repair

ABSTRACT—Diseases of the human brain and spinal cord are common and often progressive since, unlike peripheral nerve, the adult human central nervous system does not spontaneously repair itself. Studies on development and cell lineages in the nervous system have started to elucidate the scientific basis for this lack of regeneration, and have suggested ways of enhancing repair. At the same time, improved understanding of neurodegenerative processes has provided a rationale for treatments which limit neuronal and glial damage. Cell implantation has been tested, experimentally and in man, and with some prospects for successful restoration of normal cell arrangements in the central nervous system. Taken together, a coherent strategy for limiting the damage and repairing the brain is beginning to emerge. The translation of these ideas into clinical practice is timely and eagerly awaited.

Neurological medicine has its share of diseases which are common, affect the quality of life for affected individuals and in which, despite the availability of increasingly sophisticated diagnostic methods, the impact on treatment has been disappointing. In 1543, Andreas Vesalius first showed how the human nervous system is arranged by opening the head and depicting what was inside; 450 years later, still too little can be done to influence recovery from head and spinal injury or from stroke; the management of neurodegenerative disorders such as Parkinson's disease is confined to symptomatic treatment; and slow progress has been made in influencing the course of multiple sclerosis. These are the common disorders which target the nervous system of young adults and the ageing population. They represent an enormous burden for individual patients, their carers and society.

In each of these situations, the underlying problem is that the damaged brain does not spontaneously undergo repair. However, contemporary neuroscience no longer regards the brain as incapable of change and a more dynamic view of adaptation and plasticity is beginning to emerge. To understand why the central nervous system (CNS) does not routinely repair itself, and to enhance regeneration of nerve fibres and replenishing brain cells is now seen as a realistic goal which should, in time, have general implications for neurological medicine. Rapid progress is being made in defining the causes and mechanisms of brain disease, many of which will soon be understood at the genetic level. It may be some time, however, before this knowledge reduces the frequency of common neurological diseases, and other disorders will turn out to be the result of such a complex interplay of genetic and environmental factors that prevention may never be an option. The more immediate prospect for success lies in developing strategies for limiting the consequences of brain injury and for repairing the damage.

An essential starting point is an understanding of the rules of development in the nervous system, on the assumption that many obstacles surrounding repair would be overcome if developmental processes could be restored in the context of disease. Possible explanations for the lack of repair in the adult brain include a relative deficiency of precursor neurons and glia, and growth factor conditions which do not support repair. Unlike peripheral nerves, central axons do not regenerate. One hopeful concept emerging in neuroscience is that the mature CNS has the potential for axonal recovery but that this is actively inhibited. Where substantial cell loss has occurred diffusely throughout the nervous system, in chemically defined systems or non-specifically within one region, structural and functional repair may require replacement of cells. Enhancing their regeneration through the release of endogenous processes or cell implantation is a major occupation of the brain repair strategist, but repair makes poor sense if the degenerative process remains uncontrolled. Limiting the damage will reduce the size of the task and protect structures which have successfully been repaired. As the slow transition is made from theory to practice, it will be essential to deploy sophisticated methods of assessment such as positron emission tomography (PET) and functional magnetic resonance imaging (MRI), to confirm that structure and function have both been improved, and to correlate these measurements with behavioural and clinical improvements. The strategy for brain repair must therefore address and link the separate disciplines of development, degeneration, regeneration, application and assessment if there are to be dividends for the individual patient from the efforts of those working in the basic and applied neurosciences.

Development

Studies of cell and molecular biology provide a coherent but as yet incomplete account of development in the CNS. As the neural tube forms in the embryo, multipotential stem cells are found in the ventricular and subventricular zones around the lateral ventricles. Exactly when stem cells become irreversibly commit-

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ted to a particular lineage is uncertain, but early in embryogenesis bipotential precursors of neurons and glia adjust their identity by responding to environmental cues. The principle of growth factor expansion of preterminally differentiated stem cells will prove essential for successful brain repair. *In vitro*, precursor cells can be recovered from the adult (murine) brain [1–3] which retain the ability to differentiate either into neurons or glia. They grow in response to combinations of growth factors (basic fibroblast factor (bFGF), nerve growth factor (NGF) and epidermal growth factor (EGF)), and differentiate only when these factors are withdrawn. Most cells found in the subventricular zone are neuronal with a low rate of division. This may be because the glial lineage has a greater capacity for mitosis and migration so that at any one time fewer cells are to be found in these germinal zones.

Neuronal lineages

Neurons develop from the most anterior vesicle of the neural tube, and cells destined for the cortex migrate along radial columns [4]. To some extent there is significant topographical and lineage scatter, which reflects the developmental plasticity provided by local environmental factors including afferent activity on neurons in the developing cortex [5,6]. Elongation of axons occurs through the extension of growth cones which must find their targets and form connections at remote sites in the developing brain. The awkward path taken by many axons indicates that guidance is provided *en route*. Subplate cells provide signposts for growing axons by switching their orientations, especially at points where bundles of axons converge. Axons appear to grow along preformed scaffolds fashioned by reciprocally innervating afferent and efferent pathways going to and from the same brain regions. Although many growth cones use guidance channels vacated by fibres which once passed this way, it remains uncertain how pioneering axons find their targets. Boundaries and orientations seem to be established by regional variation in the expression of genes which govern cell surface adhesion and repulsion molecules, and by regulating molecules which determine cell differentiation schedules. Simply stated, if a given cell is made to differentiate it will lose migratory potential and stay in place, whereas a stem cell which does not receive these instructions remains preterminally differentiated and free to grow on. Alterations in the local environment and contact-mediated inhibition induce stop signals, terminal differentiation and the development of dense dendritic arborisations. Growth then ceases, with calcium-dependent changes in the actin cytoskeleton and tubulin polymerisation of the advancing growth cone.

