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Mechanisms of Process Outgrowth by Oligodendrocytes: *Astrocytes, Protein Kinase C* and *Matrix Metalloproteinase-9*

by

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Abstract

In the central nervous system, oligodendrocytes (OLs) form myelin that facilitates conduction of impulses along axons. An individual OL has the unique capability to myelinate up to 50 axons and to produce dramatic quantities of myelin proteins and lipids. The importance of myelin is well reflected in a number of neurological pathologies such as multiple sclerosis, a human demyelinating disease, where severe damage/loss of myelin and OLs are apparent. Although numerous studies have provided evidence of the possibility of remyelination in experimental models, successful remyelination in the demyelinated lesions has not been achieved.

One approach to enhance the remyelinating capability of OLs maybe to stimulate OL process outgrowth, a prerequisite event for myelin formation. In this thesis, I have tested three hypotheses to elucidate the mechanisms of process outgrowth by OLs. The first hypothesis is that other glial cell types provide stimulatory factors to promote process outgrowth by OLs. In this regard, I have found that astrocytes promote OL process outgrowth through the interaction between bFGF and astrocyte extracellular matrix (ECM). The second hypothesis that I tested is that protein kinase C activation in OLs is involved in mediating the promoting effects of astrocytes. My results describe that the inhibition of PKC activity attenuates the promoting action of bFGF and astrocyte ECM on process outgrowth by OLs. The final hypothesis that I tested is that matrix metalloproteinases (MMPs) are utilized in OL process outgrowth, since the advancement of OL processes would require proteases to remodel the pericellular

microenvironment. My results suggest that the production of MMP-9 by OLs, downstream of PKC activation, allows process outgrowth by OLs.

Overall this thesis has addressed the mechanism of process outgrowth by OLs. I propose that astrocytes in close contact to OLs in vivo produce the proper stimuli (i.e. bFGF and ECM molecules) to OLs for PKC activation and that this leads to up-regulation of MMPs, particularly MMP-9. This latter effector molecule is then utilized by OLs to advance their processes across a matrix to reach target axons. Although many questions still remain to be resolved to achieve satisfactory remyelination, the current portrait of the mechanism of OL process outgrowth should lead to a better appreciation of OL biology and myelin formation.

Resumé

Dans le système nerveux central, les oligodendrocytes produisent la gaine de myéline qui facilite la conduction de l'impulsion éléctrique le long des axones. Un seul oligodendrocyte peut produire la gaine de myéline d'environ 50 axones, ceci représente une production énorme des protéines et lipides qui composent la myéline. L'importance de la myéline est illustrée dans un certain nombre de maladies neurologiques caractérisées par une destruction de la gaine de myéline, comme c'est le cas dans la sclérose en plaques où de sévères dommages au niveau de la myéline et des oligodendrocytes sont apparents. Bien que de nombreuses études aient fourni des preuves de l'existence d'un processus de re-myélination dans des modèles expérimentaux, la re-myélination de lésions démyélinées n'a jamais été observée.

Une approche possible pour augmenter la capacité des oligodendrocytes a remyéliner des lésions serait de stimuler la croissance des pseudopodes des oligodendrocytes, l'extension de pseudopodes est un pré-requit pour permettre la re-myélination. Au cours de ce projet de thèses, j'ai testé trois hypothèses pour élucider le mecanisme de croissance des pseudopodes par les oligodendrocytes. Premièrement, la possibilité que d'autres cellules d'origine gliales produisent des facteurs de croissance qui stimulent l'extension de pseudopodes par les oligodendrocytes. J'ai pu demontrer que les astrocytes stimulent l'extension de pseudopodes par les oligodendrocytes grace a la production concertée du facteur de croissance bFGF et de matrice extracellulaire (MEC). La seconde hypothèse testée etait que l'activation de PKC dans les oligodendrocytes etait impliquée dans la mediation des effets promoteurs de croissance

fourni par les astrocytes. Mes resultats indiquent que l'inhibition de l'activité de PKC atténue l'effet promoteur de bFGF et de la MEC des astrocytes sur la croissance des pseudopodes des oligodendrocytes. L'hypothèse finale que j'ai testé etait que les métalloproteinases de la matrice (MMPs) jouent un role dans la croissance des pseudopodes des oligodendrocytes; en effet, l'extension des pseudopodes par les oligodendrocytes nécessiterait la production de protéases pour remodeler le micro-environnement extracellulaire. Mes résultats indiquent que la production de MMP-9 par les oligodendrocytes, en conséquence de l'activation de PKC, permet la croissance des pseudopodes des oligodendrocytes.

Globalement, cette thèse fourni le mécanisme d'extension des pseudopodes des oligodendrocytes. Je propose que les astrocytes à proximité des oligodendrocytes, fournissent les stimulis requis (bFGF et MEC) par les oligodendrocytes pour l'activation de PKC, qui, a son tour mène a l'augmentation de la production de MMPs, en particulier MMP-9. Ce dernier effecteur est utilisé par les oligodendrocytes pour étendre leurs pseudopodes à travers la matrice extracellulaire jusqu'à atteindre les neurones qui sont leurs cibles. Bien qu'il reste de nombreuses questions à resoudre pour permettre une complète re-myélination des lesions, les informations presentées ici sur les mecanismes d'extension des pseudopodes par les oligodendrocytes devrait mener a une meilleure comprehension de la biologie des oligodendrocytes et du processus de formation de la myéline.

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Acknowledgements

In life, there are important turning points coming across to every individual. I was told that later in life when I look back on the way I have come along, I shall see these turning points more clearly. I believe one of my turning points was when I entered Dr. Yong's laboratory, which has led me to become a neuroscientist.

Among many things that I appreciate in life, one thing I like to mention is that I have been fortunate to meet great teachers during my education. Dr. Yong was not an exception but the one of most influential teachers I have ever met. He has motivated, guided, and cheered me throughout my Ph.D study. In addition, members in Dr. Yong's laboratory have been helpful, stimulating, and importantly good friends. I deeply thank Dr. Yong and all these great friends.

I believe family is an important source to form good foundations for each individual. To be whom I am now there have been my parents' endless love and support. It is hard to describe how much I appreciate them. I also like to give thanks to my only sister.

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Contributions by authors

Throughout my time as a doctoral candidate, many great individuals have provided valuable supports. Within the active and dynamic environment of Dr. Yong's laboratory, I have pursued the projects to elucidate mechanisms of process outgrowth by oligodendrocytes. I have learned, designed, and participated in a number of in vitro and in vivo experiments with neural cells from different species. In this thesis, I have included three principal publications that directly address mechanisms of OL process formation.

In the first publication {Oh and Yong (1996) Astrocyte promote process outgrowth by adult human oligodendrocytes in vitro through interaction between bFGF and astrocyte extracellular matrix. Glia 17:237-253}, Dr. Yong provided the initial hypothesis. With the guidance of Dr. Yong, I conducted all the experiments and identified physiological stimuli to enhance process forming ability of OLs. In the second publication {Oh et al. (1997) The promoting effects of bFGF and astrocyte extracellular matrix on process outgrowth by adult human oligodendrocytes is mediated by protein kinase C. Brain Res. 757:236-244}, I determined the central role of PKC in the signal pathway of astrocytic factors (i.e. bFGF and extracellular matrix). Dr. Goodyer and Dr. Olivier provided important comments and materials such as human fetal and adult brain tissues, respectively. In the third publication {Oh et al. (1999) Matrix metalloproteinase-9/Gelatinase B is required for process outgrowth by oligodendrocytes, submitted}, I designed and performed all of the experiments with the guidance of Dr. Yong. Dr. Krekoski and I developed a novel in situ zymography method to localize the activity of matrix metalloproteinases. Dr. Donovan generously

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Besides the names that are mentioned in the publications, numerous individuals in Dr. Yong's laboratory and research unit have provided important tips, critiques, and advice. Within such an interactive and motivated environment, it has been a great opportunity and privilege for me to be part of a research team.

List of abbreviations

BBB	Blood brain barrier
CNP	2',3'-cyclic nucleotide 3'-phosphodiesterase
CNS	Central nervous system
CP	Calphostin C
CSPG	Chondroitin sulphate proteoglycan
DAG	Diacylglycerol
EAE	Experimental allegic encenphalomylitits
ECM	Extracellular matrix
FAK	Focal adhesion kinase
FGF	Fibroblast growth factor
GC	Galactocerebroside
GFAP	Glial fibrillary acidic protein
HSPG	Heparin sulphate proteoglycan
IP3	Inositol 3,4,5-trisphosphate
LDH	Lactate dehydrogenase
MAG	Myelin associated protein
MBP	Myelin basic protein
MMP	Matrix metalloproteinase
MS	Multiple sclerosis
MT-MMP	Membrane type-matrix metalloproteinase
OL	Oligodendrocyte

PDB	4β-phorbol-12,13-dibutyrate
PDGF	Platelet derived growth factor
PKC	Protein kinase C
PL	Poly-l-lysine
PLP	Proteolipid protein
RGD	Arginine-glycine-aspatic acid
TDT	Terminal deoxynucleotidyl transferase
TIMP	Tissue inhibitor of matrix metalloproteinase

CHAPTER I

General Introduction to Thesis

GENERAL INTRODUCTION

The central nervous system (CNS) is the most complex and least understood organ of the human body. The CNS, consisting of the brain and spinal cord, processes sophisticated psychological and physiological functions including consciousness, emotion, sensory and motor functions. Although the human brain constitutes only 2 % of the total weight of the body, it receives about 15% of the cardiac output and its oxygen consumption is approximately 20% of that for the total body; these statistics show that the brain is a highly active and dynamic organ. In the CNS, neurons and glial cells form intensely interconnected networks in a complicated but organized manner implicating their intimate relations. Failure or defect of any cell type can affect other cells critically, and possibly lead to severe damage of neurological functions with little chance of recovery or regeneration.

Electrical impulses are transmitted along axons to allow signaling to occur from one neuron to another. Transmission of impulses along axons is facilitated by a structure called myelin, which is a multi-lamellar membrane that surrounds axons. The presence of myelin allows rapid propagation of an action potential along the axon by increasing its diameter. Although myelin decreases the capacitance of the axon, the action potential gradually diminishes as it spreads passively down the axon. To prevent such decrement of action potentials, the myelin sheath is interrupted every 1-2 mm by bare, non-myelinated segments (about ~2 μm) of axon, called the node of Ranvier (Fig. I-1). The node of Ranvier contains high density of voltage-gated Na^+ channels and thus can generate an intense depolarizing Na^+ influx in response to the passive spread of

action potential from the axon upstream. These regularly distributed nodes boost the amplitude of the action potential periodically, preventing it from dying out. The action potential, which spreads quite rapidly along the internode (i.e. myelinated region of axon), slows down as it passes the high capacitance region of the node of Ranvier. Consequently, as the action potential moves down the axon, it appears to jump from node to node. This is called saltatory conduction (from the Latin "saltare", which means "to leap"). Thus, the myelin sheath is critical in facilitating saltatory conduction of an action potential along the axon.

Oligodendrocytes (OLs) are the cells that enwrap and myelinate the axons to facilitate the conductivity of nerve impulses. Successful myelin formation by OLs is a very critical event during development. Although the overall steps are complex, myelin formation can be reduced to the following steps: process extension by OLs to contact axons, the appropriate OL process-axon recognition and interaction, the ensheathment of the axon by OL process, the extrusion of cytoplasm (i.e. compaction), and completion of myelin sheath (reviewed in McLaurin and Yong, 1995). In so doing, specific myelin proteins are localized to particular areas of the myelin lamella. They include proteolipid protein (PLP) as a major intrinsic protein, myelin basic protein (MBP) in the major dense line, and myelin-oligodendrocyte glycoprotein in the outer lamella (Fig. I-1). Defective developmental myelination or demyelination can render severe phenotypes in animals (e.g. those occurring in shiverer and jimpy mice), and blindness and paralysis in patients with multiple sclerosis (MS), which is the prototype of human demyelinating diseases.

Recent years of research in OL biology and myelination have uncovered many aspects of OLs. Particularly, observations of limited remyelination in demyelinated lesions in MS brain (see section on Myelination and Remyelination) have suggested the potential for greater recovery from demyelinating disease. Since OL process outgrowth is an early critical step to complete myelin formation/remyelination, a potential approach to enhance remyelination is to understand the mechanism of process outgrowth by OLs. Such studies could uncover new strategies or drugs aimed at promoting process extension to allow regeneration. At the very least, these studies are relevant to understanding an early step in developmental myelin formation, that of process extension.

The main aim of my thesis is to understand the mechanisms of process outgrowth by OLs. Given that glial cells in the brain are intimately related to each other, I have tested the hypothesis that astrocytes promote OL process outgrowth through the elaboration of growth factors and that these promoting effects are mediated through the protein kinase C (PKC) signal transduction pathway in OL. As downstream effectors of PKC activation, I have tested the hypothesis that matrix metalloproteinases (MMPs) are involved since these proteinases are involved in the physiological remodeling of the extracellular matrix (ECM), which may be necessary for the OL processes to advance. The main aims of my thesis, which will be clarified further on page I-32, can be summarized in Figure I-2.

In order to provide the reader with sufficient background to the aims of my thesis, I will review these topics in the following sections: 1. Development of OLs, 2.

Myelination and remyelination, 3. The interaction of OL with other cell types, 4. Growth factors and OL biology, 5. Protein kinase C, and 6. Matrix metalloproteinases.

DEVELOPMENT OF OLIGODENDROCYTES

In the CNS, cells that resemble OLs were first described in the late 19th century (Robertson, 1899; Ramon y Cajal, 1913). Later, using metal impregnation based on silver carbonate or silver nitrate modified from Golgi's technique, Del Rio Hortega characterized and named oligodendroglia (from Greek oligo: few, dendro: tree, and glia: glue) (Del Rio Hortega, 1921; 1928). Several decades after, using electron microscopy, the ultrastructure of OL was described. The mature OL was shown to have multiple processes extending from a cell soma (10~20 μm in diameter) (Wood and Bunge, 1984). Within the round or oval nucleus, the substantial amounts of heterochromatin occupying the nuclear periphery contribute to its dense appearance. No unique organelles are found, although the rough endoplasmic reticulum is well developed, and the cisternae appear to be empty, flattened, and arranged in stacks. Elaborate studies based on morphology revealed OLs to be the myelinating cells that generate myelin sheets around axons (Bunge et al., 1960;1961;1962; Wood and Bunge, 1984)

OLs originate mainly from the subventricular zone where immature neuroectodermal cells expand substantially in late gestation and early postnatal CNS development (Goldman, 1992). Cells similar to OL progenitor cells have been isolated from other regions of the neonatal rodent brain including the cerebral cortex and cerebellum (Levi et al., 1987; Levine and Stallcup, 1987; Grinspan et al., 1990;

Grinspan et al., 1993). Others described a pluripotent stem cell isolated from subventricular zone or adult mouse striatum, which can produce neurons, astrocytes or OLs in culture (Halliday and Cepko, 1992; Reynolds and Weiss, 1992). Recently, using chick-quail chimeras, it was demonstrated that OLs in the spinal cord are specifically derived from ventral neuroepithelium while astrocytes are from both ventral and dorsal regions (Pringle et al., 1998). While they proliferate and differentiate, OL progenitor cells migrate from their original locations into the designated regions of grey and white matters. Mature OLs extend their processes to the target axons and myelin formation takes place.

The development of techniques to isolate and maintain OLs in culture for prolonged periods has contributed significantly to the understanding of the OL biology (McCarthy and de Vellis, 1980; Suzchet et al., 1980; Lisak et al., 1981) since OLs in culture extend multiple processes and express all the major myelin proteins and lipids (Szuchet, 1987; Vartanian et al., 1992). Moreover, in the absence of neurons and astrocytes, the appearance of myelin proteins and lipids on isolated OLs follows a time course corresponding to that in vivo: CNP and galactocerebroside (GC), then MAG, MBP and PLP (Zeller et al., 1985; Dubois-Dalcq et al., 1986; Zalc et al., 1987; Amur-Umarjee et al., 1990).

Extensive studies have generated a number of antibodies to classify OL development in a stage-specific manner; these antibodies recognize cell surface lipids and myelin proteins. An OL progenitor cell, also called O-2A cell, was first found in cultures of the developing rat optic nerve (Raff et al., 1983); the O-2A cells is recognized by the A2B5 antibody directed against gangliosides. Antibodies (e.g. NSP-

4) that bind to specific glycoproteins (e.g. NG-2) or intermediate filaments (i.e. vimentin) also recognize O-2A cells. O-2A cells can develop into either OL or type 2 astrocytes depending on culture conditions (Raff et al., 1983). In the presence of serum, O-2A cells develop into type 2 astrocytes, while they become OLs in the absence of serum. OL progenitors express PDGF-α receptor during development, and an antibody targeting PDGF-α receptor is used to characterize OL progenitors in vivo (Hart et al., 1989). O-2A cells have been found not only in neonatal rodent cell cultures but also in the cultures of adult rodent brain (ffrench-Constant and Raff, 1986; Dubois-Dalcq, 1987; Wolswijk and Noble, 1989). Analyses of semi-thin frozen sections and associated cell suspensions indicate that O-2A progenitors comprise less than two percent of the total cell population in adult rodent CNS (Miller et al., 1985; Wood and Bunge, 1991). These adult brain progenitor cells have lower capability to migrate and proliferate compared to their neonatal couterparts (Wolswijk and Noble, 1989; Wren et al., 1992; Engel and Wolswijk, 1996). Administration of growth factors such as PDGF and FGF can convert adult progenitor cells into cells reminiscent of neonatal O-2A cells: high proliferative rate, bipolar morphology, and increased motility (Wolswijk and Noble, 1992; Engel and Wolswijk, 1996).

Whether the O-2A cell exists in vivo has been controversial, particularly with the difficulty of using the A2B5 antibody to reliably recognize antigens in vivo (William and Price, 1992; Ludwin, 1997). Furthermore, the transplantation of O-2A cells into the normal neonatal rat brain yielded only OLs (Espinosa de los Monteros et al., 1993). In addition, it was demonstrated that astrocytes and OLs were generated in distinct and different phases in vivo (Skoff et al., 1991). To further complicate cell

lineage analysis, markers such as those directed against the GD3 ganglioside, that was thought to be expressed by OL precursors in vivo (Curtis et al., 1988; Reynold and Wilkin, 1988), have been shown in more recent studies to label also ramified and amoeboid microglia. Indeed, a significant portion of GD3+ cells in developing cerebellum appears to belong to the microglia lineage (Wolswijk, 1995; Ludwin, 1997). In parallel, microglia can produce carbonic anhydrase II (CAII) suggesting that antibodies targeting CAII are not appropriate to identify OL lineage in vivo (Nogardi, 1993).

A pro-oligodendrocyte, which is thought to be an intermediate stage between O-2A progenitor and mature oligodendrocyte (Fig. I-3), is characterized by the presence of multiple processes from the cell soma, capability to migrate and proliferate (Warrington and Pfeiffer, 1992), and by the expression of sulfated glycolipids, recognized by the monoclonal antibody O4 (Sommer and Schachner, 1981; Gard and Pfeiffer, 1990). GC is not expressed and vimentin immunoreactivity is absent in these cells. The pro-OL and O-2A cell have collectively been referred to as an oligodendrocyte precursor cell (Wang et al., 1998).

Mature OLs express myelin proteins and GC, which is recognized by the monoclonal antibody O1. Although there are other antibodies (e.g. R-mAb) that bind to GC, they tend to bind other surface molecules (i.e. seminolipid and sulfatide) that are expressed by pro-OLs (Bansal et al., 1989; Bansal and Pfeiffer, 1992). Mature O1 positive OLs are considered as post-mitotic cells while O-2A progenitors and pro-oligodendrocytes are proliferative (Gard and Pfeiffer, 1990). The monoclonal antibody,

RIP, directed against an unknown antigen on OL, is used to identify a broad range of OL lineage cells in vivo as well as in vitro (Friedman et al., 1989).

MYELINATION

In humans, myelination in the brain occurs within the first year after birth; in the spinal cord, it begins at 11 to 12 week of fetal life (Niebroj-Dobosz et al., 1980; Choi et al., 1984). Light and electron microscopic studies showed that the amount of myelin increases rapidly between 12 and 24 weeks of gestation in all areas of the spinal cord except the lateral cortical spinal tract (Weidenheim et al., 1992). Nonetheless, studies of human fetal tissues of various gestational ages have implicated significant variations in the onset and rate of myelination (Gilles et al., 1983; Poduslo and Jang, 1984).

Developmental myelination in rodent brain generally proceeds from rostral to caudal direction. Within the brain, the brain stem is myelinated first, followed by the cerebellum and then propagates to other area (Monge et al., 1986; Foran and Peterson, 1992; Bjartmar et al., 1994). Onset of myelination is region-specific; in the corpus callosum, it begins at around postnatal day (P) 10 to 12, while in the optic nerve it starts at P5 (Foran and Peterson, 1992). Active myelination in the brain is usually between 14 to 30 days. During this time, the myelinating OL is able to produce three times of its own weight of myelin per day (Norton and Poduslo, 1973). In the rat optic nerve, the estimated surface area of the myelin that an OL sustains is over 500 fold of the surface area of its soma (Raine, 1981). Morphological studies showed that OLs extend multiple processes with secondary, tertiary branches prior to contacting the target axons. Once

processes contact and enwrap the axon, many un-contacted processes retract resulting in the organized structure of myelin (Braun et al., 1988). Recent studies revealed that during myelination, up to 30-50% of the total OLs fail to complete myelin formation and subsequently undergo programmed cell death (Braun et al., 1988; Barres et al., 1992; Trapp et al., 1997).

As alluded to above, a number of events have to occur in a precise sequence for myelin formation. Initially, OLs extend processes from their soma to the target axons. Among OLs with multiple processes, only those that make proper contact with axons allowing the next events to occur survive and proceed with the wrapping of an axon. As an OL process encircles its axon several times forming inner and outer mesaxons, its cytoplasm gets extruded so that the membranes condense into a compact paracrystalline structure in which each unit membrane is closely apposed to the adjacent one (Fig. I-1). The inner or cytoplasmic surfaces of the membrane of the OL processes involved in myelin formation fuse to form a major dense line, while the intraperiod line results from close apposition of the external leaflets of the same unit membrane (reviewed by McLaurin and Yong, 1995). Although the mechanisms by which an OL enwraps an axon is speculative, the most accepted model proposes that the inner leaflet continues to migrate around the axon by making and breaking ionic interactions with molecules on the axonal surface (Peters and Vaughn, 1970; Raine, 1984). In this manner, the outermost layer is the initial wrap and new ones are added at the periaxonal space.

The molecular events that control myelin formation are still largely unknown. Recently, signaling through the notch receptor has been implicated in inhibiting the differentiation of OL precursor cells into OLs (Wang et al., 1998). When this pathway

is quiescent, OL differentiation and maturation promptly occur. The fyn tyrosine kinase was found to regulate the initiation of myelin formation as a signaling molecule downstream of MAG (Umemori et al., 1994) and by stimulating MBP transcription (Umeomori et al., 1999). Essential growth factors that appear to signal the initiation or enhance the rate of myelination include transferrin (Espinosa de los Monteros et al., 1999), glucocorticoids and progestins (Chan et al., 1998).

Myelin proteins and lipids maintain the integrity of myelin structure and help to propagate efficient nerve impulse conduction. Myelin basic protein (MBP), a major constituent of myelin proteins, is a family of alternatively spliced variants ranging in size from 14 to 21.5 kDa. Unlike other myelin proteins, MBP is exclusively intracellular, its expression begins mainly in the myelin sheath, at the major dense line (Morell et al., 1994)(Fig. I-1). The function and importance of MBP is revealed from a mutant that is deficient in MBP. Shiverer mice develop severe seizures early in life and have short life span (Schwartz, 1991). In these mice, myelin compaction is incomplete at the major dense line suggesting a role of MBP in intracellular membrane compaction in myelin.

Proteolipid protein (PLP) is the predominant intrinsic protein in CNS myelin constituting about 50 % of the total myelin protein content (Fig. I-1). There is a smaller (26.5 vs. 30 kDa) variant of PLP, termed DM-20, which is derived by alternative splicing. PLP has been reported to play a role in compaction of the intraperiod line of myelin (Hudson et al., 1989; Morell et al., 1994) whereas DM-20 may function in the onset of myelinogenesis (Ikenaka et al., 1992). Among several PLP mutant mouse models with varying phenotypes, the jimpy mouse suffers from seizures and dies

prematurely (Klugmann et al., 1997). It is of interest that the PLP null mouse lacks any phenotype and abnormal myelination although dysmyelination may be manifest in aged mice (Klugmann et al., 1997). There are related human diseases including Pelizaeus-Merzbacher disease, in which point mutations of the PLP gene result in dysmyelination and mental retardation. Although the defined functions of PLP are still unclear, the lack of intraperiod lines in PLP mutants compared to normals (Duncan et al., 1989) suggests that PLP functions in the interaction and stabilization between opposing sheets of membranes in the myelin sheath.

2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) is an important component in myelinogenesis, which is implicated in roles in morphological differentiation of OLs and in the interaction with axons (Yin et al., 1997). The CNP protein can be detected at the time of the appearance of GC in OLs (Braun et al., 1988; Pfeiffer et al., 1993). CNP is localized in the OL cytoplasm where it is involved in signal transduction and binds to cytoskeletal proteins such as actin (DeAngelis and Braun, 1996). In vitro, the application of CNP antisense oligonucleotides interferes with OL process formation implicating the participation of CNP in the differentiation of OL (Bernier and Braun, 1991). Furthermore, a CNP isoform is phosphorylated by protein kinase C (Vartanian et al., 1986) which is proposed as a critical mediator in OL process extension (see section on Protein kinase C). Although CNP overexpressing mice do not show behavioral abnormalities, myelin proteins are expressed earlier than those in normal control mice (Gravel et al., 1996). Dramatic vacuolation in the brain sections of CNP overexpressing mice (Gravel et al., 1996) and the lack of major dense line in the myelin

sheath caused by inhibiting MBP accumulation (Yin et al., 1997) implicate a role of CNP in myelinogenesis.

Myelin associated protein (MAG) is a transmembrane glycoprotein localized in the periaxonal OL membrane (Trapp et al., 1989) where it is thought to function in OL-axon interaction during myelination (Tropak et al., 1988; Sato et al., 1989; Quarles, 1997). Several studies show that MAG interacts with a number of signal transduction molecules such as fyn tyrosine kinase, FAK, and phopholipase Cγ (Jaramillo et al., 1994; Umemori et al., 1994). Moreover, MAG influences neurite outgrowth (McKerracher et al., 1994), and axonal abnormalities are observed in MAG null mice (Fruttiger et al., 1995; Yin et al., 1997).

There are several other minor myelin proteins which are not fully characterized yet. Myelin/oligodendrocyte glycoprotein (MOG) constitutes a small percentage of total myelin protein but is highly concentrated on the outermost surface of myelin sheath (Brunner et al., 1989). Although little is currently known about its function, MOG may be involved in transmitting signals to the OL soma from extracellular factors. MOG has received attention as an important antigen in autoimmune demyelinating diseases in the CNS (Amor et al., 1994; Quarles et al., 1997). The oligodendrocyte-myelin glycoprotein (OMgp) is a 120 kDa glycoprotein expressed on the surface of OLs in culture. Its function is unknown although OMgp is shown to be anchored to membrane through a phosphatidyl inositol linkage suggesting its involvement in signaling, adhesion, and recognition (Quarles et al., 1997).

Lipids constitute 70 % of the dry weight of total myelin, and consist of three major components: cholesterol (25%), phospholipids (40%) and glycolipids (30%) with

some variations depending on age, species and pathological condition (Wood and Moscarello, 1989; reviewed by McLaurin and Yong, 1995). Cholesterol is thought to regulate the fluidity of fatty acyl chains in the membrane (Rumbsy, 1984). It is of note that the total amount of cerebroside in the brain is directly proportional to the amount of myelin present (Norton and Poduslo, 1973), suggesting a role in myelinogenesis. Although the significance of the cerebroside is not clear yet, it may be involved in the control of fluidity in the membrane. The compositional asymmetry of myelin lipids between the extracellular and intracellular membranes has been demonstrated (Rumbsy, 1984). The asymmetry of the lipid phase in myelin is GC, cholesterol and phosphatidylcholine and sphingomyelin in the outer leaflet, while cerebrosides, ethanolamine, phosphoglycerides and phosphatidylserine are located at the cytoplasmic side (reviewed by McLaurin and Yong, 1995). The presence of certain lipid components including inositol phospholipids and GC suggests a role of myelin lipids in signal transduction during myelin formation (see section on Protein kinase C). GC has been demonstrated to affect calcium influx and cytoskeleton organization in cultured OLs (Dyer and Benjamins, 1990; Dyer et al., 1997).

REMYELINATION

Remyelination by OLs in demyelinated lesions would require a number of interacting factors including the availability of OLs, the proper synthesis and turnover of OL/myelin components, and the interaction between the ensheathing OLs and the axons (Ludwin, 1989; reviewed by McLaurin and Yong, 1995). These factors are

present to some degree in the lesions in human demyelinating diseases such as MS, since the presence of healthy OLs and limited remyelination around the lesion of the MS brain have been observed (Raine et al., 1981; Ghatak et al., 1989; Prineas et al., 1989; Prineas et al., 1993; Raine and Wu, 1993). The occurrence of remyelination in MS lesions was first suggested by ultrastructural studies almost 30 years ago (Suzuki et al., 1969). Later extensive studies appreciated the significant extent of spontaneous myelin repair; approximately 40 % of plaques exhibit remyelination extending over 10 % of the lesion area during ongoing inflammation (Prineas et al., 1993; Raine and Wu, 1993)

The origin of the remyelinating cells in MS has remained obscure and controversial. In animal models of demyelinating insults, several reports demonstrated that OL progenitors have significant roles in remyelination (Ludwin and Bakker, 1988; Godfraind et al., 1989; Rodriguez et al., 1991; Gensert and Goldman, 1997). Gensert and Goldman (1997) showed that OL progenitor cells are capable of significant remyelination; when dividing cells in the subcortical white matter of adult rats were tagged by injection of a replication-deficient lacZ-encoding retrovirus, and the animals then subjected to a focal demyelination induced with lysolecithin, significant numbers of β-galactosidase labeled cells differentiated into myelinating OLs and engaged in repair of the lesion, implicating the contribution of endogenous OL progenitor cells in remyelination. The transplantation of glial cells into the CNS has been studied to understand the mechanisms of remyelination; OL progenitor cells transplanted into the normal CNS did not survive well whereas those introduced into the demyelinated site caused by X-irradiation or injection of ethidium bromide migrated long distances and

remyelinated (Franklin et al., 1995; Franklin and Blakemore, 1997). Transplantation of OLs and OL progenitors into the CNS of myelin deficient canines or into the demyelinated lesions of the spinal cord of adult rat produced extensive remyelination (Groves et al., 1993; Archer et al., 1997). Although functional improvement following remyelination by transplanted OLs is lacking, normal conductivity of nerve impulse is restored (Utzschneider et al., 1994).

While few progenitor cells were found in the CNS of adult normal mice, the numbers increased significantly following demyelinating injuries; moreover, progenitor cells at various stages of remyelination showed a higher potential for proliferation than cells from normal animals (Armstrong et al., 1990; Lucchinetti et al., 1996).

The presence of OL progenitor cells or pro-OLs has been demonstrated in normal adult human CNS and in the lesions of MS brain by using antibodies to PDGF-α receptor or O4 antibody (Armstrong et al., 1992; Gogate et al., 1994; Scolding et al., 1998; Wolswijk, 1998). Pro-OLs in the chronic lesions of MS brain bear notable processes and do not proliferate (Wolswijk, 1998) whereas OL progenitor cells (i.e. PDGF-α receptor+), without notable processes, are found in both acute and chronic lesions in MS brains (Scolding et al., 1998). Although it is not yet known that these cells contribute to remyelination several studies suggest that OL proliferation occurs in MS lesions (Raine et al., 1981; Prineas et al., 1989; Lucchinetti et al., 1996).

While adult human OL progenitor cells have the capacity to divide in vitro, they do not respond to PDGF and bFGF (Armstrong et al., 1992; Scolding et al., 1995), which are potent mitogens for adult rodent OL progenitor cells (Richardson et al., 1988;

Hart et al., 1989; Wolswijk and Noble, 1992), suggesting that the capacity of OL progenitors to regenerate is dependent on the species.

A number of studies revealed that a relatively large number of OLs are preserved in the acute lesion of MS (Rodriguez et al., 1993; Rodriguez and Scheithauer, 1994; Lucchinetti et al., 1997). These morphologically preserved OLs in the lesion may be disrupted in function without being dead and possibly may participate in remyelination. These cells may dedifferentiate into proliferative cells to replenish OLs in the lesion which then effect remyelination (Ludwin and Bakker, 1988; Wood and Bunge, 1991; Duncan et al., 1992; Wu and Raine, 1992; Ludwin and Szuchet, 1993; Raine and Wu, 1993).