Cell survival

The overproduction of cells and axons in the developing nervous system, and the need to select those that are best suited to make stable connections, involve programmed cell death. Cell debris is removed by microglia which have the phagocytic properties of bone marrow-derived macrophages. Survival is probable for those cells which receive adequate amounts of trophic factors and for those that are electrically active. Growth factor dependence is further conditioned by intracellular signals, including calcium, such that cell

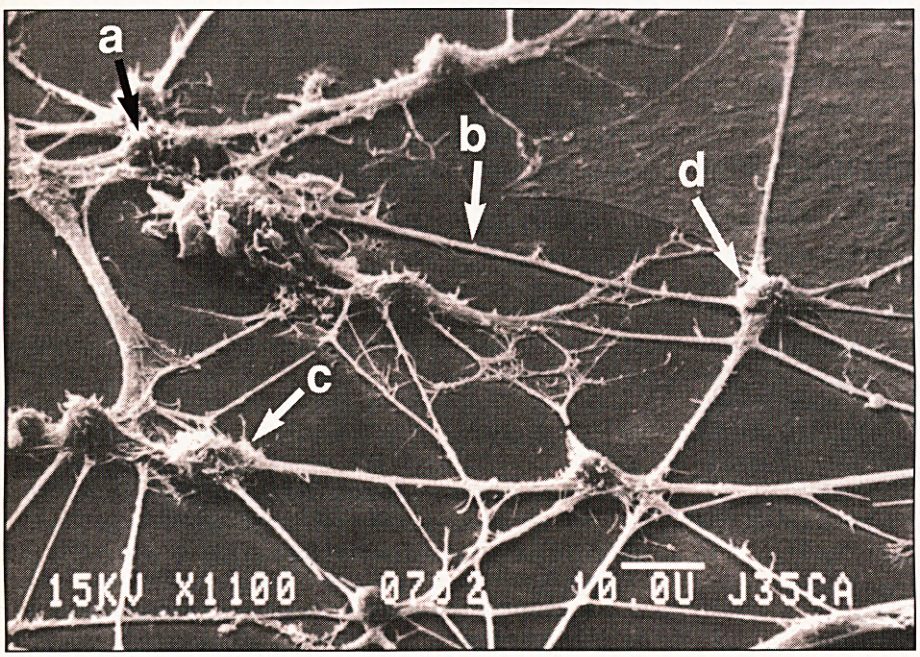

Fig 1. *Scanning electron micrograph showing cellular arrangements in the developed nervous system: a) nerve cell body and b) its axon; c) oligodendrocyte myelinating an axon; d) an astrocyte extending processes to neighbouring axons.*

death may occur despite the presence of trophic factors in concentrations sufficient for survival under more favourable conditions. These interactions illustrate that (at the cellular level) survival, growth, injury and repair are closely related states. In the worm *Caenorhabditis elegans*, the *ced-3* and *ced-4* genes programme for cell death, and *ced-9* regulates these suicide genes by activating the endonuclease which digests DNA and so causes apoptosis [7]. Of equal interest is the mammalian mitochondrial product Bcl-2 which protects cells that would otherwise undergo programmed cell death [8].

Nerve growth factors

A family of factors determining growth, survival and protection of neurons has been described. It includes NGF, brain-derived nerve growth factor (BDNF), neurotrophin NT-3, NT-4 and NT-5. These are produced by the targets that axons seek. Only those fibres which bind and achieve sufficient retrograde transport of growth factors during development will survive. Growth factor dependence continues during the life of that axon and its cell body, although it may diminish with time [9]. The early expression of receptors specific for one or other of the defined NTs makes it likely that developmental diversity within neuronal lineages is determined by whether neurons express specific NT receptors [10] and not by regional variation in their availability. Without growth factor support cells die by apoptosis but can be rescued in the interval between deprivation and activation of the nuclear endonuclease. This protection is calcium-dependent. NGF, BDNF and NT-3 all act as survival factors for neural crest-derived sensory neurons and some motor neurons. Sympathetic neurons require NGF, BDNF supports retinal ganglion cells, and both NGF and BDNF are survival factors for cholinergic neurons [11]; b-FGF promotes the survival of tyrosine hydroxylase-positive neurons derived from the ventral mesencephalon and influences the rate of division of their precursors [12]. Ciliary neurotrophic factor (CNTF), produced by peripheral nerve Schwann cells and some astrocytes, has effects *in vitro* on O-2A progenitor differentiation (see below), and acts as a survival factor for motor neurons. Removal of CNTF production by gene deletion (homologous recombination), leads to steady loss of motor neurons in the postnatal period [13,14]. Both CNTF and leukaemia inhibitory factor (LIF) promote cholinergic differentiation of sympathetic neurons and influence the survival of motor and sensory neurons. The most recently described factor is glial cell-line derived neurotrophic factor (GDNF) which stimulates fetal dopaminergic neurons in tissue culture [15].

Glial lineages

The first morphological change in glia is the appearance of radial cells which form parallel arrays from the subventricular zone to the subpial brain surface [16,17]. This radial network serves as a scaffold for nerve cells as they migrate from their germinal zones towards the developing cortex. Radial glia differentiate as nerve cells switch from migration to establishing synaptic connections. They show morphological variety, form the glial limiting membrane, contact blood vessels and the nodes of Ranvier, and have structural, immunological and metabolic functions in the developing nervous system.

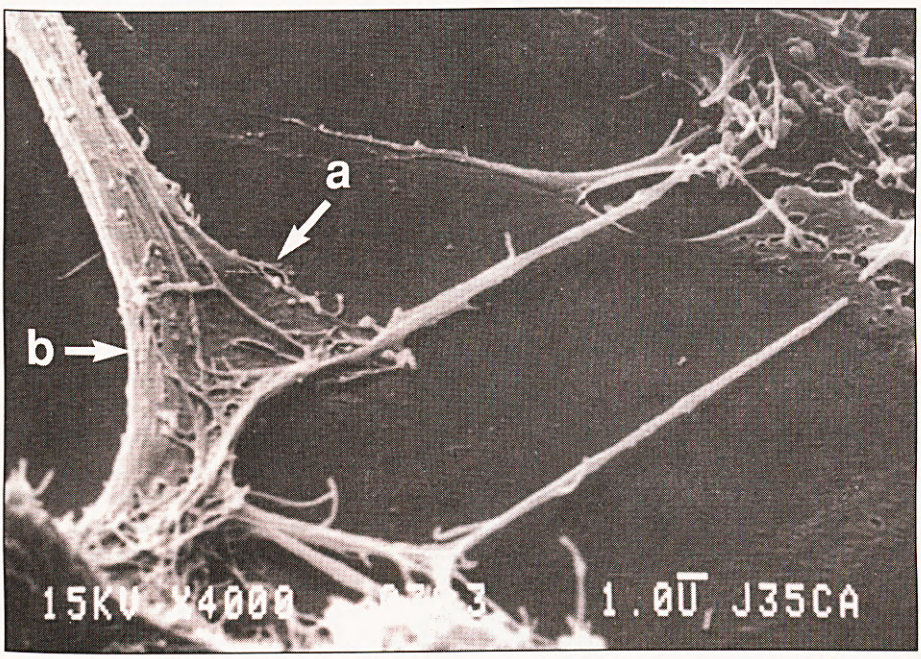

Fig 2. *Scanning electron micrograph showing a) the myelin sheath around b) an axon.*