Although recovery from demyelination in animal models is generally satisfactory (Ludwin, 1981), it is not the case in human demyelinating diseases such as MS. The unresolved question is why the extent of myelin repair is incomplete in MS patients, and this may be related to the immune abnormalities of the disease. However, as aforementioned, limited remyelination occurs in MS despite the prevailing immune irregularities suggesting that a better knowledge of OL biology can provide novel avenues to treat the disease.

THE INTERACTION OF OLS WITH OTHER CELL TYPES

Astrocytes have been known to be very versatile in function. They can provide a wide range of growth factors and extracellular matrix (ECM) components that are involved in maintenance of CNS homeostasis, regeneration, and formation of blood

brain barrier (BBB) (Muller et al., 1995). Extensive studies provide evidence that astrocytes are good substrates and produce a number of neurotrophic factors that are favorable for neuron survival and neurite outgrowth (Noble et al., 1984; Fallon, 1985; Hatten et al., 1988; Liesi and Silver; 1988; Ang et al., 1992; Ang et al., 1993; Giulian et al., 1993; Muller et al., 1995). The role of astrocytes in OL development and myelination has been studied extensively but substantial controversy still exists. Although OLs in culture survive, differentiate, and even myelinate axons in the absence of astrocytes, a large literature (see below) provides evidence that astrocytes regulate OL biology, and support OL development and myelin formation. Conditioned medium from astrocyte cultures or ECM deposited by astrocytes is beneficial for OL survival and differentiation (Bhat and Pfeiffer, 1986; Gard et al., 1995).

While astrocytes have been known to produce a number of ECM molecules that provide favorable substrates for adhesion, neurite outgrowth, and neuronal migration (Fallon, 1985; Doherty and Walsh, 1989; Matthiessen et al., 1989; Hatten, 1990; Miller and Smith, 1990; Johnson-Green et al., 1991), the role of astrocyte-derived ECM molecules in OL development is poorly understood (Ovadia et al., 1984). Among cell surface adhesion and ECM molecules, astrocytes express N-CAM, L1, laminin, fibronectin, and tenascin as well as various proteoglycans (Liesi et al., 1986; Sanes, 1989; Muller et al., 1995). Given the local and transient nature of the expression of ECM components and growth factors in the CNS, the interaction between growth factors and ECM constituents may be an important issue in OL development as well as regeneration (see also section on Growth factors and OL biology).

Several astrocyte-derived growth factors have been identified to promote OL development and these include platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), ciliary neurotrophic factor (CNTF), transforming growth factor (TGF)- β, insulin-like growth factors (IGFs) (Richardson et al., 1988; Hart et al., 1989; McKinnon et al., 1990; Komoly et al., 1992; Barres et al., 1993; Eckenstein, 1994; Mayer et al., 1994; Gard et al., 1995; Muller et al., 1995). In contrast, other studies showed inhibitory effects of astrocytes on the myelin forming capacity of OLs (Rosen et al., 1989; Amur-Umarjee et al., 1993).

In the normal brain, immunohistochemistry studies show that astrocytic processes are in close interaction with processes of OLs and surround the OL soma (Suzuki and Raisman, 1992; Butt and Ransom, 1993; Butt et al., 1994). In situ, OLs and astrocytes are coupled by gap junctions suggesting the potential intercellular communication between these two cell types (Massa and Mugnaini, 1982). Transplantation of astrocytes into demyelinated lesions enhances remyelination, and where astrocyte density is high, remyelinated sheaths tend to be thicker (Franklin et al., 1993). Furthermore, in demyelinated lesions OL soma are often invested by astrocytic processes (Prineas et al., 1990; Ghatak, 1992; Wu et al., 1992). Whether astrocytes are detrimental or beneficial to OLs in the lesion is still uncertain. Nevertheless, it is evident that astrocytes play important roles in OL biology during development and in response to injuries.

Neurons also regulate myelinogenesis. In vitro, conditioned medium of neuronal cultures is mitogenic to OL progenitors (Wood and Bunge, 1986; Gard and Pfeiffer, 1990; Hardy and Reynolds, 1993). Like astrocytes, neurons are capable of

producing several growth factors which can be facilitatory to OL maturation. Upon the contact between OL and neuron, the expression of myelin proteins such as MBP and PLP is elevated (Ludwin and Suzchet, 1993; Matsuda et al., 1997). In vivo, OL development and gene expression of major myelin protein are modulated by the presence of axons (Kidd et al., 1990; McPhilemy et al., 1990; Barres et al., 1992; Trapp et al., 1997). The electrical activity of neurons appears to affect OL survival and proliferation (Barres and Raff, 1993), and myelin formation (Stevens et al., 1998) which influences the distribution of Na^+ and K^+ channels on the axons (Kaplan et al., 1997). The notch receptor on OL precursor cells and expression of the jagged ligand on retinal ganglion neurons, are thought to regulate the timing of OL differentiation (Blaschuk and ffrench-Constant, 1998; Wang et al., 1998). In demyelinated lesions in the brain, initial damages occur in myelin and OLs, but in chronic lesions, naked axons are also severely impaired. Recently, the phosphoinositide pathway in myelin was found to require an interaction with axon (Chakraborty et al., 1999).

Microglia can produce a variety of inflammatory cytokines and neurotoxic factors such as tumor necrotic factor (TNF)-α, which can be toxic to OLs. In co-culture, microglia are cytotoxic to OLs (Merrill and Zimmerman, 1991; Merrill et al., 1993). Nitric oxide that is produced by rat amoeboid microglia induces necrotic death of OLs, while the promoting effect of microglia on myelin protein synthesis has also been observed (Hamilton and Rome, 1994; Loughlin et al., 1994). The conditions under which microglia become neurotoxic or oligodenrocyte-trophic are not known.

GROWTH FACTORS & OL BIOLOGY

In the last two decades, several studies have identified a number of growth factors that are involved in OL development. PDGF and basic FGF are potent mitogens of OL progenitors (Richardson et al., 1988; Hart et al., 1989). Studies with mice that are genetically manipulated have shown that PDGF influences the number of OL progenitor cells and myelin formation in the brain and spinal cord (Calver et al., 1998; Fruttiger et al., 1999). Basic FGF stimulates proliferation and blocks maturation of rodent OL progenitors in culture. When bFGF and PDGF are given together, OL proliferation is enhanced, and cells eventually differentiate into mature OLs (McKinnon et al., 1991). The presence of astrocytes or astrocyte-conditioned medium can override the inhibitory effect of bFGF on OL differentiation in vitro (Mayer et al., 1993). Interestingly, as described on page I-15, studies with OL progenitors derived from human do not proliferate in response to PDGF and bFGF suggest the presence of species difference (Yong et al., 1988; Armstrong et al., 1992; Gogate et al., 1994; Satoh and Kim, 1994; Scolding et al., 1995).

Other growth factors besides bFGF and PDGF also influence OL development. Insulin and IGFs regulate OL development and myelination (McMorris et al., 1986; Barres et al., 1992; Carson et al., 1993), whereas CNTF and leukemia inhibitory factor (LIF) promote OL survival (Louis et al., 1993; Mayer et al., 1994). Neurotrophin-3 (NT-3) stimulates proliferation of OL progenitors (Barres et al., 1993), while TGF-β inhibits it and allows differentiation (McKinnon et al., 1993). Effects of growth factors are stage-specific in OL development; mature OLs do not proliferate in response to

PDGF or bFGF treatment. Rather, survival and differentiation of mature OL are promoted by PDGF and bFGF (Gogate et al., 1994; Gard et al., 1995; Yasuda et al., 1995).

Not all growth factors are favorable for OL survival and development. Apoptosis of OLs is induced when TNF-α is introduced to the culture (Mayer et al., 1994; D'Souza et al., 1996), and antibodies to TNF-α or soluble TNF receptor, prevented OL death (Wilt et al., 1995). Injection of TNF-α antibodies to mice primed for experimental allergic encephalomylitis (EAE) suppressed EAE development (Selmaj et al., 1991). Several pro-inflammatory cytokines such as interleukin-2 and interferon-γ are also detrimental to OLs, and in vivo, levels of TNF-α and pro-inflammatory cytokines are elevated in active lesions of MS and EAE (Selmaj et al., 1991; Cannella and Raine, 1995).

The ECM is a multifunctional complex of proteins. A large volume of literature provides evidence that ECM can trigger signal transduction by interacting with cell surface receptors such as integrins and eventually influence gene expression (Hynes, 1992; Juliano and Haskill, 1993; Schwartz et al., 1995). Numerous ECM components are assembled in a highly organized manner that contributes to the structural integrity and biological processes including development, tumor metastasis, inflammation and tissue remodeling. The fundamental role of ECM in these processes is to modulate cellular behaviors such as proliferation, adhesion, migration, differentiation and apoptosis (Venstrom and Reichardt, 1993).

Immunohistochemistry studies revealed that in the adult normal brain parenchyma, ECM molecules such as laminin, heparin sulfate proteoglycan (HSPG) and

chondroitin sulfate proteoglycan (CSPG) are distributed in a diffuse and amorphous pattern, while injury such as that induced by a neurotoxin injection induces astrogliosis accompanied by up-regulation of several ECM components (Liesi et al., 1984; Bertolotto et al., 1990). During development, a number of ECM molecules are transiently and locally expressed, and are involved in migration, axon guidance, synapse formation (Liesi and Silver, 1988; McLoon et al., 1988; Liesi and Risteli, 1989; Rogers et al., 1989; Sanes, 1989; Sheppard et al., 1991). For instance, laminin is transiently expressed and colocalized with GFAP immunoreactivity in developing rat optic nerve (McLoon et al., 1988). In vitro, laminin that is expressed by astrocytes stimulates neurite outgrowth and migration of neuronal cells (Liesi, 1985; Calof and Lander, 1991; Lentz et al., 1997). HSPG and laminin deposited by astrocytes stimulate neurite outgrowth (Ard and Bunge, 1988) while an anitbody to an HSPG-laminin complex was inhibitory (Matthew et al., 1985). Certain ECM components exert inhibitory influence on neurite outgrowth (Letourneau et al., 1992); for instance, CSPG and tenascin have shown to limit regeneration of CNS axons after injury (McKeon et al., 1991).

Bartsch and colleagues demonstrated that the expression of janusin, an isoform of tenascin, is coincident with the period of myelination in the mouse optic nerve. With the observation that janusin is localized on the outer aspect of myelin sheet and cell surface of OLs, these authors have proposed a functional role of janusin during myelination (Bartsch et al., 1993; Wintergerst et al., 1993).

In vivo, many ECM molecules are interconnected by building web-like structures with distinct 3D conformation of each constituents (Luckenbill-Edds, 1997). Moreover, a number of growth factors are able to bind ECM complexes rendering the

notion that ECM is a reservoir of growth factors. ECM constituents regulate the stability and diffusion of growth factors such as bFGF, PDGF, and TGF-β (Venstrom and Reichardt, 1993). In addition, adhesion of cells to ECM influences growth factor expression and activity by the cell. Matrix composition and cell surface expression of adhesion receptors are in turn modulated by growth factors.

Accumulating literature indicates that integrin receptors on the cell surface bind to ECM components and mediate signal transduction in the cell. The integrin receptors are a family of heterodimers consisting of various α and β subunits that are non-covalently linked. Different integrin receptors are expressed on diverse cell types, which interact with specific ECM molecules. The intracellular domains of integrin subunits function as docking or binding sites for numerous cytoskeletal proteins and signal transduction molecules. Cell-ECM interaction via integrin receptor thus elicits a number of cellular responses such as platelet aggregation, tumor metastasis, and neurite extension. In addition, cells at different stages (e.g. floating versus adherent) express different profiles of integrins. It has been shown that OL lineage cells express several integrin receptors including αvβ3, αvβ5, αvβ8 and α6β1 (Milner and ffrench-Constant, 1994; Milner et al., 1997), and astrocytes produce ECM molecules that enhance adhesion of OLs (Cardwell and Rorne, 1988). Other studies reported that OL adhesion to substratum and their migration are integrin-dependent mechanisms (Malek-Heyadat and Rome, 1994; Milner et al., 1996). When OLs are cultured on ECM derived from bovine corneal endothelial cells, proliferation and survival of OLs are enhanced (Lubetzki-korn et al., 1983; Ovadia et al., 1984).

Therefore it is likely that growth factors and ECM components via integrin receptors orchestrate to regulate OL biology such as proliferation, migration, differentiation and myelin formation.

PROTEIN KINASE C

Protein kinase C (PKC) is a ubiquitous family of phospholipid-dependent isozymes that are abundant in the CNS. Biochemical and molecular cloning analyses have shown that PKC comprises a large family with at least 12 isozymes that are categorized into three subgroups depending on structural differences and mode of activation. Cellular response upon PKC activation can vary depending on several factors including subcellular localization of the isozyme and the availability of the substrates (Nishizuka, 1992; Newton, 1995).

The PKC isozymes (reviewed in Nishizuka, 1995) can be divided into three groups and these are conventional PKCs (α,βI,βII and γ), novel PKCs (δ, ε, μ, θ and η), and atypical PKCs (ζ and ι; the mouse homolog of ι is called λ). The structure of PKC consists of a single polypeptide chain that contains an amino-terminal regulatory region and a carboxy-terminal kinase region (Fig. I-4). There are distinct domains in the regulatory and catalytic regions. In the regulatory region, the C1 domain has cysteine-rich sequences that coordinate two Zn^{+} atoms. This domain binds to ligands such as phorbol esters and diacylglycerol, and contains a pseudosubstrate motif, which exerts an autoinhibitory activity. In atypical PKC isozymes, modification of the C1 domain leads to these being unresponsive to phorbol esters or diaglycerol. The C2

domain in the regulatory region of the conventional PKC isozymes binds Ca^{2+} for activation. The novel and atypical PKC isozymes lack the Ca^{2+} binding C2 domain and are therefore not regulated by Ca^{2+}. The catalytic domain of PKC isozymes has high homology to that of protein kinase A, and does not differ significantly between the PKC isozymes. The C3 domain of the catalytic region contains an ATP-binding sequence, while the C4 domain binds to pseudosubstrate and protein substrate (Newton, 1995).

Upon stimulation of cell surface receptors, phospholipases hydrolyze phospholipids to diacylglycerol (DAG) and other products such as inositol-3,4,5-triphosphate (IP3) and cis-unsaturated fatty acid. IP3 leads to increases in Ca^{2+} concentration, and DAG binds to PKC to activate it. Although, in general, activated PKC is translocated to the plasma membrane, certain isozymes are translocated to the nuclear envelope. Since DAG disappears quickly, whether sustained PKC activation occurs had been in question. However, it has become evident that depending on the source of the DAG molecule, and regulation of DAG kinase and lipase in the cell, the activity of PKC can be sustained for prolonged periods (Nishizuka, 1995). In fact, unlike neuronal cells, PKC activity in OLs is sustained for up to 12 days after phorbol ester treatment (Yong et al., 1994). Activation of PKC results in a number of cellular responses including gene transcription, proliferation, and differentiation. For instance, in many cell types, PKC activation induces the expression of gene transcription factors such as the Jun/Fos complex, which in turn initiates gene expression by binding to the AP-1 binding sites in the promoter region. Extensive studies in signal transduction have revealed that intense cross talk between signaling cascades occurs (Heidecker et al., 1992; Nishizuka, 1995); for example, PKC activation has been shown to directly

phosphorylate Raf-1 kinase which in turn activates MAP kinase cascade (Kolch et al., 1993). Recently, more evidence has emerged that PKC activation leads to the active complex formation of Raf-1 and Ras (Marais et al., 1998) while the MAP kinase cascade is regulated by multiple PKC isozymes (Schonwasser et al., 1998). A number of growth factors including epidermal growth factor and FGF stimulate receptor tyrosine kinases which eventually activate MAP kinase; it has become evident that receptor tyrosine kinases also lead to activation of the PKC by stimulating phospholipases. For instance, in endothelial cells, PKC is activated in response to bFGF and induces proliferation (Presta et al., 1991; Kent et al., 1995).