Oligodendrocyte precursors arise from subventricular zones of the lateral and fourth ventricles. They proliferate, migrate and, about the time of birth, mature into oligodendrocytes which form the myelin membrane. *In vitro*, the bipotential glial progenitor differentiates into either an oligodendrocyte or an astrocyte (type 2) depending on culture conditions [18], but controversy surrounds its status *in vivo*. Myelination depends upon the expression of cell surface and extracellular matrix molecules which ensure interactions between oligodendrocytes, myelin and axons [19]. Differential regulation in the expression of adhesion molecules, including janusin, tenascin, laminin and fibronectin, occurs during cell–cell contact and is required for stability of the emerging glial-neuronal unit [20].

Evidence from *in vitro* studies, with circumstantial support from the analysis of experimental and human disease [21–23], suggests that O-2A progenitor cells are also present in the adult CNS [24]. Adult O-2A progenitors divide slowly, but can re-enter a more proliferative phase for a limited period, perhaps by resuming the phenotype, growth factor responsiveness and behaviour of their perinatal O-2A counterparts [25].

Analysis of glial progenitors within the rat cerebellum has shown that cells with the O-2A phenotype develop only into oligodendrocytes, whereas the same cells recovered from the cerebellum and grown in tissue culture display phenotypic plasticity by differentiating into astrocyte and oligodendrocyte progeny [26]. The development of glial precursor cell lines, to exclude contamination of mixed cultures as an explanation for the appearance of astrocytic and oligodendrocytic progeny from O-2A progenitors, has made it possible to demonstrate their differentiation into oligodendrocytes and astrocytes when transplanted into the rat spinal cord [27,28]. Other workers have shown that cells migrating away from germinal zones retain developmental plasticity and differentiate either into astrocytes or oligodendrocytes [29].

Even this evidence leaves unresolved the question of whether a bipotential glial precursor, equivalent to the O-2A cell described *in vitro*, exists in the neonatal and adult nervous system. Its status is important since manipulating growth factor conditions might create an environment in which areas of demyelinated axons could be reconstituted by proliferative and migratory glial cells, and so be remyelinated. There is evidence that the nervous system does repair following oligodendrocyte depletion and demyelination. Gliotoxic lesions of the rat brain stem are accompanied by the accumulation of GD3-positive oligodendroglia around the lesion site [30]. However, in this and other experimental models of demyelination in which proliferating oligodendrocytes are found it remains a matter of controversy whether they are the progeny of migrating progenitors or de-differentiated mature oligodendrocytes [25,31].

Glial growth factors

Growth, migration, survival and proliferation of glial progenitors and their progeny are influenced by platelet-derived growth factor (PDGF), bFGF, insulin-like growth factor (IGF-1), interleukin (IL)-6 and LIF. In some situations, a pot pourri of factors is required to optimise growth and survival *in vitro* [32], but the full orchestration of these factors, either *in vitro* or *in vivo*, awaits notation. Astrocytes act as a source of glial growth factors, especially PDGF. However, this and

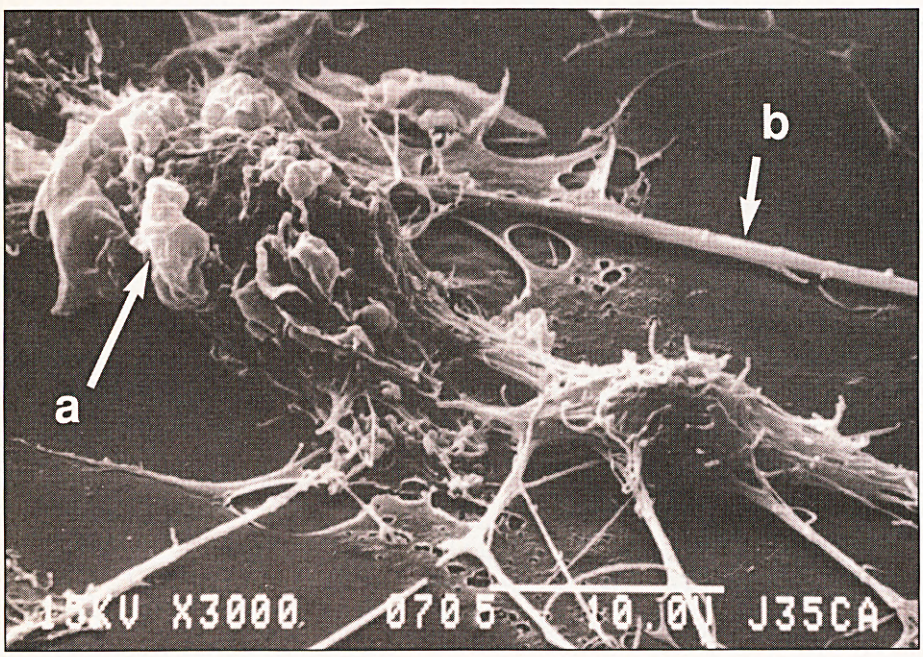

Fig 3. *Scanning electron micrograph showing a) a microglial cell phagocytosing a redundant neuron and b) its axon.* During development the nervous system is shaped by programmed cell death with microglial removal of surplus neurons and glia.

other unidentified soluble or cell surface-bound molecules which influence glial differentiation are also produced by neurons [33–35]. In addition, oligodendrocytes secrete autocrine factors that regulate their own development, which could be one means of maintaining a pool of undifferentiated oligodendrocyte precursors in the adult nervous system [36].