PKC in OL biology can be first inferred from studies that demonstrate the corpus callosum, a brain area especially rich in OLs, to have high density of phorbol ester-binding sites (now known to be PKC itself) when compared to other brain areas (Shoyab et al., 1976; Girard et al., 1985). Later in vivo studies revealed that the levels of PKC rise during the period of myelination in rodent brain (Shoyab et al., 1976; Hashimoto et al., 1988). Moreover, activity of PKC was correlated with the deposition of myelin proteins during development (Yoshimura et al., 1992). OLs express a range of PKC isozymes, which affect myelin gene expression during OL maturation in vitro; at the mRNA level, PKC γ is high initially and then decreases to negligible levels by P12, whereas PKC α and β increase progressively in neonatal rat derived OL cultures (Asotra et al., 1993; Asotra and Macklin, 1994). The adhesion of floating OLs to the substratum initiates PKC-mediated signal transduction which leads to myelinogenic metabolism in OLs (Malek-Hedayat and Rome, 1986; Szuchet, 1987; Vartanian et al., 1992). In vitro, several myelin proteins including MBP and MAG have been shown to

be phosphorylated by PKC (Turner et al., 1982; Vartanian et al., 1986; Kirchhoff et al., 1993; Yim et al., 1995). At the level of OL progenitor cells, Bhat and colleagues provided evidence by using PKC inhibitors that the regulation of proliferation of these cells may be through PKC (Bhat et al., 1992). Phorbol ester induces a transient reversion of pro-OLs (O4+, vimentin-, A2B5+) to less mature cells (O4-, vimentin+, A2B5+). The less mature OLs then proliferate and differentiate to mature OLs (Avossa and Pfeiffer, 1993).

Evidence from Dr. Yong's laboratory implicates PKC in regulating OL process extension (Yong et al., 1988; 1991; 1994). Activation of PKC in human, bovine and rodent OLs by an active phorbol ester {4β-phorbol-12,13-dibutyrate (PDB) or phorbol-12-myristate-13-acetate} enhanced process extension while an inactive phorbol ester (4α-phorbol-12,13-didecanoate) that binds but does not stimulate PKC activation did not have any effects on OL process outgrowth (Yong et al., 1991). The role of PKC in OL process formation was further tested by employing PKC inhibitors ranging from those with low selectivity (e.g. polymixin B and staurosporine) to intermediate selectivity (chelerythrine and H-7) to those that are highly selective for PKC (calphostin C and CGP 41 251). These inhibitors block the basal- and phorbol ester- induced process outgrowth. Moreover, measurements of PKC enzyme activity, using a histone phosphorylation assay, has supported the role of PKC in OL process outgrowth. The treatment of adult human OLs with PDB results in the translocation of PKC from the cytosol to the particulate fraction of cell, in accordance with the mode of PKC activation (Kraft and Anderson, 1983; Chida et al., 1986). Following PDB treatment, PKC enzyme activity in the particulate fraction has also been found to be elevated for

prolonged periods (up to 12 days) whereas in other cell types including astrocytes and fibroblasts, down-regulation of PKC was readily observed (Yong et al., 1994). Although the rate of process formation is slowest in adult human OLs among many species tested, PKC stimulation dramatically enhances process extension by adult human or rat OLs to an extent which is comparable to that of neonatal OLs (Yong et al., 1994). Studies using isoform-specific agonists suggest PKC α as a major determinant in process formation by OLs (Schmidt-Schultz and Althaus, 1994; Yong et al., 1994).

MATRIX METALLOPROTEINASES

Matrix metalloproteinases (MMPs) are potent Zn^+ -dependent proteinases that can degrade most, if not all, ECM components. Initially it was thought that individual MMPs have unique substrate specificity, and they were categorized according to it. Subsequent studies revealed that MMPs share significant substrate specificity overlap and current classification is based primarily on structural difference and secondarily on substrate preference: collagenases, gelatinases, stromelysins, and membrane type (MT)-MMPs (Chambers and Matrisian, 1997; Shapiro, 1998). In the N-terminus of all MMPs, there is an active site containing a Zn^+ ion, and a short signal peptide at the extreme end. Typically, the Zn^+ atom is associated with three histidine residues in a highly conserved sequence in the active site, and this is present in all MMP members. The C-terminal, with a high level of homology to members of the hemopexin family, confers substrate-binding capacity. This C-terminal is absent in MMP-7 (matrilysin). The MT-MMPs have an additional transmembrane domain which results in them being

membrane bound (see for reviews Murphy and Knauper, 1997; Yong et al., 1998). At least 20 MMP members are currently known.

Because of their potent proteolytic activity, MMPs are involved in many developmental processes such as bone growth/remodeling, tooth eruption, and other physiological events that require ECM turnover including ovulation, embryogenesis, angiogenesis and wound healing. Uncontrolled MMP activity can be potentially destructive and detrimental as demonstrated in a number of pathological processes including cancer metastasis, rheumatoid arthritis, gastric ulcer, atherosclerosis and several neurological diseases (see for reviews Woessner, 1994; Yong et al., 1998).

The powerful degrading action of MMPs necessitates cells to acquire stringent control mechanisms to modulate the activity of MMPs. First, in many cell types, MMPs are not constitutively expressed but are induced upon stimulation with growth factors, ECM molecules and phorbol esters. Often, these stimuli induce the expression of the transcription activator protein (AP)-1 complex consisting of homo- or hetero-dimers of c-jun and c-fos proto-oncogene products. An AP-1 complex binds to specific conserved sequences called AP-1 binding site or phorbol ester responsive element (TRE) in the promoter region and modulates transcription. While the majority of genes encoding MMPs have AP-1 binding site in their promoter region, the MMP-2 gene lacks AP-1 binding site and normally its expression is constitutive rather than inducible. There are other transcriptional binding elements (e.g. PEA-3, Sp1, NFκB) that are present in several MMPs that can affect the expression of MMPs (Matrisian, 1994).

A second means to control MMP activity is that MMPs are expressed as inactive zymogens, which have to be activated. All MMPs known to date contain a pro-peptide

at the N-terminal; a cysteine residue in the propeptide region ligates back to the Zn^+ atom at the active site, thus blocking the exposure of the active site. Once this pro-peptide is dissociated from the active site, MMPs become proteolytically active (Springman et al., 1990; Van Wart et al., 1990). Activating factors such as plasmin, plasminogen activator and other MMPs disrupt the cysteine-zinc interaction at the active site producing partially activated MMPs. These intermediate MMPs can be further activated by cleaving the pro-peptide by autoproteolysis or by other activating factors (Nagase, 1997).

Thirdly, there are physiological MMP inhibitors, called tissue inhibitors of metalloproteinases (TIMPs), to regulate MMP activity (Murphy and Knauper, 1997). Currently, four TIMPs have been identified. Their inhibitory property comes from the capacity to bind the catalytic active site of MMPs to render inactivation. Recent studies have shown that TIMPs can not only inhibit activity of MMPs but also may facilitate the activation of MMPs (Emmert-Buck et al., 1995; Murphy and Knauper, 1997). For instance, TIMP-2 coordinates the interaction between pro-MMP-2 and MT1-MMP, which activates pro-MMP-2 (Strongin et al, 1995). Thus, the concentration of TIMPs may be significant in the regulation of MMP activity; at low concentration they may facilitate the activation of MMPs while high levels of TIMPs inactivate MMPs. In many cases, the up-regulation of MMPs is often accompanied by up-regulation of TIMPs (Marshall et al., 1995; Kapila et al., 1996; Kossakowska et al., 1998). Recent reports provide evidence that TIMPs are also involved in other cellular processes. TIMP-1 and -2 have been demonstrated to promote cell proliferation (Yamashita et al., 1996). TIMP-3 inhibits cells from undergoing programmed cell death by protecting the

degradation of basement membrane of cells (Basbaum and Werb, 1996). On the other hand TIMP-3 may promote apoptosis in some cell types (Ahonen et al., 1998).

In the CNS, the participation of MMPs in pathology has been demonstrated in stroke, malignant gliomas, Alzheimer's disease and MS (reviewed in Yong et al., 1998). A number of reports have revealed that breakdown of the blood brain barrier (BBB) and demyelination in diseases such as MS is contributed by MMPs. The intracerebral injection of MMP-2 resulted in BBB breach accompanied by perivascular infiltration, edema, and hemorrhage (Rosenberg et al., 1992). In vitro studies have provided evidence that several metalloproteinases are able to degrade myelin proteins such as MBP (Gijbels et al., 1993; Millichip et al., 1998). Recently, a series of studies demonstrated that MMPs are up-regulated in the active lesion and in the cerebrospinal fluid of MS patients. Immunohistochemistry studies showed that in general, MMP-2 and -9 are localized to the perivascular space and to infiltrated macrophages/monocytes in lesions (Cuzner et al., 1996; Maeda and Sobel, 1996; Chandler et al., 1997). In animal models, by using quantitative polymerize chain reaction analyses, it was shown that matrilysin (MMP-7) was elevated by over 500 fold in EAE while MMP-9 increased about five fold (Clement et al., 1997). Application of synthetic hydroxamate inhibitors of metalloproteinase suppressed or reduced the symptoms and course of EAE (Gijbels et al., 1994; Hewson et al., 1995; Matyszak and Perry, 1996).

On the other hand, the beneficial property of MMPs in the CNS is becoming evident with accumulating findings that MMPs are involved in removal of debris from the injury site, the release of growth factors anchored on ECM, or the degradation of inhibitory substances. Early OL progenitor cells, the O-2A cells, migrate on myelin in

vitro by utilizing MMPs (Amberger et al., 1997), and it is possible that several neural progenitor cell types require MMPs to migrate into lesion sites to attempt to repair. A number of studies have demonstrated that MMPs and other MMP activating proteases are involved in neurite outgrowth and the migration of neural cells (Krystosek et al., 1981; Monard, 1988; Machida et al., 1989; Muir, 1994; Zuo et al., 1998). The nerve section becomes permissive to neurite extension by treatment with MMP-2 which degrades inhibitory molecules such as CSPG and exposes laminin, a neurite promoting component (Zuo et al., 1998).

AIMS OF THESIS

My overall thesis seeks to understand the mechanisms that regulate oligodendrocyte process outgrowth, an early and prerequisite event in myelin formation.

Specifically, for Aim 1 (described in Chapter II), I have sought to identify physiological stimuli that promote OL process outgrowth, given that previously identified stimulators of OL process outgrowth are phorbol esters (Althaus et al., 1991; Yong et al, 1991; 1994), which are exogenous compounds. In particular, I tested the hypothesis that other glial cell types enhance OL process formation, and addressed the mechanisms involved.

In Aim 2, following the identification of physiologic stimuli that enhance OL process outgrowth (from Aim 1), I addressed the signal transduction pathway in OL that regulates the process forming capacity of these stimuli. Specifically, I tested the

hypothesis that PKC activation in OL is involved in mediating the effects of these stimuli. The results are described in Chapter III.

Finally, in Chapter IV, regarding the need for proteases to remodel the pericellular microenvironment as OL process outgrowth occurs, I tested the hypothesis that MMPs are involved in OL process outgrowth. These results are described in Chapter IV.

Figure I-1: A schematic representation of an OL/myelin/axon unit (top) and a cross-sectional representation of the myelin sheath (bottom). Abbreviations are: proteolipid protein (PLP), myelin basic protein (MBP), 2'3'-cyclic nucleotide 3'-phosphodiesterase (CNP), myelin associated protein (MAG), and myelin/oligodendrocyte protein (MOG). These proteins and the intraperiod and major dense lines, are described further on pages I-9~12. (Adapted from McLaurin and Yong, 1995; Newman et al., 1995; Quarles et al., 1997)

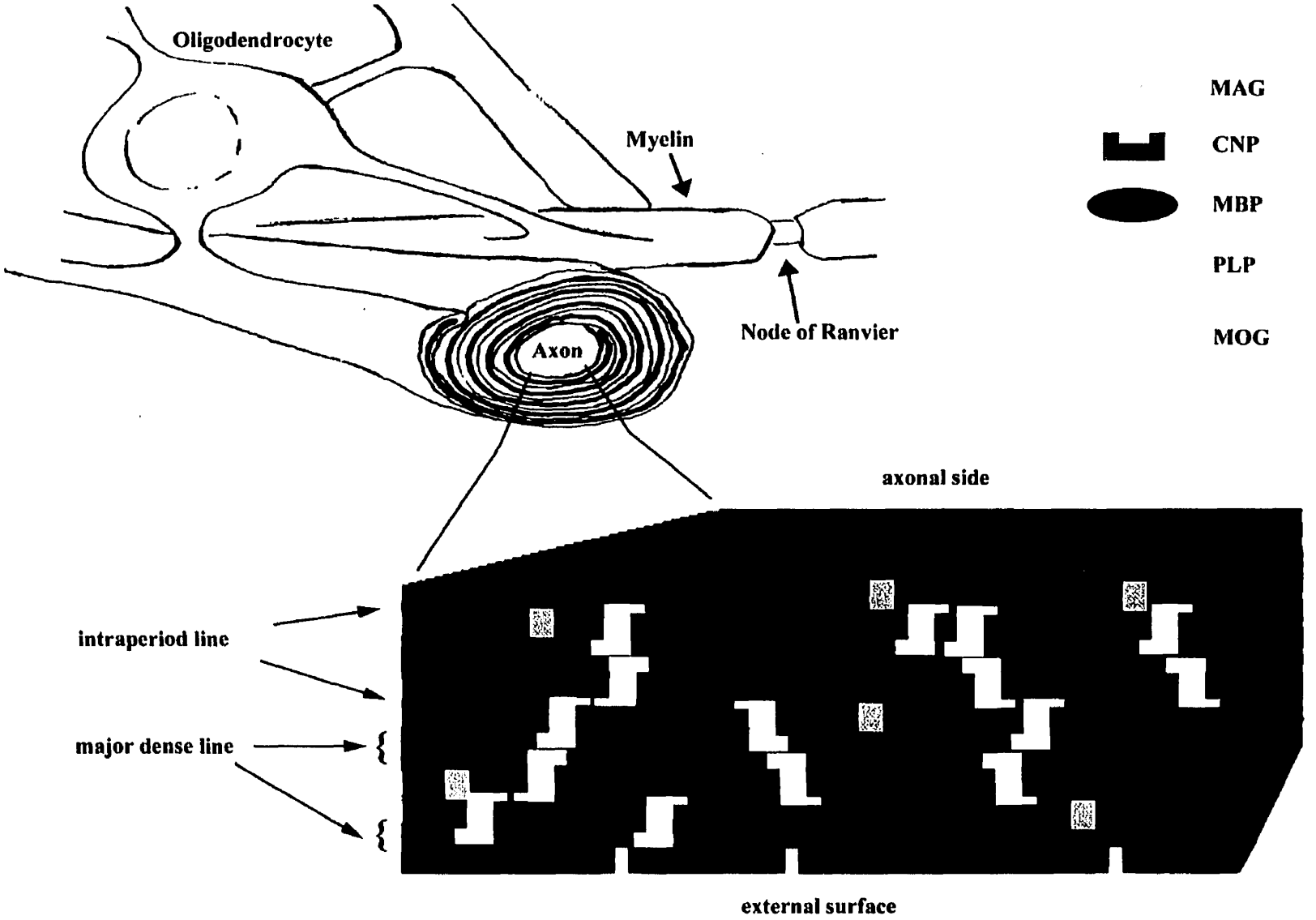

Oligodendrocyte
Myelin
Node of Ranvier
Axon
MAG
CNP
MBP
PLP
MOG
axonal side
intraperiod line
major dense line
external surface

Figure I-2: Main aims of my thesis can be summarized as a schematic, and include:

1. Whether and how astrocytes promote process outgrowth by OLs.

2. Whether PKC activation is involved in mediating promoting effects on OL process outgrowth.

3. Whether and which matrix metalloproteinases (MMPs) are involved in process extension by OLs.

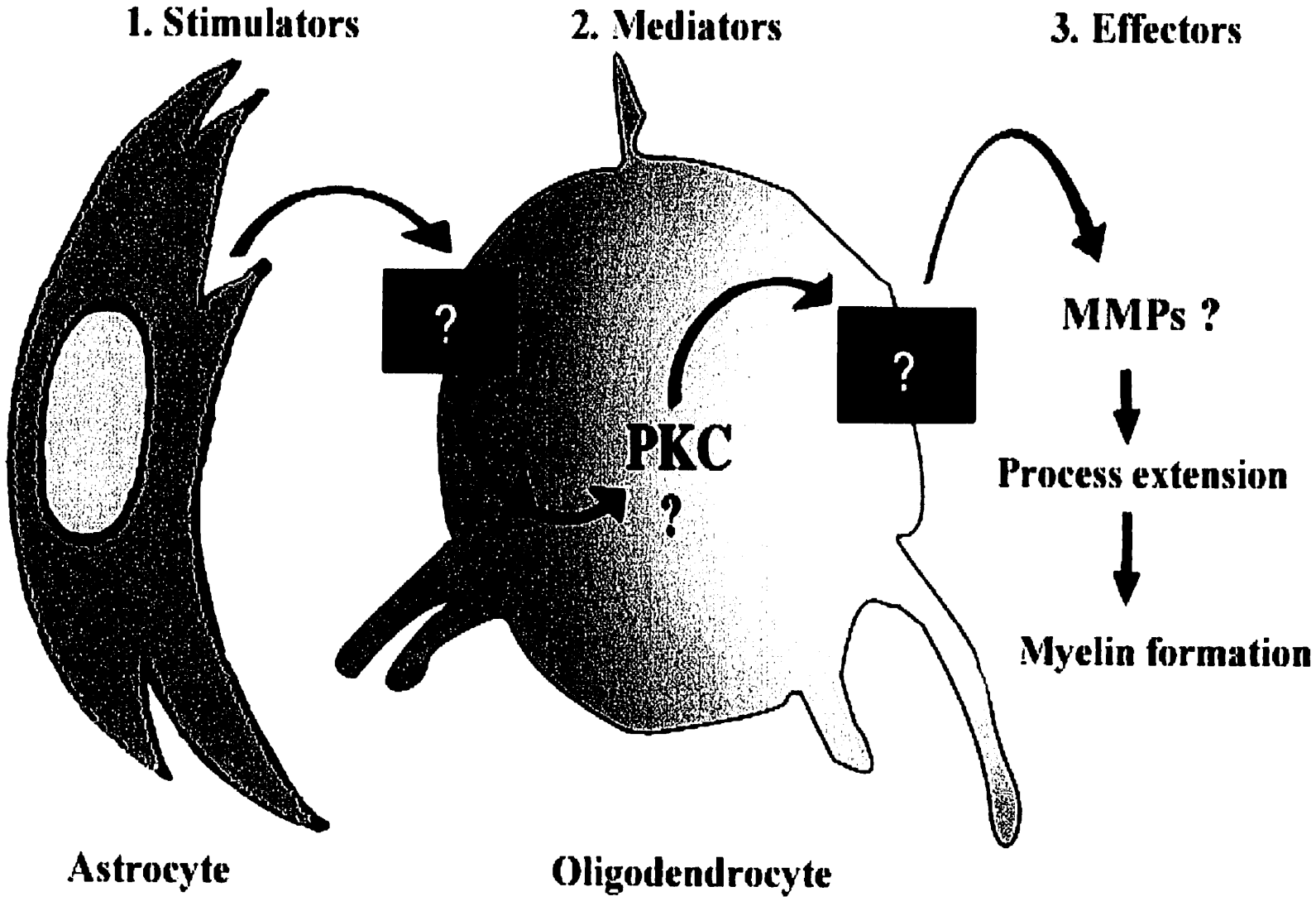

1. Stimulators
2. Mediators
3. Effectors
?
?
PKC
?
MMPs ?
Process extension
Myelin formation
Astrocyte
Oligodendrocyte

Figure I-3: A schematic diagram of the development of an oligodendrocyte from its progenitor cell. Antigenic markers and antibodies are described in text.
(Adapted from Pfeiffer et al., 1993; McLaurin and Yong, 1995).

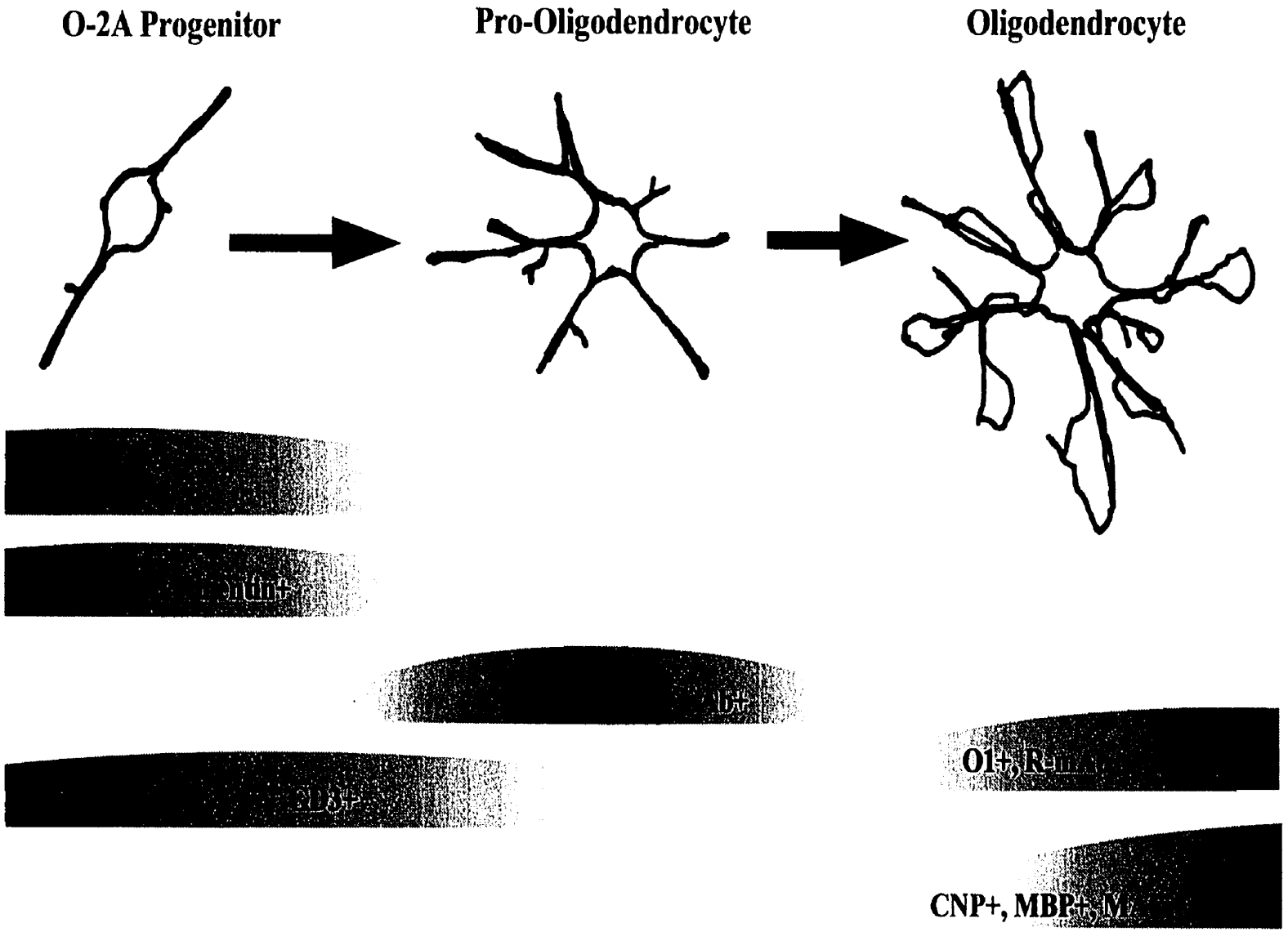
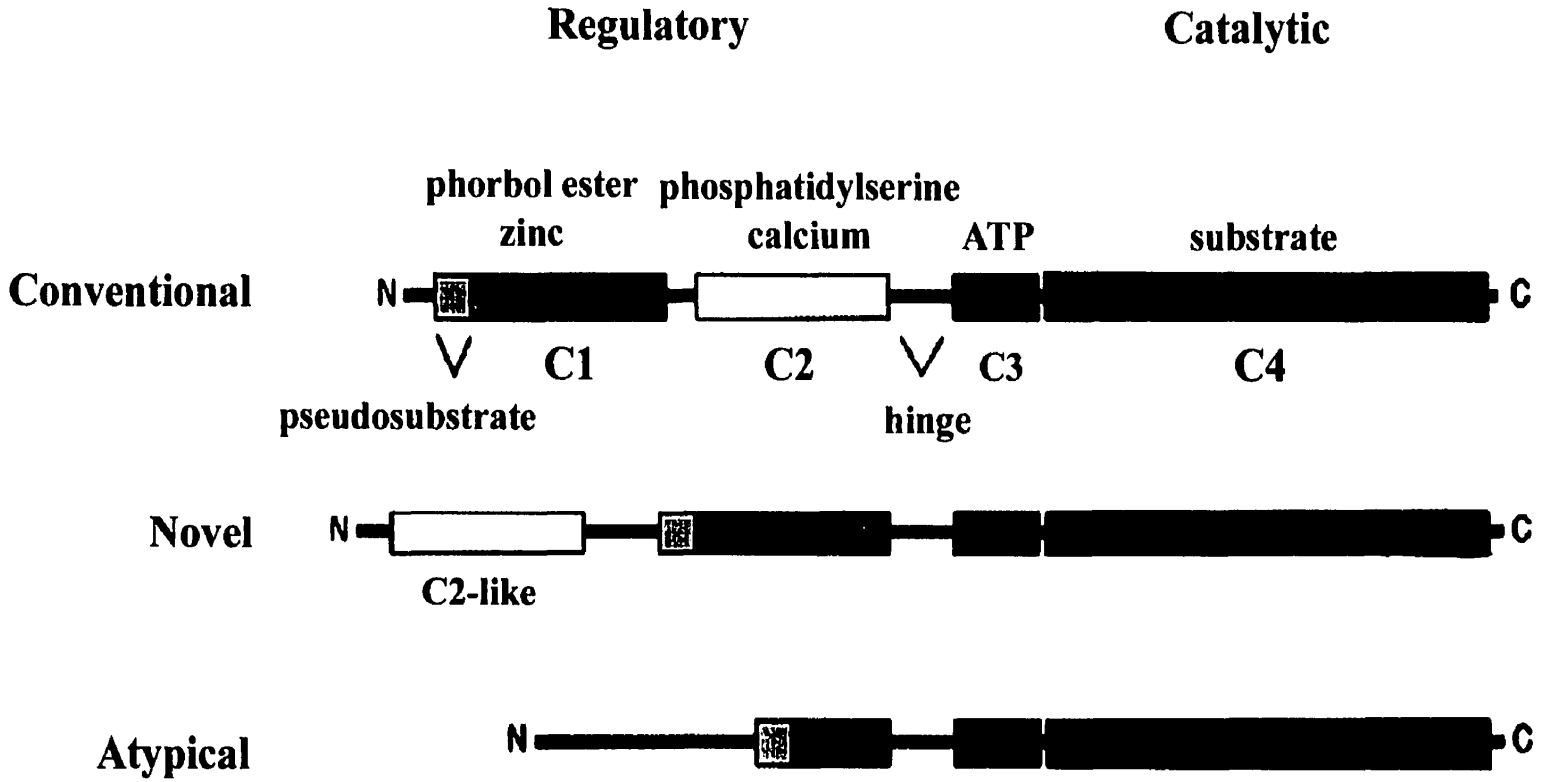

O-2A Progenitor
Pro-Oligodendrocyte
Oligodendrocyte
O1+, R
CNP+, MBP+, M

Figure I-4: A schematic representation of PKC structure. In regulatory region, C1 domain (red) contains phorbol ester and zinc binding sites. Calcium binds to the C2 domain (yellow) while the exact location where phosphatidylserine binds remains unclear; it is thought that this molecule can bind to multiple sites of the C1 and C2 domains. The pseudosubstrate (green) motif at the N-terminal binds the substrate docking region of the C4 domain (purple) to keep the enzyme inactive. In the catalytic region, ATP binds the C3 domain (pink) whereas the C4 domain binds substrate. (Adapted from Newton, 1995).


Regulatory
Catalytic
phorbol ester
zinc
phosphatidylserine
calcium
ATP
substrate
Conventional
N
C
C1
C2
C3
C4
pseudosubstrate
hinge
Novel
N
C
C2-like
Atypical
N
C

In chapter II, physiological stimuli that can promote process outgrowth by OLs were investigated. Previous studies demonstrated that OL process formation was enhanced by using phorbol esters which are exogenous compounds (Althaus et al., 1991; Yong et al., 1994). Since many important functions within the CNS are regulated by cell-cell interactions, I addressed whether and which glial cell type can enhance OL process formation and by which mechanisms promoting action occurs. I tested the effects of other glial cell types on OL process formation in co-culture. Moreover, a number of growth factors and ECM components were examined for the promoting effects on process outgrowth by adult human OLs, which is an early critical event to complete myelin formation.

CHAPTER II

Astrocytes Promote Process Outgrowth by Adult Human Oligodendrocytes in vitro through interaction between bFGF and Astrocyte Extracellular Matrix

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Abstract

Cell-cell interactions regulate many important functions within the CNS. In this report, we demonstrate that process outgrowth by adult human oligodendrocytes(OLs) in vitro, an early event of myelinogenesis in vivo, is promoted by astrocytes. To elucidate the mechanisms by which astrocytes might exert this effect, we tested several growth factors known to be produced by astrocytes and found that only basic fibroblast growth factor (bFGF) could enhance process extension by the OL. In correspondence, the treatment of astrocytes with a neutralizing antibody to bFGF decreased their effects in promoting oligodendroglial process outgrowth. The potency of bFGF, however, was only one-third that of astrocytes, and since bFGF did not synergize with other soluble growth factors, we investigated the potential facilitatory role of the extracellular matrix (ECM) deposited by astrocytes. The astrocyte ECM was found to be a promoter of oligodendroglial process extension, and significantly, bFGF synergized with astrocyte ECM to match the potency of live astrocytes. The astrocyte ECM was found in Western blot analyses to contain fibronectin, vitronectin and laminin. These purified ECM components, and heparan sulfate proteoglycan, did not promote oligodendroglial process extension by themselves, although laminin and fibronectin potentiated the effects of bFGF. We conclude that process outgrowth by OLs is guided by astrocytes; the mechanism of the astrocyte effect appears to be due to the combination of bFGF and an unidentified ECM component.

Introduction

Remyelination by oligodendrocytes (OLs) in demyelinating states depends on a number of interacting factors including the availability of OLs, the morphological differentiation of OLs to allow for process extension from their soma, the proper synthesis and turnover of OL/myelin components, and the adequate interaction between the ensheathing oligodendroglial process with an axon to form myelin (Ludwin, 1989; McLaurin and Yong, 1995). That these factors are present to some degree in the prototype human demyelinating disease multiple sclerosis (MS), despite the prolonged scarring and chronic demyelination, is evident by the finding that remyelination can occur in the disease although incomplete in its extent (Raine et al., 1981; Ghatak et al., 1989; Prineas et al., 1989; 1993; Raine and Wu, 1993; Bruck et al., 1994). Stimuli to enhance OL formation, maturation or function may improve the remyelinating capacity of OLs. One such strategy is to promote surviving OLs, or OLs formed from progenitors, to extend their processes to enwrap axons. This approach to enhance the extent of process formation is particularly relevant for MS since substantial number of OLs are documented around the edges of lesion in the disease (Raine et al., 1981; Prineas et al., 1989; 1993; Raine and Wu, 1993).

OLs in culture have served as useful models for their in vivo counterparts. In vitro, OLs extend multiple processes and the plasma membrane of the cultured OL contains all the major proteins and lipids of myelin (Szuchet, 1987; Vartanian et al., 1992). The

emergence of myelin proteins and lipids in vitro, even in the absence of neurons or astrocytes, follows a time course that corresponds to that in vivo (Zeller et al., 1985; Dubois-Dalcq et al., 1986; Zalc et al., 1987). Furthermore, OLs isolated from mutant animals with defective myelination show corresponding abnormalities in vitro (Bartlett et al., 1988).

Using cultured OLs, we and others have reported that biologically active phorbol esters could promote process outgrowth by OLs through the stimulation of oligodendroglial protein kinase C (PKC) activity (Yonget al., 1988; 1991; 1994; Althaus et al., 1990; 1991). As phorbol esters are exogenous compounds, it would be advantageous to identify physiologic growth factors that could promote process extension by OLs. We report here that adult human OLs in mixed culture with astrocytes extended processes readily when compared to OLs in purified culture. While astrocytes have long been known to be excellent substrates for neurite extension by many different populations of neurons in vitro and in vivo (Lindsay, 1979; Silver and Ogawa, 1983; Noble et al., 1984; Fallon et al., 1985; Wujek and Akeson, 1987; Neugebauer et al., 1988; Tomaselli et al., 1988; Kliot et al., 1990; Johnson-Green et al., 1992), a similar role for astrocytes in promoting process formation by OLs, a critical early event in myelinogenesis, has not been reported. We therefore investigated the mechanisms of the astrocyte effect and report here that the facilitatory role of astrocytes on oligodendroglial process extension is due to a synergistic effect between basic fibroblast growth factor (bFGF, FGF-2) and a component of the astrocyte extracellular matrix (ECM).