In vitro, O-2A progenitors differentiate into type 2 astrocytes under the influence of CNTF, extracellular matrix molecules and other, as yet unidentified, signals [37]. PDGF is mitogenic for O-2A cells but they escape from this stimulus after a number of divisions; and PDGF and bFGF together indefinitely suspend O-2A progenitor differentiation. IGF-1 and IGF-2 stimulate proliferation of oligodendrocytes, especially at the O4 stage: cells with the O4 phenotype remain capable of division but are not motile [38].

The interactive and complex effects of bFGF and PDGF [39] have been further defined by studies showing that motility is reduced in O-2A cells exposed to bFGF alone. With both growth factors, motility increases and the morphology simplifies, but differentiation into oligodendrocytes occurs only after growth factor removal. In addition, the inhibitory effect of bFGF on differentiation can be overridden by astrocyte-derived factors which promote differentiation of O-2A cells without inhibiting the proliferative drive of bFGF in low concentrations [40].

These results taken together illustrate that the separate phases of proliferation, migration, survival and differentiation of glia are under the control of separate growth factors [41]. In addition to their role as growth promoting factors, IGFs act as long term survival factors for O-2A progenitor cells and oligodendrocytes. As with neurons, cell numbers are regulated *in vivo* through changes in the availability of survival factors. This leads to strategic loss of a high proportion of newly formed oligodendrocytes in parts of the developing nervous system [42]. Less is known concerning astrocytes, but transforming growth factors-β inhibit astrocyte proliferation [43].

Axon regeneration

Axon regeneration depends both on the intrinsic ability of nerve cells to extend new growth cones and on the permissive nature of the environment in which growth is set to occur. Axonal potency seems to diminish with age and, as expected, is most prominent during embryogenesis. Human cells have been shown to cover vastly greater distances than rodent embryonic striatal axons transplanted to an equivalent environment [44,45], providing a clear demonstration of species differences and the enhanced ability of embryonic neurons to grow. These differences may relate to variations in the amount of microtubule-associated protein (MAP) available in the growing axon, which in turn affects the cytoskeleton of the growth cone, and adhesion molecules expressed by the advancing axonal tip, especially nerve cell adhesion molecule (N-CAM). The enhanced ability of frog retinal axons and mammalian olfactory neurons to regenerate correlates with persistent expression of embryonic forms of MAP and N-CAM. Although these switches in gene expression may not be restored in the adult brain, growth factors have been shown to enhance regeneration of those axons which they nurture during development [46].

Motor nerves regenerate slightly better through the CNS environment than do sensory neurons. Substantial differences in regeneration are also seen within and between species: neurons regenerate well in the frog visual system (but not in neighbouring non-visual pathways), and in situations where the environment has been made permissive by grafting peripheral nerve into the axotomised brain (see below); gamma-aminobutyric acid (GABA) neurons grow better than other thalamic neurons; and deep cerebellar nuclear axons grow better than cortical axons.

One other feature of glial neurobiology that relates to axonal regeneration is the inhibitory effect of glia on neuronal regeneration. The astrogliosis that is a feature of chronic injury in the CNS seems to limit axonal penetration, preventing access of regenerative elements into damaged areas. Although axons will grow across sheets of astrocytes in culture, this does not reliably model glial arrangements *in vivo*. Three-dimensional cultures of astrocytes present a physical barrier which regenerating axons will not cross, in contrast to a matrix assembled from Schwann cells [47].

The inhibitory properties of astrocytes have not been fully characterised, but the production of astrocytic cell lines that express only one or a few of several extracellular matrix molecules normally present on astrocytes and in glial scars has been correlated with inhibition of neurite outgrowth and impaired O-2A progenitor migration [48]. Differences in the extent to which embryonic and adult growth cones penetrate their surrounding matrix depend on local secretion of protease [49], which is increased by bFGF and IL-1, and indirectly on the availability of serine protease inhibitors. The astrocyte factor that inhibits regeneration of adult axons has a high concentration in grey matter and shows regional specificity, enhancing growth at one site whilst inhibiting it at another [50].

Apart from the physical barrier of the glial limiting membrane and networks of astrocytes, mature oligodendrocytes inhibit neurite outgrowth [47]. This results from expression of two molecules designated NI-35 and NI-250, but there may be others [51]. These are the molecules which contribute to axonal guidance during development [52] and optimise the ratio of neurons arriving at target zones to glia in the developing nervous system. *In vitro*, neurites will skirt around oligodendrocytes, and their growth cones collapse through contact inhibition if they meet the surface of an oligodendrocyte or its processes.

The repulsive and permissive properties of certain non-mammalian glial cells are also revealed by the ability of neurons from different species to grow large distances across fish oligodendrocytes [53]. One suggestion is that the enhanced regenerative ability of fish optic nerves relates to the release of a factor with IL-3 like properties. It is cytotoxic to oligodendrocytes [54], and so removes the inhibitory effects of their cell surface molecules.

Degeneration

A detailed discussion on the causes and pathogenesis of individual disorders is beyond the scope of this review, but many forms of injury converge on the same final common pathway of cell death. Those biochemical systems which subserve physiological functions in health are overwhelmed by the pathological process. When nerve and glial cells are physiologically active, they respond to molecules which bind to receptors, open ion channels and transduce signals across the cell membrane, leading to changes in intracellular calcium, induction of immediate early genes and transcription of cell-specific products.

The threat to cell survival and recovery of cell homoeostasis requires rapid alterations in gene expression and protein synthesis. Immediate early genes are usually expressed at low or undetectable levels in quiescent cells but are rapidly induced and transcribed within minutes of extracellular stimulation. Immediate early gene induction is stimulated by growth factors and other events occurring at the cell membrane, leading to calcium entry. Thereafter, activation pathways converge, increasing gene transcription and expression [55] which, in turn, establish the conditions needed for induction of cell-specific genes and structural repair. A sustained but transient rise in intracellular calcium lasting several minutes is sufficient to induce immediate early gene expression, but the transcription of individual genes may be differentially sensitive to intracellular calcium [56]. An uncontrolled rise may destabilise the cell, committing it to irreversible injury. Several immediate early genes have been identified. The best characterised is the proto-oncogene *c-fos* [57], transcription of which increases more than sevenfold within 15 minutes of stimulation with 12-0-tetradecanoyl phorbol-13-acetate (TPA). mRNA remains elevated for at least four hours, but transcripts are still detected several days after stimulation, indicating that repair mechanisms operate over a prolonged period. Immediate early gene transcription is followed by increased expression of genes that secure cell survival and of those that restore specialist or luxury properties of the oligodendrocyte.

The entry of calcium and associated intracellular signals shows different temporal and spatial organisation when physiological events switch to calcium overload and cell injury. These changes can be imaged in single cells responding to physiological or potentially damaging stimuli. Manipulation of this final common pathway may provide one means for protecting cells from injury, but attention has also turned to events occurring earlier in the biochemical cascade of cell death at the cell membrane.

Growth factor protection

One exciting development is that growth factors are now known to protect from injury the same neurons and glia as those they themselves support during devel-

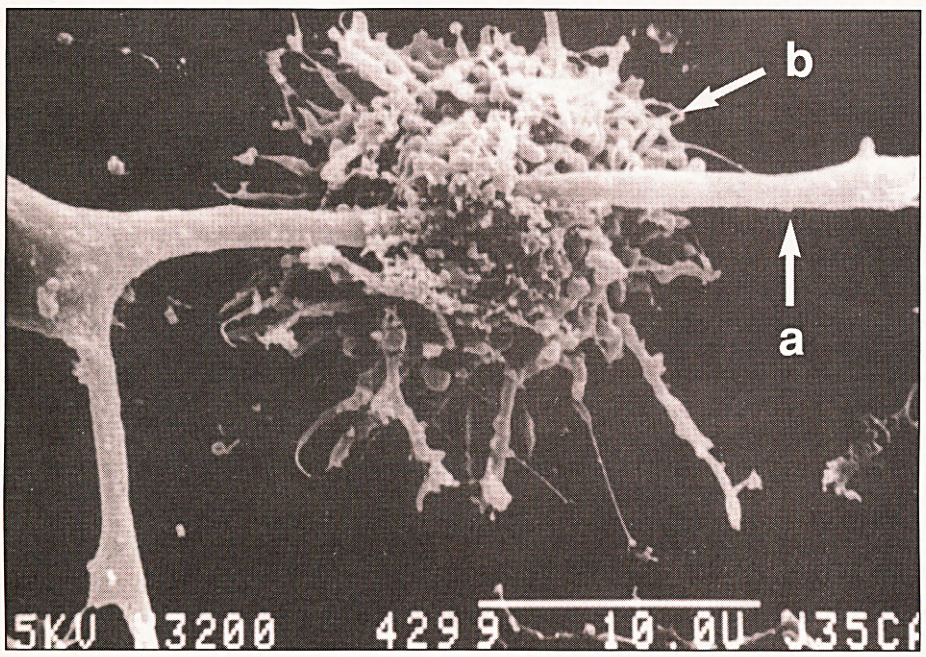

Fig 4. *Scanning electron micrograph showing a) oligodendrocyte phagocytosis by b) a microglial cell.* Following injury, microglial cells adhere to and remove damaged tissue.

opment and maintain in the post-mitotic state. For example, BDNF protects dopaminergic neurons from the toxic metabolite 1-methyl-4-phenylpyridinium (MPP^+) [58] and from axonal injury [59], and hippocampal neurons are saved from excitotoxic injury by bFGF. The latter and several other growth factors protect a range of hippocampal, septal and cortical neurons from hypoglycaemic and hypoxic injury; and their tissue expression increases following ischaemic and other insults *in vivo*. Many of these effects are mediated by stabilising the rise in intracellular calcium which characterises excitotoxic and other potentially lethal mediators of cell injury [60]. NGF and bFGF released close to the striatum reduce the excitotoxic effects of glutamate receptor analogues in a model of Huntington's disease [61]. CNTF may also be important as a survival factor in response to injury: it is transported in increased amounts to the cell body in axotomised nerve [62]. This and other growth factors influence the fate of nerve fibres threatened by injury, including motor neurons [63,64]. CNTF also appears to protect oligodendrocytes from injury by tumour necrosis factor (TNF)-α [65]. From this analysis emerges a scheme for protecting brain cells from injury whilst at the same time providing environmental cues that may be needed to restore normal cellular arrangements.

Neuronal injury and repair

The demonstration of mutations in the gene for superoxide dismutase (SOD) on chromosome 21 in familial motor neuron disease has suggested that selective motor neuron death results from oxidative stress [66], and similar mechanisms have been proposed to account for cell death in Parkinson's disease. Free radicals cause local damage by capturing nearby electrons to fill their own unpaired electrons, leading to lipid peroxidation and other changes that precipitate cell death [67]. Protection from free radicals occurs physiologically through dismutation by SOD and can be increased by scavengers such as vitamins C and E. Certain molecules, such as metals (including iron), donate electrons more readily than others, and free radical stress is enhanced if these are regionally concentrated.

In motor neuron disease, oxygen radicals might, through their ability to react with conserved nitrate tyrosine residues, interact with tyrosine kinase receptors and so induce survival factor deprivation involving the growth factors BDNF and NT-3. Other metabolic interactions are of importance in the generation of excitotoxicity. Oxygen radicals and nitric oxide are generated in response to a rise in intracellular free calcium. Tissue damage resulting from excitotoxicity can be modified by excitotoxin antagonists and by inhibition of oxygen radical and nitric oxide formation.

This metabolic cascade has been proposed as a final common pathway of damage occurring in a variety of neurodegenerative situations including ischaemia, Huntington's, Parkinson's, Alzheimer's and motor neuron diseases. In Parkinson's disease the combination of local iron accumulation in affected tissue [68] and the inherent capacity for dopaminergic neurons to generate oxygen radicals have been emphasised as specially inducive to neuronal injury. Clearly, there are rival, but not necessarily competing, theories for the mechanism of tissue damage in Parkinson's disease. These include exposure to toxic metabolites analogous to 6-hydroxydopamine or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its MPP^+ metabolite [69], and hypotheses generated from experimental evidence for a decrease in complex I of the mitochondrial respiratory chain [70].