Materials and Methods

Sources of Materials

The following reagents were purchased from UBI (Lake Placid, NY): recombinant human (rh) platelet derived growth factor AB (PDGF), rh epidermal growth factor (EGF) and rh insulin-like growth factor-1 (IGF-1). Rh brain-derived neurotrophic factor (BDNF), rh neurotrophin-3 (NT-3), rh neurotrophin-4 (NT-4) and rh glial derived neurotrophic factor (GDNF) were bought from PeproTech Inc. (Rocky Hill, NJ). 2.5S nerve growth factor (NGF) was from Cedarlane (Hornby, Ontario). Rh basic fibroblast growth factor (bFGF) was from either Becton Dickinson (Bedford, MA) or UBI and both were of similar potency. 4β-phorbol-12,13-dibutyrate (PDB) was from LC Laboratories (Woburn, MA) while recombinant rat CNTF was provided by Dr. P. Richardson (McGill University). Extracellular matrix components including heparan sulfate proteoglycan (HSPG), EHS laminin, vitronectin, collagen Type IV and fibronectin were purchased from Becton Dickinson. Monoclonal antibodies to bFGF (Type I: neutralizing, and Type II: non-neutralizing) were from UBI. The polyclonal rabbit antibody to fibronectin was obtained from Bio/Can Scientific (Mississauga, Ontario) while the source of polyclonal rabbit antibodies to laminin and vitronectin was Gibco BRL (Burlington, Ontario).

Cell culture

Adult human cells were derived from brain biopsy specimens from 9 patients

(average age = 27 ± 3 years, range 15 to 36 years) who underwent surgical resection to amelioratedrug intractableepilepsy. Samples were of areas adjacentto, but not containing, the epileptic focus. Dissociated cells were obtained using trypsin digestion and Percoll gradient centrifugation and this protocol is described in detail elsewhere (Yong and Antel, 1992).

Upon obtaining dissociated cells, these were either plated directly onto substrates (Lab-Tek 16 well chamber slides, Nunc, Inc., Naperville, IL) coated with 10 μg/ml poly-l-lysine (PL) or onto uncoated T-25 cm^2 flasks. When plated directly onto PL, all cell types adhered to result in a mixed culture containing OLs, astrocytes or microglia (see below for immunohistochemical identification of cell types). In our experience with over 400 adult human samples, while some degree of variability exists, mixed cultures contain on averageapproximately 40% OLs, 40% microgliaand 20% astrocytes. When plated onto uncoated flasks, adult human OLs were poorly adherent in contrast to astrocytes and microglia; floating cells were removed the next day and, when subjected to another round of differentialadhesion, resulted in OL cultures of over 90% purity (Yong and Antel, 1992) - these are henceforth called *enriched*OL cultures to differentiate them from OLs in *mixed* culture. Enriched OLs or OLs in mixed culture were seeded at a density of 10^4 cells per well (in 250 μl volume of feeding medium) of 16 well chamber slides.

In experiments where adult human OLs were seeded with a purified culture of adult human microglia, the latter was obtained as we have previously described (Williams et al., 1992). Briefly, adherent cells following the removal of floating OLs were allowed to

develop morphologically for 7 days (this resulted in astrocytes stratifying themselves above the microglia) and the astrocytes were then floated off by rotary shaking (150 rpm, 5 hours, room temperature). The adherent microglial population was detached by trypsinization and then plated onto PL-coated 16 well chamber slides. Purity of microglia was over 95% (Williams et al., 1992).

In experiments where adult human OLs were seeded onto a monolayer of fetal human astrocytes, the latter were derived using procedures that have been previously described (Yong et al., 1992). Purity of fetal human astrocytes was in excess of 98%. Although it would have been ideal to plate OLs onto adult human astrocytes, the low yield and purity (70% at best) of adult human astrocytes (Yong and Antel, 1992) precluded such an experiment.

To obtain an astrocyte extracellular matrix (ECM), the protocol of Cardwell and Rome (1988) was followed. Fetal human astrocytes were seeded at a high density of 25,000 cells/chamber of PL-coated 16 well slide in order to achieve confluency by 2 days post-plating. Four days post-plating, cells were treated with water for 2 hours at 37°C (with 3 changes of water during this duration). Under a light microscope, the removal of the cell layer was verified. Purified OLs were then seeded at 10^4 density per well.

Feeding medium in all cases was Eagle's minimum essential medium supplemented with 5% fetal bovine serum, 20 μg/ml gentamicin, and 0.1% dextrose. All culture reagents were purchased from Gibco BRL.

Administration of test agents

Unless otherwise stated, purified OLs were treated with test agents two days following plating onto PL. Treatment was performed twice spaced two days apart, and cells were harvested two days following the second treatment. In experiments that addressed whether the effects of live astrocytes or astrocyte ECM could be neutralized by antibodies to bFGF, live astrocytes or their ECM were pre-incubated with antibodies contained in feeding medium for 1 hour at 37°C. The solution was then removed and OLs with fresh antibodies were then added. Antibodies were replenished after two days and cells were analysed two days later.

Immunohistochemistry and assessment of extent of process formation in vitro

Chamber slides with live cells were incubated with hybridoma supernatant containing a mouse monoclonal antibody designated O1 (Sommer and Schachner, 1981), which recognizes galactocerebroside (GalC) and related lipids (monogalactosyldiglyceride and psychosine) which are specific to OLs (Bansal et al., 1989). Following 45 min at room temperature, goat anti-mouse immunoglobulin conjugated to rhodamine (1:150 dilution, Cappell, Lexington, MA) was added for another 45 min. Cells were fixed with 95% ethanol-5% glacial acetic acid (v/v) for 15 min and then labelled with Hoescht dye to label nuclei. The whole chamber slide was then coverglassed with gelvatol. Using an immunofluorescence microscope, the % of GalC+ (i.e. O1+) cells with processes more than 3 soma diameters in length was tabulated as previously described (Yonget al., 1991; 1994). The 3 soma diameter cutoff point was used because our previous experience had shown

that less than 10% of control purified OLs would attain this criterion; thus, the facilitatory effects of agents could be effectively assessed.

For the identification of astrocytes in vitro, cells following the acetic acid-ethanol fixation were incubated with a rabbit polyclonal to glial fibrillary acidic protein (GFAP, Dako Corporation, Scarborough, Ontario, 1:100 dilution) for 45 min followed by a goat anti-rabbit Ig conjugated to FITC (Cappell, 1:100). Microglia were labelled using Leu-M5 (mouse anti CD11c, Beckon-Dickinson) immunoreactivity of live cells, as previously described (Williams et al., 1992), or with an anti-KP-1 antibody (anti CD68) following fixation.

Western blot analyses of the astrocyte ECM

Fetal human astrocytes in 100 mm plate were lysed with water as described above to leave behind the astrocyte ECM. A 1x SDS sample buffer (1 ml) was then added to the plate to solublize the ECM; total protein content of the ECM was analysed by a BCA protein assay kit (Pierce) that utilized bovine serum albumin as a standard. Thirty μg astrocyte ECM was then electrophoresced on SDS-polyacrylamide gels with appropriate standards (fibronectin, vitronectin and laminin). Fibronectin and vitronectin were resolved using 8% and 15% polyacrylamide gels respectively, while laminin was electrophoresced using a 4-20% gradient gel. Expected molecular weights for fibronectin, vitronectin and laminin were, respectively, 220, 66 and 200 kDa.

Following electrophoresis, proteins were transferred onto nitrocellulose membranes.

The membranes were reacted with skim milk powder to saturate sites of non-specific protein binding. Blots were then incubated with polyclonal antibodies to fibronectin, vitronectin or laminin (all were used at 1:500 dilution). Subsequent incubation with ^{125}I-Protein A (1 μCi diluted in 10 ml of Blotto) for 2 h ensued. Membranes were then washed and exposed on phosphoimager casettes. Protein bands were visualized using a phosphoimager (Molecular Dynamics).

Results

Processextension by adult human OLs in mixed versusenriched culture: The differential effects of astrocytes and microglia

We have previously reported that enriched adult human OLs have a very slow rate of process extension compared to OLs derived from adult or neonatal *rat* brain (Yong et al., 1994). Figure II-1 demonstrates that process outgrowth by enriched adult human OLs did not become readily apparent even at 2 weeks following plating onto PL coating. However, in mixed culture with astrocytes and microglia, the rate of process extension was significantly enhanced and the majority of OLs had processes over 3 soma length by 6 days post-plating (Fig. II-1).

To address which cell type in the mixed culture promoted process formation by OLs, OLs were seeded onto microglia or astrocyte substrates. Figure II-2 shows that astrocytes promoted survival and process outgrowth of OLs while microglia were toxic to OLs. Of the surviving OLs in co-culture with microglia, their rate of process extension was also retarded when compared to controls. Thus, we concluded that astroglial-derived growth factors facilitated process extension by OLs.

As aforementioned, phorbol esters such as PDB are very potent stimulators of oligodendroglial process extension (Yong et al., 1991; 1994). Figure II-3 demonstrates that under the same experimental conditions, the efficacy of live astrocytes matches that of PDB at 50 ng/ml, a concentration that provides maximal stimulation of oligodendroglial process extension (Yong et al., 1991).

Effects of purified growth factors on process extension by adult human OLs

We considered whether soluble factors elaborated by astrocytes could enhance oligodendroglial process outgrowth. A range of growth factors known to be produced by astrocytes (Ferrara et al., 1988; Finklestein et al., 1988; Gadient et al., 1990; Yoshida and Gage, 1991; Araujo et al., 1992; Gomez-Pinilla et al., 1992; Han et al., 1992; Rudge et al., 1992; Carroll et al., 1993; Chernausek, 1993; Seniuk et al., 1994) was added to purified OLs. Of these, only bFGF and aFGF could significantly enhance process extension by adult human OLs (Fig. II-4 and -5, Table II-1). However, the potency of bFGF was not as marked as that of PDB (Table II-1). Furthermore, a range of concentrations of bFGF, added for varying periods of time, did not result in process extension of the magnitude that was elicited by PDB (Fig. II-6).

Althaus et al. (1992) have previously reported that NGF could cause adult bovine OLs to extend long processes. In our experience, NGF did not promote adult human OLs to extend processes (Fig. II-5); other neurotrophins tested (BDNF, NT-3 and NT-4) similarly did not promote process outgrowth by human OLs. To demonstrate that the NGF preparation that we utilized was bioactive, the NGF-responsive PC12 cell line was incubated with NGF. At all concentrations of NGF used (5 to 500 ng/ml), PC12 wells underwent neuritic outgrowth (Fig. II-5).

It has been previously observed that bFGF treatment of rodent OLs caused these cells to dedifferentiate in maturity (Grinspan et al., 1993; Fressinaud et al., 1993; 1995). To evaluate whether such occurs also for human cells, adult OLs were treated with bFGF

and then subjected to double immunofluorescence for O1 and myelin basic protein (MBP). Figure II-7 demonstrates that in contrast to the reports for rodents cells, human OLs did not dedifferentiate in response to bFGF treatment.

Because the potency of bFGF in eliciting process extension is less than that of PDB or of live astrocytes (see Fig. II-11 below), we tested whether bFGF could synergize with other soluble growth factors. Figure II-8 reveals that the effects of bFGF could not be augmented by other soluble growth factors tested including aFGF.

Astrocytes promote process outgrowth by human OLs: Modulation by bFGF antibodies and the role of the astrocyte ECM

To determine whether the process promoting actions of bFGF and live astrocytes were mechanistically linked, the live astrocyte-OL co-culture was treated with a neutralizing antibody (Type I) to bFGF; the results show that the facilitatory role of astrocytes could be attenuated by antibody but not eliminated (Fig. II-9). Type II bFGF antibody, which is non-neutralizing (Matsuzaki et al., 1989), as well as an irrelevant Ig of the same isotype as both bFGF antibodies, did not reproducibly alter process outgrowth.

Medium conditioned for two days by astrocytes, whether non-concentrated or concentrated, failed to promote process extension by OLs (Fig. II-10). These negative results included fractions of astrocyte conditioned medium between 10 to 30 kDa (where bFGF is expected to be) which were concentrated 50 or 100 times.

Since OLs have integrin receptors that would allow them to bind ECM molecules (Malek-Hedayat and Rome, 1994; Milner and ffrench-Constant, 1994), and given a

previous report that rat OLs extended processes on ECM derived from bovine corneal endothelial cells (Ovadia et al., 1984), we plated adult human OLs onto ECM of astrocytes. Under this condition, process extension was found to be enhanced (Fig. II-11) when compared to cells on PL although the magnitude was not as potent as that of live astrocytes. However, the co-administration of bFGF and astrocyte matrix promoted process outgrowth to the same extent as that of live astrocytes (Fig. II-11), underscoring the interplay between bFGF and a component(s) of the astrocyte ECM.

To attempt to identify which component(s) of the astrocyte ECM was responsible for facilitating process outgrowth, the astrocyte matrix was solubilized into SDS buffer and probed in Western blot analyses for proteins known to be constituents of ECM made by astrocytes or other cell types. It was found that fibronectin, laminin and vitronectin were present in the astrocyte matrix (Fig. II-12). When OLs were seeded onto purified ECM components, none of the agents tested (HSPG, laminin, vitronectin and fibronectin) facilitated process extension by themselves; however, laminin and fibronectin, but not vitronectin or HSPG, enhanced the effect of bFGF (Fig. II-13). Tenascin was also tested but this ECM molecule did not allow OLs to adhere.

An interesting morphological feature that was observed when OLs were plated onto live astrocytes or astrocyte ECM was that while the live astrocytes promoted process extension, the process formation was not of the form of membranous sheets, which probably represents the most mature form of oligodendroglial processes (Pfeiffer et al., 1993). In the astrocyte matrix, however, where live cells have been removed, OLs with membranous expansions were frequently encountered (Fig. II-14). Thus, while astrocytes

promoted processes to extend from the oligodendrocyte soma, some component(s) of the live state does not allow the morphological maturation of oligodendroglialprocesses into membranous sheets.

Discussion

Certain characteristics of cells of the OL lineage have been shown to be dependent on astrocyte function. Thus, growth factors from astrocytes are reported to cause the proliferation of OL precursors and to modulate their survival and differentiation into OLs (Noble et al., 1988; Richardson et al., 1988; McKinnon et al., 1990; Barres et al., 1992; 1993; Komoly et al., 1992; Mayer et al., 1993). The survival of mature oligodendrocytes in vitro is also promoted by astrocytes (Gard et al., 1995). Rome and colleagues(Cardwell and Rome, 1988; Malek-Hedayat and Rome, 1994) have demonstrated that OLs adhered well to an astrocyte matrix via integrin-dependent mechanisms. In lesion areas of MS, OLs have been observed to be invested within hypertrophic astrocytes (Prineas et al., 1990; Ghatak, 1992; Wu and Raine, 1992); the role of such glial associations remains controversial and may represent a protective mechanism for OLs by astrocytes (Raine and Wu, 1992) or destruction of OLs by astrocytes (Prineas et al., 1990).

The role of astrocytes in myelin formation by OLs has been the subject of few studies, and some of the results have been conflicting. Bhat and Pfeiffer (1986) reported that soluble extracts from astrocyte cultures increased myelin proteins of OLs. However, astrocytes have been demonstrated to inhibit the myelination of dorsal root ganglion axons by adult rat OLs (Rosen et al., 1989) and to prevent the translocation of MBP mRNA from soma into the processes of OLs (Amur-Umarjee et al., 1993). Nonetheless, in the ethidium bromide model of demyelination, transplants of astrocytes improved remyelination by OLs (Franklin et al., 1993). In vivo, astrocytic processes are closely associated with the OL

soma and processes (Butt et al., 1995), suggestive of intimate interactions.

While astrocytes have long been known to be excellent substrates for *neurite* extension by many different populations of neurons in vitro and in vivo (references cited earlier), a similar role of astrocytes in promoting process formation by OLs, a critical early event in myelinogenesis, has not been reported. The present study addresses such a role, and the results demonstrate that astrocytes are good substrates for process extension by adult human OLs; indeed, the magnitude of the astrocyte effect was comparable to that produced by the potent activator of PKC, PDB. Of growth factors known to be elaborated by astrocytes, only bFGF and aFGF were effective in causing process outgrowth. The effects of bFGF and aFGF are likely through the same receptor or pathway, since no synergy was observed when the two were used simultaneously. Furthermore, the potency of aFGF was less than that of bFGF, in line with the observations that in most systems, bFGF is more potent than aFGF and that the receptor for FGF binds bFGF with higher affinity than aFGF (reviewed in Baird and Bohlen, 1991). The effects of astrocytes were attenuated by a neutralizing antibody to bFGF, in accordance with the postulate that the astrocyte effect was partially due to bFGF. Nonetheless, bFGF by itself, or in conjunction with other soluble factors, did not mimic the potency of live astrocytes in promoting oligodendroglial process extension, and thus our attention became directed to other factors, in particular, the ECM of astrocytes. The results demonstrate that the astrocyte ECM could also promote process extension, and that together with bFGF, could mimic the potency of live astrocytes.