Excessive postsynaptic stimulation has been related to anoxic-ischaemic neuronal injury in neural systems as a result of calcium influx, and the same mechanism can damage white matter tracts experimentally and after trauma. Attention to the combined neuronal and white matter tract damage occurring after focal ischaemia or trauma requires a more sophisticated approach since subtle differences exist in the mechanisms involved [71]. Many of these changes are reversible during the early phase of injury and are therefore amenable to strategies for cell protection.

Excitotoxicity caused by calcium overload and mediated by glutamate or its analogues operating through ligand-gated calcium channels has been suggested as an alternative mechanism of cell injury in motor neuron disease. One stimulus to this line of reasoning has been the toxicity of motor neurons that occurs following exposure to the cycad nut in the dementia-motor neuron disease disorder seen in the Pacific island of Guam. This hypothesis is not universally accepted and has been modified by the suggestion that an immunological, or some other primary insult, first injures motor neurons and changes their threshold for excitotoxic injury [72]. Others have shown that glutamate transport is defective in motor neurons from affected regions of the nervous system in patients with motor neuron disease, and that this is both disease- and region-specific. Defective transport would lead to glutamate accumulation and calcium overload, thus amplifying cell damage [73].

Injury of glia

Immune injury of the CNS follows damage to the blood-brain barrier, bringing inflammatory cells and their mediators into the brain. The movement of inflammatory cells involves alterations in the expression of selectins on the endothelial cell surface. At the same time, integrins are induced on the surface of infiltrating lymphocytes, with reciprocal expression of their receptors on endothelial cells. Both events are cytokine stimulated [74]. Inflammation involving cellular penetration of the blood-brain barrier occurs in acute insults of the nervous system such as trauma and

contributes to neuronal loss and astrocytic scarring, but it is also a feature of chronic and repetitive inflammatory injury. In multiple sclerosis, it culminates in removal of oligodendrocytes and their myelin sheaths by microglia.

The opportunity to study cells *in vitro* has helped to illuminate injury and repair mechanisms in oligodendrocytes. Rodent oligodendrocytes activate complement present in fresh autologous serum through the classical pathway [75]. Cells exposed to sublytic concentrations of complement show a transient increase in intracellular calcium. During this increase pores formed in the membrane are gathered into vesicles and shed from the cell surface; this restores membrane integrity and leaves the oligodendrocyte metabolically intact. Inhibiting the intracellular calcium activated protein calmodulin experimentally with W7 lowers the threshold for complement lysis by blocking vesiculation. In these circumstances, concentrations of serum from which oligodendrocytes are normally protected by vesicle formation prove lethal [76]. The evidence points to a pivotal role for calcium and calmodulin in orchestrating the response of oligodendrocytes to membrane injury. Single-cell studies show that a delicate balance exists between injury and repair, with oligodendrocytes showing heterogeneous responses in broadly similar experimental conditions. The ability to recover from inflammatory injury is associated with an oscillatory calcium response, whereas other forms of injury from which recovery may not occur feature different spatial and temporal calcium responses [77].

Complement activation of rat oligodendrocytes occurs because oligodendrocytes lack a factor, the complementary regulatory protein CD59, which normally protects cells from autologous complement injury by regulating assembly of the membrane attack complex [78]. Complement activation leads to breakdown of C3, releasing membrane-bound and fluid-phase products which determine interactions between oligodendrocytes and macrophages or microglia. These phagocytic cells possess receptors for C1q, C3b (CR1) and iC3b (CR3) which bind ligands on target cells. Some are constitutively expressed, whereas others require activation by cytokines, including interferon (IFN)-γ. Cell activation with IFN-γ increases the number, mobility and affinity of these receptors; with opsonisation of oligodendrocyte targets by appropriate ligands, adherence occurs between the cell types which culminates in phagocytosis. The lethal cytotoxic signal is delivered by local release of TNF-α and depends upon the high concentration of this factor available at the cell surface [79].

Although this combination of receptor and ligand has proved informative with respect to microglial-oligodendrocyte interaction *in vitro* using neonatal rat oligodendrocytes, preliminary evidence (unpublished observations) suggests that complement activation is not a property of human oligodendrocytes. Oligodendrocytes cultured from samples obtained at craniotomy are not lysed by contact with normal human serum and they possess CD59, which is missing from the surface of neonatal rat cells. Other receptor-ligand interactions may therefore be more important in mediating damage to human oligodendrocytes by microglia. Of particular relevance to multiple sclerosis is the demonstration that antibody in low concentration, coating the surface of the oligodendrocyte or its myelin sheath, opsonises the target cell for lytic damage by microglia using their Fc receptors [80].

Cell implantation

It may be unrealistic to hope that limiting the damage and stimulating endogenous repair will together be sufficient to restore structure and function in disorders in which large numbers of nerve and glial cells have been lost through the disease process. Cell implantation may be required in these situations.

Implantation of neurons and glia works experimentally and can be used as a source of neurotransmitter release. In this situation, drugs could be delivered using a surrogate brain cell such as an autologous fibroblast. However, to restore sophisticated functions, grafted cells have to interact with their environment, and more specialised material will need to be transplanted for this purpose. Once connectivity has been achieved, afferent stimulation may be necessary to drive the grafted material [81]. Experimentally, cell implantation has been used to compensate for endocrine deficits, restore fronto-striatal function in dopaminergic and cholinergic pathways, replace retinal and retino-tectal circuitry, reconstruct cerebellar circuits, replace spinal motor neurons or support endogenous axonal regeneration and restore glial-neuronal arrangements [82]. Dopaminergic neurons have been implanted successfully in patients with Parkinson's disease [83,84]. In many situations extensive connectivity needs to be established in the appropriate receptor zone between grafted neurons and their targets. Grafts should therefore ideally be placed at the site of origin of the degenerate neurons they are intended to replace. These grafted cells must then extend their axons and grow long distances through an inhospitable environment for appropriate connectivity to be restored. Ectopic placement of grafts overcomes this requirement for growth, but limits the extent to which implanted cells can explore their target area and connect appropriately.

The ability of Schwann cells to enhance regeneration of central axons has been exploited by introducing peripheral nerve bridges to connect sites of implantation of specific neurons to their targets in the septo-hippocampal projection, spinal cord [85] and retina [86]. As expected, the viability of transplanted neurons is enhanced by local infusion of growth factors which promote growth and survival of these neurons *in vitro*, especially bFGF.