A cautionary note is in order since the majority of the experiments utilized

astrocytes from *fetal* brains and oligodendrocytes from *adult* specimens. Astrocyte maturational state can be important for astrocyte function and differencesdo exist between fetal and adult human astrocytes in vitro, such as the low basal rate of proliferation of adult astrocytes in contrast to the high mitotic rate of fetal astrocytes (Yong et al., 1992). For the experiments described herein, it was not possible to obtain *adult* human astrocytes in sufficient quantity and purity to elucidate the interplay between astrocytes and oligodendrocytes;hence,*fetal* astrocytes were used. Despite this caveat, it should be noted that the enhanced growth of processes was also noted when adult oligodendrocytes were in co-culture with *adult* astrocytes (Fig. II-1).

Although bFGF lacks a leader sequence for secretion by "classical" pathways, it is generallythought that bFGF is exported out of cells (Rifkin et al., 1989; Kandel et al., 1991; Baird and Bohlen, 1991; Araujo et al., 1992; Mason, 1994). Thus, if the facilitation of process outgrowth by astrocytes in the present study were partially due to bFGF, why then did the conditioned medium of astrocytes not promote process extension by OLs? It is possible that the secreted bFGF became bound to the ECM upon release, thus decreasing its concentration in the conditioned medium. This contention is supported by the results that the process extendingefficacy of live astrocytes (Fig. II-9), as well as of its matrix (unpublished observations), was decreased by a neutralizing antibody to bFGF. Furthermore, it has been well documented that bFGF is found in the ECM and that the matrix bindingof bFGF provides a reservoir for this growth factor (Vlodavsky et al., 1987; Rifkin et al., 1989; Gonzalez et al., 1990).

In reviewing the literature, it is evident that bFGF has many functions on cells of

the OL lineage. bFGF is known to be a mitogen for O-2A cells and to block their differentiation into OLs (Noble et al., 1988; McKinnon et al., 1990; Bansal and Pfeiffer, 1994; Fressinaud and Vallat, 1994) although this blockade could be overcome by other factors made by astrocytes (Mayer et al., 1993). In cultures from neonatal rodent brain containing more differentiated GalC+ OLs and their precursors, bFGF was shown to increase the proliferation of both cell types (Eccleston and Silberberg, 1985; Saneto and deVellis, 1985; Deloulme et al., 1992; Vicks and DeVries, 1992). In even more differentiated rodent OL cultures (from neonatal brain and expressing MBP), bFGF administration induced cell proliferation and dedifferentiation of OLs into precursor cells (Fressinaud et al., 1993; 1995; Grinspan etal., 1993). bFGF was recently found to prevent the apoptosis of OLs (Yasuda et al., 1995). Finally, of relevance to this study and in direct contrast, bFGF inhibited process formation of cultured rat (Besnard et al., 1989) and canine (Hoffman and Duncan, 1995) OLs. In our hands, bFGF not only promoted process formation by adult human OLs, as has been reported by Gogate et al. (1994), but bFGF also did not produce proliferation (Yong et al., 1988) of OLs and did not cause MBP+ cells to dedifferentiate (Fig. II-6). These differences are likely accounted for by species factors. In concordance with this argument, Armstrong et al. (1992), Gogate et al. (1994) and Yong et al. (1988) reported that bFGF was not a mitogen for adult human white matter derived OLs; Satoh and Kim (1994) reported that bFGF was not a mitogen for OLs from fetal human brains. Finally, we have also observed that astrocytes (Yong et al., 1990; 1992) and microglia (William et al., 1992) from human brains are different from their rodent couterparts in their morphology, expression of major histocompatibility antigens and

response to mitogenic factors. Species difference may also account for the result that NGF promoted process extension by adult pig (Althaus et al., 1992) but not by adult human OLs (this study).

Astrocytes are known to synthesize a number of ECM molecules that include laminin, HSPG, chondriotin sulfate proteoglycan, fibronectin, thrombospondin, tenascin, hyaluronan and vitronectin (Leisi et al., 1983; Price and Hynes, 1985; Selak et al., 1985; Asch et al., 1986; Gallo et al., 1987; Ard and Bunge, 1988; Giftochristos and David, 1988; Grierson et al., 1990; Snow et al., 1990; Johnson-Green et al., 1991); astrocytes are not known to make collagens (Rutka et al., 1986). In the current study, we could demonstrate the presence of fibronectin, laminin and vitronectin on the astrocyte ECM. OLs plated on purified ECM substrates alone did not show enhanced process outgrowth, but laminin and fibronectin could potentiate the effect of bFGF (Fig. II-13). It remains unclear whether the effects of the astrocyte ECM in promoting oligodendroglial process outgrowth is due to laminin or fibronectin. In the absence of bFGF, laminin and fibronectin, unlike the astrocyte ECM, did not promote process extension. This differential effect, however, could be due to interactions between ECM substrates and the bFGF anchored on the astrocyte ECM; such bFGF would be absent from the purified preparations of ECM molecules tested in isolation. It is also probable that there is a requirement for interactions between ECM molecules in the astrocyte matrix to promote oligodendroglial process extension; again, these interactions would be absent in purified ECM substrates tested in isolation. The use of antibodies with neutralizing activity to particular ECM molecules would be necessary to elucidate the role of laminin and fibronectin, or related molecules, in

the astrocyte ECM as a facilitator of process outgrowth by OLs.

A prior prediction had been that OLs plated onto HSPG and given bFGF would bear extensive processes, since HSPGs are involved in the promotion of FGF activity, either by facilitating the interaction of the bFGF-HSPG complex with the high affinity FGF receptor (Yayon et al., 1991; Aviezer et al., 1994), or by HSPGs causing the oligomerization of FGF molecules to allow the FGF-HSPG complex to bind and activate several receptor molecules (Spivak-Kroizmann et al., 1994). That HSPG did not promote the bFGF-induced process extension by OLs could be due to the experiments being conducted in medium containing serum. Serum has a variety of soluble HSPGs which could complex to bFGF and thus further plating on HSPG substrate would not increase this interaction further.

With respect to the maturity of oligodendroglial processes, our experience with cells from over 400 biopsy specimens has been that on a PL substrate, membranous sheets indicative of mature processes do not occur. Thus, it is remarkable that the astrocyte ECM allowed the maturation of OLs into those with membranous expansions (Fig. II-14). Even more remarkable is that the live astrocytes, while a promoter of process outgrowth, prevented the morphological maturation of the oligodendroglial processes. For the astrocyte to promote myelin formation in vivo, some other factors, or cells, will have to override the signal(s) from live astrocytes that does not allow the oligodendroglial processes to become membranous expansions that subsequently ensheath axons.

With further regards to cell-cell interactions, enriched microglial cells have an opposite effect to astrocytes in their influence on oligodendroglial process extension (Fig.

II-2). Microglial cells were also toxic to OLs, supporting the observations of Merrill and colleagues(1991) that rat microgliaare toxic to rat OLs; in certain cases, however, microglia have been reported to stimulate the myelinogenic program of OLs (Hamilton and Rome, 1994; Loughlin et al., 1994).

In conclusion, process outgrowth by adult human OLs is promoted by astrocytes through the actions of bFGF and a component of the astrocyte ECM that appears related to laminin and fibronectin. The findings are relevant to the understanding of myelin formation in vivo since FGFs are expressedat several stages of brain developmentincluding the postnatal period when myelination occurs (Thomas et al., 1991; Giodano et al., 1992). Furthermore, receptors for FGF have been localized by in situ hybridization studies to myelinated process tract of the rat CNS (Asai et al., 1993). Finally, astrocytes appear chronologically earlier than OLs and the close promixity of both cell types favors interactions that likely include the facilitation of process formation by OLs, an early event in myelinogenesis.

Acknowledgements

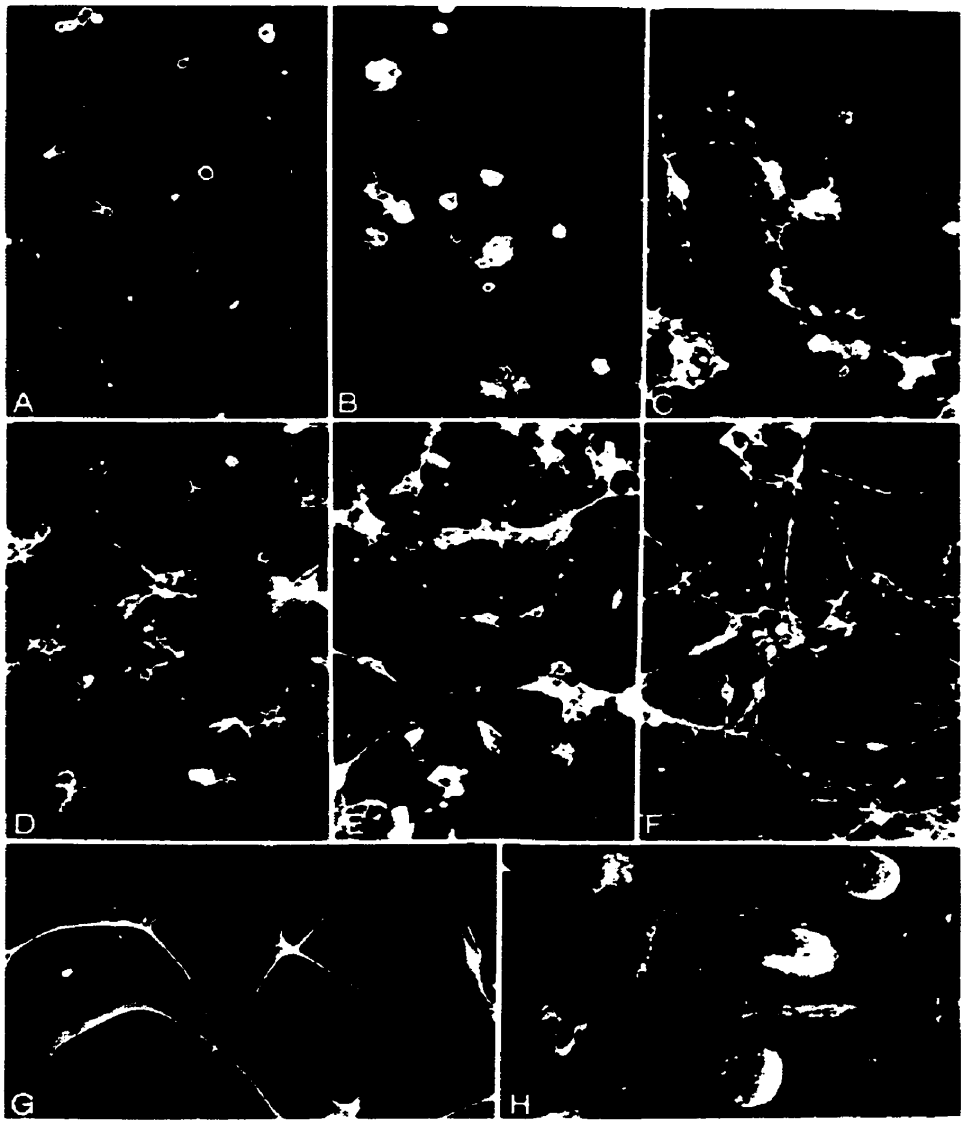
We wish to thank the Multiple Sclerosis Society of Canada for their support of operating funds. Luke Oh is supported by a studentship from the Multiple Sclerosis Society of Canada.

Table 1. Effects of bFGF and aFGF compared to PDB on different series of Adult human OLs

Series	% of O1+ cells with processes over 3 soma units				
	Control	bFGF 20	PDB 50	aFGF 20	aFGF 50
W678 HOL	3.2 ± 0.9	14.5 ± 1.9[a,b]	63.8 ± 3.9[a]	9.9 ± 2.4	10.2 ± 4.7
W686 HOL	5.3 ± 1.7	23.3 ± 5.5[a,b]	61.6 ± 9.4[a]	N.D.	N.D.
W695 HOL	13.5 ± 2.4	22.6 ± 6.3[a,b]	68.8 ± 4.8[a]	N.D.	N.D.
W717 HOL	4.4 ± 1.6	27.9 ± 6.0[a,b]	47.4 ± 4.4[a]	24.0 ± 5.3[a]	10.4 ± 4.0
W721 HOL	8.1 ± 2.9	29.8 ± 3.5[a,b]	61.8 ± 1.8[a]	21.1 ± 2.1[a]	22.7 ± 4.4[a]
W729 HOL	9.3 ± 2.0	63.9 ± 5.8[a]	78.0 ± 2.6[a]	35.8 ± 4.2[a]	52.8 ± 7.1[a]

All cultures were treated for 6d and were given 2 treatments of test factors. Concentration of test factors are in ng/ml.
All values are mean ± SEM from 3 or 4 coverslips of cells; on average, 154 ± 12 O1+ cells per coverslip were analyzed.
[a]$p<0.05$ compared to control, [b]$p<0.05$ compared between bFGF and PDB (1 way ANOVA with Duncan's multiple comparisons). In nearly all series, the effects of bFGF or aFGF did not reach to that of PDB.
N.D. = not determined.

Figure II-1: Process extension on poly-l-lysine (PL) by adult human OLs is enhanced in mixed versus purified culture. In the top panel, micrographs A to F are of O1+ cells where A, B and C are of purified OLs at 4, 11 and 32 days in vitro; frames D, E and F are of OLs in mixed culture at 6, 10 and 34 days in vitro respectively. Note that process extension is rapidly observed in OLs in mixed versus purified culture. Frames G (GFAP+ astrocytes) and H (Leu-M5+ microglia) show the other cell types in the mixed culture with OLs. In the bottom panel, the growth of processes of OLs is tabulated as a function of time in vitro and each value is mean $\pm$ SEM. The magnification of frames A to G is 100x while that of frame H is 250x.


A
B
C
D
E
F
G
H

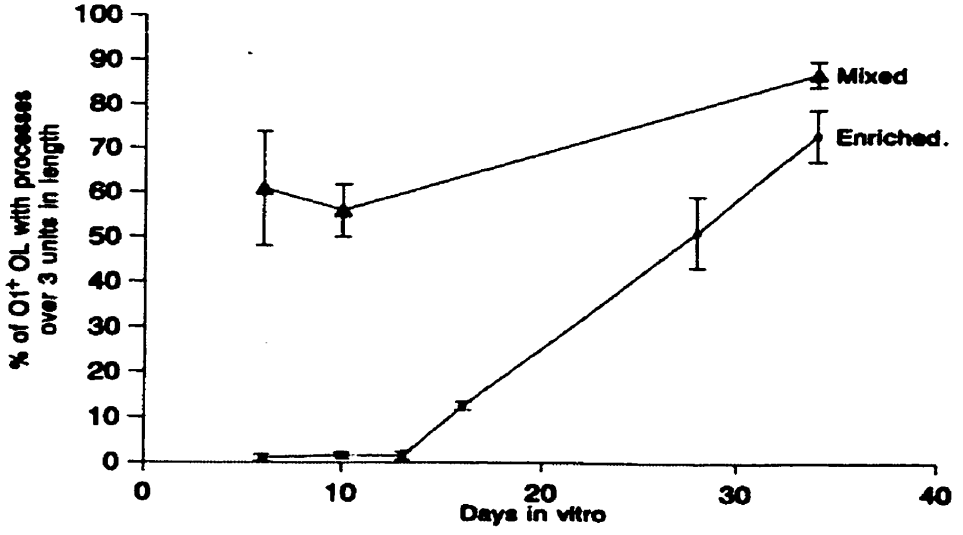
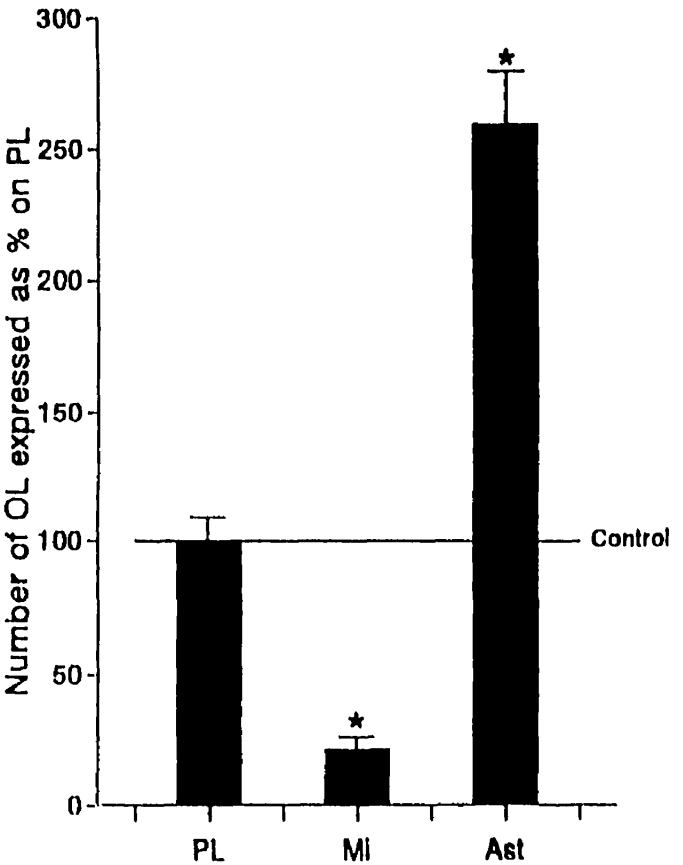

100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days in vitro
% of O1+ OL with processes over 3 units in length
Mixed
Enriched.