More recent developments include the generation of cell lines for transplantation engineered to tailor-make growth factors. The assessment of grafts has required the development of ingenious behavioural tasks that can reliably be reproduced in experimental animals and mimic, with reasonable accuracy, the functional defects of neurodegenerative disease. Grafting has been shown to restore akinesia, sensory neglect and memory, using learned tasks of motor and cognitive performance in rodents and primates.

These principles of transplantation neurobiology are also important when considering the possibility of replacing oligodendrocytes as a means of restoring myelin sheaths around axons [87,88]. Remyelination is achieved experimentally by transplanting oligodendrocyte precursors into gliopaenic regions [38,89]. Growth factors can first be used to stimulate and expand the numbers of grafted cells. This raises the possibility that cells could be maintained in the laboratory for repeated use, and overcomes some of the practical and ethical issues that arise with respect to transplantation in neurological and other branches of medicine. Aside from considerations of transplantation immunology, it is probable that three conditions have to be met for successful grafting:

1. Astrocytes are needed to create the micro-architecture of the damaged region. They provide the scaffold for cell migration and, more importantly, prevent inward migration of Schwann cells which compete successfully for naked axons in the spinal cord but fail to achieve widespread remyelination or restoration of function.
2. The grafts need to contain enough O-2A lineage cells which can differentiate into oligodendrocytes and remyelinate naked axons.
3. It seems likely that grafts also act as a local source of growth factors which ensure the potential of the implanted cellular elements to differentiate and perform their specialist roles.

Is it too much to hope that as knowledge about the nervous system increases, it will prove possible to exploit the potential for endogenous repair to eliminate completely the need for cell implantation? Even now it is not unreasonable to speculate on the possibility of engineering the expression of cell surface receptors and the proliferative potential of grafted cells, to maximise their capacity for accomplishing the biologically and metabolically complex tasks of restoring glial-neuronal arrangements in the adult CNS.

Specific applications

Against this background of knowledge on development, regeneration, degeneration and cell implantation, applications of the brain repair strategy can be contemplated in several diseases affecting the nervous system with varying degrees of confidence.

Spinal cord injury

The simplest situation is accidental injury in which the brain or spinal cord has suffered an insult that is not likely to recur and in which the main cause of disability is interruption of axons without significant cell loss. Some of the inhibitory molecules expressed on the surface of mature oligodendrocytes and other cells which stop axon growth have been characterised, so axonal regeneration can be enhanced using antibodies to block these molecules.

Experimentally, axonal regeneration through an area of spinal cord damage is greatly enhanced by infiltrating a spinal lesion with these reagents; nerve fibres then advance a substantial distance beyond the site of injury [90]. The increase in axonal regeneration which follows inhibition of repulsion molecules can be further influenced by promoting axonal growth using NT-3—but only if both strategies are adopted simultaneously and where the cord lesion is incomplete [52]. The inhibitory molecules are not found on Schwann cells which myelinate peripheral nerve. They provide a permissive environment for axon regeneration, whether in peripheral nerve or on relocation to parts of the CNS.

For some time efforts to repair the transected spinal cord have concentrated on the use of peripheral nerve or Schwann cell-infiltrated guidance channels through which axons can grow and re-establish their connectivity: this has improved central regeneration [91,92]. Implanted channels have the advantage of being designed to maximise conditions, including the availability of growth factors which will promote repair and minimise inhibitory influences on axon regeneration. More recently, the enhanced ability of embryonic neurons to grow and connect has been used to show that both ends of a fetal spinal cord graft will connect appropriately to descending (motor) and ascending (sensory) fibres, respectively, across a total spinal lesion, and restore function [93].

Motor neuron disease

The trophic and survival effects on motor neurons have prompted therapeutic studies of CNTF in animal models of motor neuron disease [13,14,62]. CNTF has a local effect in rescuing neonatal facial motor neurons following axonal section and influences genetically determined neuromuscular abnormalities in mice. Although NGF has no effect, other members of the neurotrophin family act as motor neuron protective factors: BDNF, NT-3 and NT-4 accumulate in cultured embryonic rat motor neurons and increase choline acetyl transferase activity. *In vitro*, on exposure of motor neuron cultures to CNTF and BDNF there is a synergistic induction of marker enzymes, indicating that these neurotrophic factors have a synergistic effect on the structure and function of motor neurons.

Experimental studies show that treatment with either CNTF or BDNF alone reduces the progression of genetically determined neuronal degeneration, and that these growth factors have complementary effects. They will both soon be evaluated in motor neuron disease alone or in combination, depending on the further results of experimental studies.

Neurodegenerative disease

Disability in many neurodegenerative diseases is triggered by environmental factors and results from genetic predisposition to cell injury. In Parkinson's disease, one hypothesis is that (as yet unidentified) environmental toxins selectively damage dopaminergic nerve fibres projecting from the substantia nigra to the putamen. Drugs which interfere with detoxification pathways in the brain limit the rate of degeneration and slow the progress of neurological disability. This has led to the therapeutic use of the free radical scavenger alpha-tocopherol (vitamin E) and the selective monoamine oxidase-B antagonist deprenyl in preliminary clinical trials [94]. These and other treatments which increase the availability of dopamine in the striatum have improved the quality of life for a generation of patients with Parkinson's disease but they neither cure the disease nor replace lost nerve cells.

More than 140 patients have received brain cell implants as treatment for Parkinson's disease [95–97]. Detailed analysis of six patients studied in Sweden and London, including two with MPTP-induced parkinsonism, have shown the benefits of improved techniques which have resulted from the application of principles established in animal work. Graft survival in the more recently treated patients has improved, and is matched by evidence of improved graft function using PET with fluorodopa as the ligand [83,84]. The images show that grafted neurons will survive, grow and improve disability for up to three years after transplantation.

The clinical effects can be attributed to increased dopamine activity in the treated putamen but not on the contralateral side. Even then, only a minority of the implanted neurons survive, and present estimates are that tissue from 3–4 fetuses would be needed to repair each side of the affected putamen.

The ethical and practical difficulties of using a human tissue source for each procedure have stimulated work on the development of growth factor expanded and engineered cell lines, including transfected fibroblasts. Attempts to improve graft survival and function through growth factor infusion may enhance survival of implanted tissue. It remains to be determined which cocktail of growth factors will optimise graft survival, but BDNF, bFGF, PDGF, IGF-1 and GDNF are all being evaluated.

Although much still needs to be learnt before cell implantation can be regarded as a routine treatment for neurodegenerative disease, the principle is now established and in a number of diseases, tragic in their natural state, a temporary reprieve might be achieved by replacing cells lost through the disease process. Some, such as Huntington's disease, are poised to benefit from advances in molecular genetics but, even with identification of the gene which determines disease susceptibility, prevention by screening or treatment by gene therapy will remain a distant goal and will not displace the need for brain repair. Animal models that mimic the chemically defined lesions of Huntington's and Alzheimer's diseases have demonstrated that the ability to perform learned cognitive and motor skills can be restored by neural grafting [98].

Multiple sclerosis

Superficially, the most difficult type of disease to tackle by the brain repair strategy is multiple sclerosis, in which inflammation occurs randomly, in patches, unpredictably and erratically. Disability results both from the inflammatory process and its failure to repair. Limiting the inflammatory process without attempts at repair is a poor ambition, and repair without damage limitation makes no sense. Stabilisation of the disease process should soon be possible by exploiting the opportunities of modern therapeutic immunology which avoid punishing the entire immune system for the misdemeanours of a few constituent cells.

Pulsed immune therapy can now be delivered using reagents that reduce or inhibit responding and inducer T cells without influencing other lymphocyte subpopulations (some of which may contribute to immune suppression), using monoclonal antibodies or peptides which mimic specific antigens and impair T cell responses even when the target antigen or epitope involved in the disease process is not known. Restricting the entry of immune cells into the CNS has been achieved experimentally by inhibiting the expression of adhesion molecules that underpin interactions between endothelial and inflammatory cells [99]. The effect of systemic lymphocyte depletion on disease activity has recently been assessed in a small number of patients with multiple sclerosis, using humanised antibodies in which anti-human antigen binding sites are engineered onto a xenogeneic (and hence non-immunogenic) molecule [100]. Preliminary results are encouraging.

CAMPATH-1H, which targets the CDw52 antigen present on lymphocytes and some monocytes, induces profound and sustained lymphopaenia which persists for several months. Within hours of the first infusion, patients with multiple sclerosis develop transient worsening of existing or previously experienced neurological symptoms. The most likely candidate for mediating these short term adverse effects of monoclonal antibody therapy is TNF-α, which is released in large quantities when lymphocytes are lysed (unpublished obser-

vations). These observations may prove relevant to questions concerning both the mechanism of symptom production in inflammatory brain disease and the basis for interactions between phagocytes, macrophages and microglia, which culminate in digestion of the oligodendrocyte and its myelin sheath.

CAMPATH-1H treatment significantly reduced disease activity in seven treated patients. The first patient had active disease on a single pretreatment scan and showed almost no new lesion formation on serial MRI carried out over the following 28 months. In the next six cases, gadolinium-DPTA-enhanced cerebral magnetic resonance images were obtained monthly for 3–4 months before and at least six months after treatment: new lesions were identified before, and for a short period after treatment, but beyond three months there was a substantial reduction in disease activity [101].

This was not the first study to use magnetic resonance as a surrogate for screening possible new therapies in multiple sclerosis, and an effect on new lesion formation has previously been demonstrated. The recent study of IFN-β showed a one-third reduction in new clinical episodes after three years in patients with relapsing disease, but disability was not assessed. A 60–70% reduction in the rate of accumulation of new lesions was also detected [102,103].

Apart from preventing the entry of T lymphocytes into the CNS, intervention could be targeted at:

- release of IFN-γ, which activates microglial receptors;
- synthesis of antibodies directed against any one of several antigens present on the oligodendrocyte cell surface or its myelin sheath;
- release of TNF-α in the vicinity of opsonised oligodendrocyte targets [104].

Several pharmacological and biosynthetic agents are available that inhibit macrophage function, and these could be used to limit oligodendrocyte and myelin injury occurring in areas of perivascular infiltration. Methylprednisolone, given intravenously and in high dose, inhibits the release of eicosanoids and other mediators of macrophage activity, reduces tissue oedema in the white matter of patients with multiple sclerosis, and is associated with rapid reduction in the enhanced magnetic resonance appearances of individual T2-weighted lesions.

Success in the next part of the repair strategy in multiple sclerosis depends on whether complex glial-neuronal interactions can also be restored. Preliminary studies of glial lineages in the human nervous system suggest that oligodendrocyte precursors may be present in the adult brain [105].

The failure of glial precursor cells to repopulate and usefully repair damaged nerve fibres may result from lack of appropriate growth factor signals, difficulty in penetrating the astrocytic scars that form around areas of demyelination, or inappropriate differentiation if the remyelinating cell is genuinely bipotential. *In vitro* studies show that a bias in differentiation favouring the development of astrocytes and reducing the number of oligodendrocytes, occurs as part of the complex interaction between axons and O-2A progenitors *in vitro* [35]. This raises the possibility that cells penetrating gliopaenic areas *in vivo* might encounter naked axons and, in the context of inappropriate growth factor signals, differentiate into astrocytes and not into the oligodendrocytes required for remyelination. Should lack of precursors or physical impediments to migration prove an insuperable set of obstacles limiting endogenous reconstitution of the post-inflammatory lesion, glial repair will require cellular implantation. Stem cells might be harvested from the nervous system of individuals who are themselves to benefit from transplantation, expanded with growth factors in the laboratory, and restored in increased numbers to strategically placed lesions which have been prepared so as to maximise the potential for survival and repair.

Conclusions

The strategy for restoring structure and function in the adult nervous system is already being illuminated by knowledge on normal development, and the application of techniques for limiting nerve and glial cell injury is set to reduce the size of the task. Substantial improvements may be possible through release of endogenous repair processes and the recruitment of stem cells into areas of damage. Replacing complex glial-neuronal arrangements and circuitry is likely to involve implantation of human cells, engineered to maximise their potential for integration with host tissues. Assessment will be needed at each stage and progress will probably be frustratingly slow, but in time laboratory work will translate into clinical practice and pay dividends for the many individuals with neurological disease.

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