Figure II-2: Astrocytes promote while microglia decrease the health of OLs. Adult human OLs were seeded onto PL-coated coverslips containing adult human microglia (over 95% purity as assessed by KP-1 stainings) or fetal human astrocytes (over 95% GFAP+ cells); fetal cells were used to derive astrocyte monolayers since adult human astrocytes could not be obtained in large quantity or high purity for experimental purposes (see Yong and Antel, 1992). Cells were stained for O1 immunoreactivity after 11 days on the respective cell substrates. Each value is the mean $\pm$ SEM of 4 coverslips of cells. $^{*}p<0.05$ compared to controls on PL-coated coverslips (1 way ANOVA with Duncan's multiple comparisons, p at 0.05).

Survival



Differentiation

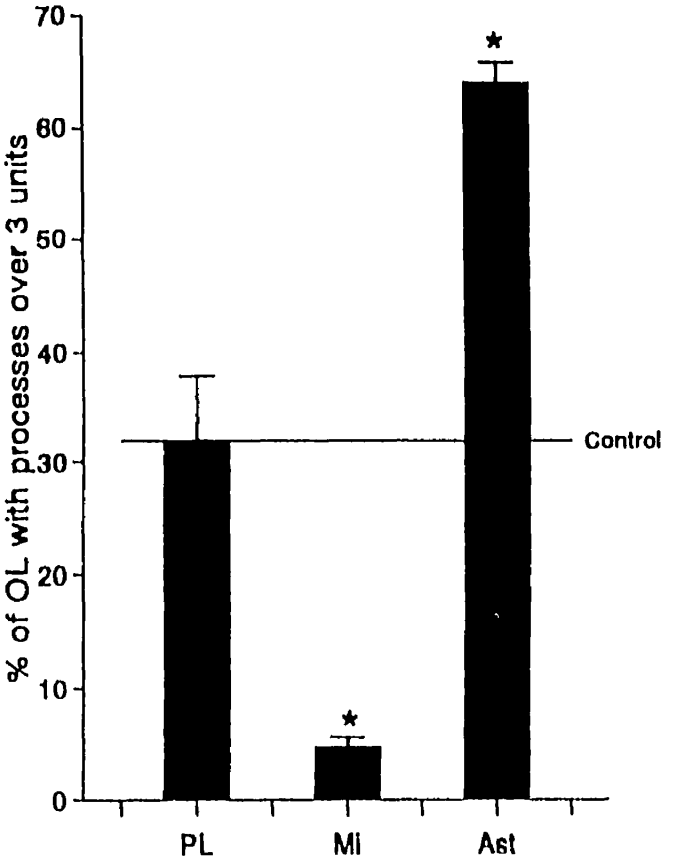


Figure II-3: Live astrocytes are equipotent with the phorbol ester, PDB, in enhancing process outgrowth by adult human OLs. OLs were seeded onto a monolayer of live fetal human astrocytes for 6 days, or onto PL. In the latter group, cells were treated with 50 ng/ml of PDB (twice, every 2 days, and harvested 2 days after the second treatment) or PBS vehicle (control).

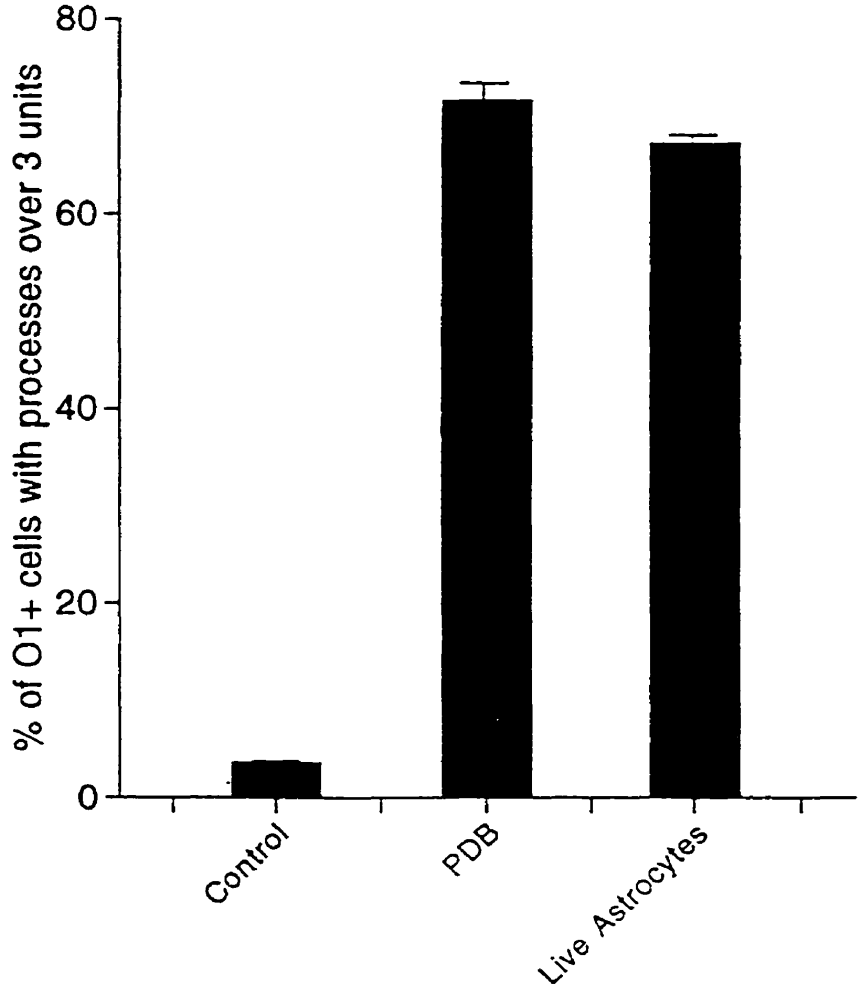
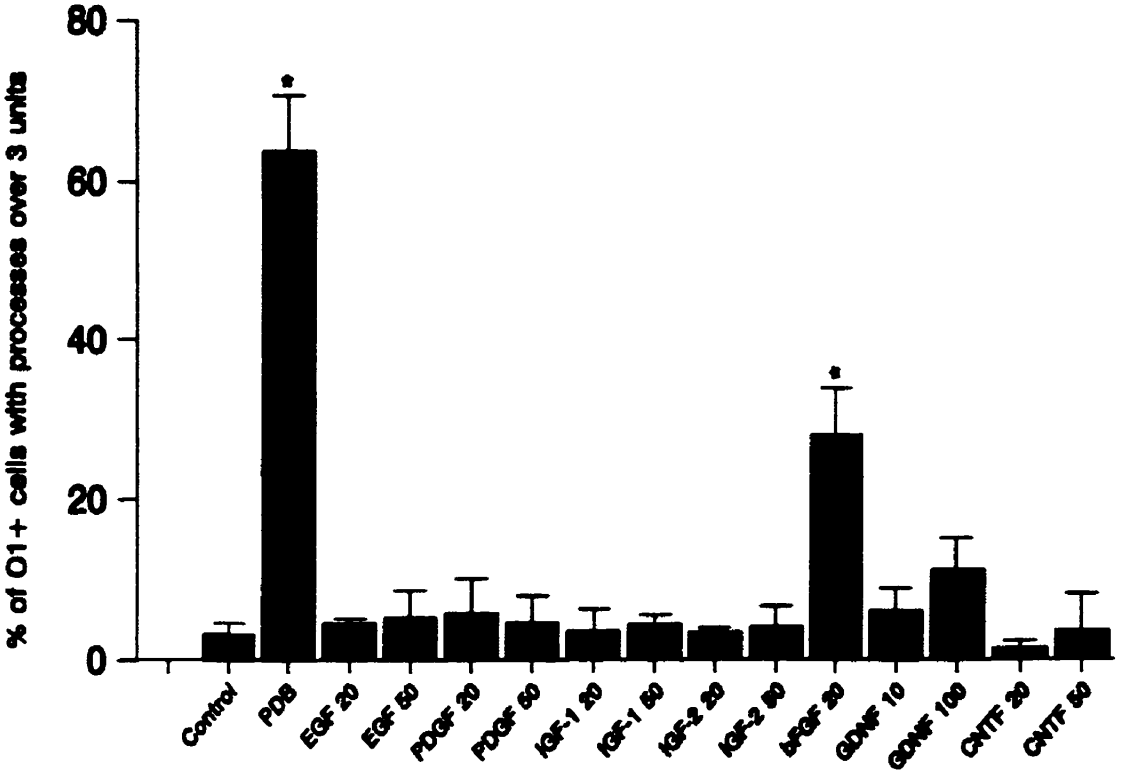

% of O1+ cells with processes over 3 units
80
60
40
20
0
Control
PDB
Live Astrocytes

Figure II-4: Of a range of growth factors tested, only bFGF and PDB (50ng/ml) significantly enhanced process extension by adult human OLs on PL. Cells were given 2 treatments with test agents (ng/ml concentrations), spaced 2 days apart, and then fixed and stained for O1 2 days after the second treatment. In the top panel, values are mean $\pm$ SEM of 4 coverslips of cells; on each coverslip, an average of 149 $\pm$ 4 O1+ OLs were analysed blind. $^{*}p<0.05$ compared to controls (1 way ANOVA with Duncan's multiple comparisons). In the bottom panel, O1 immunoreactivity of adult human OLs is shown for controls (A), PDB (B) and bFGF (C) at 6 days from the initiation of treatment.


% of O1+ cells with processes over 3 units
80
60
40
20
0
*
*
Control
PDB
EGF 20
EGF 50
PDGF 20
PDGF 50
IGF-1 20
IGF-1 50
IGF-2 20
IGF-2 50
bFGF 20
GDNF 10
GDNF 100
CNTF 20
CNTF 50

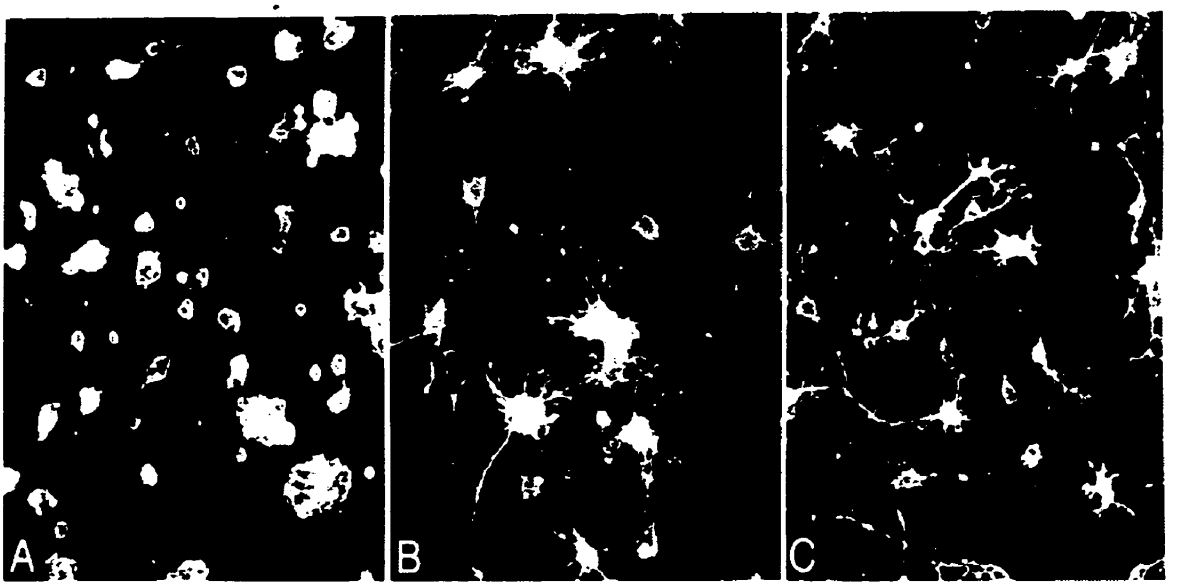
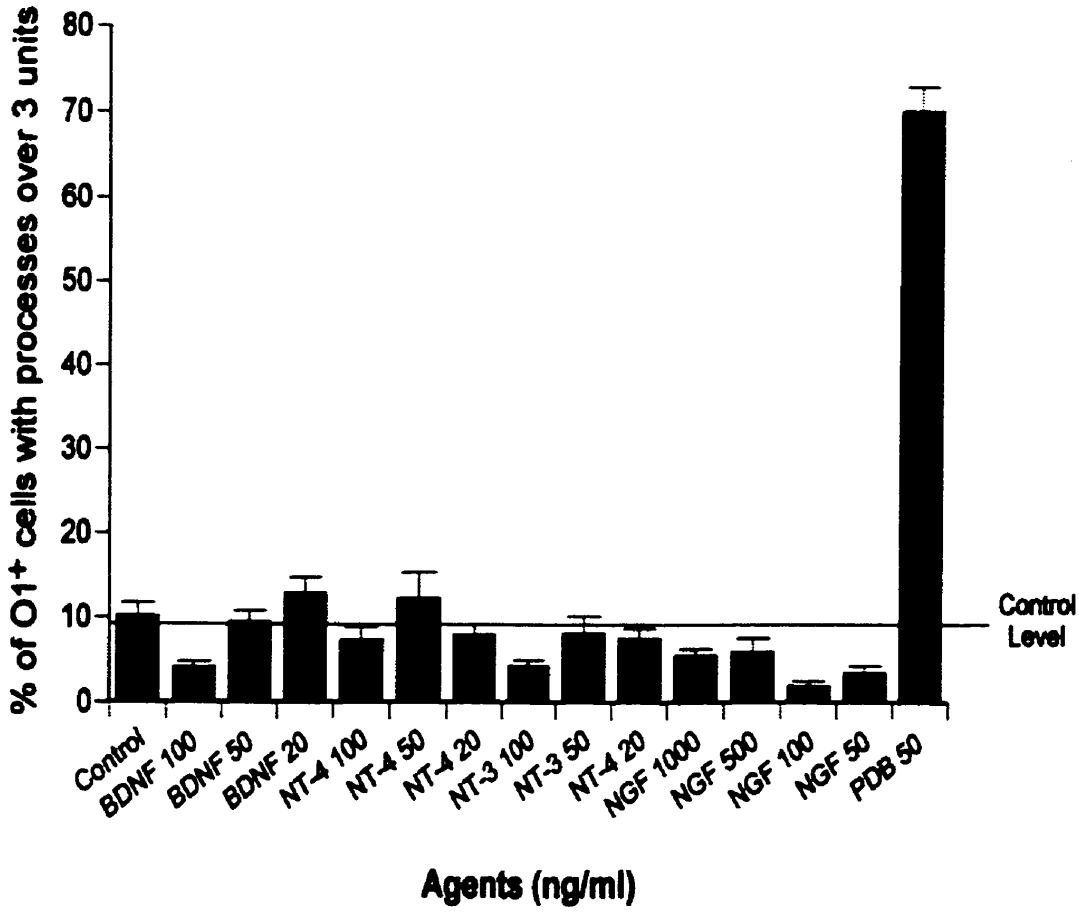

A
B
C

Figure II-5: Neurotrophins do not promote process extension by adult human OLs seeded onto a PL substrate. In the top panel, cells were treated every 2 days for 4 days and then harvested for immunohistochemistry 2 days following the second treatment with test agents (concentrations in ng/ml). Results are mean ± SEM of 4 to 11 coverslips of cells and have been pooled from 4 different experiments involving 4 human adult samples. In the lower panel, because of a previous report that NGF could promote process extension by adult bovine OLs (Althaus et al., 1992), we subjected PC12 cells to NGF (5 to 500 ng/ml) to test its efficacy. The micrographs of cells stained with toluidine blue demonstrate that NGF (from 5 ng/ml) promoted neurite formation by PC12 (B) when compared to controls (A); however, NGF did not promote process outgrowth by adult human OLs.


% of O1+ cells with processes over 3 units
80
70
60
50
40
30
20
10
0
Control Level
Control
BDNF 100
BDNF 50
BDNF 20
NT-4 100
NT-4 50
NT-4 20
NT-3 100
NT-3 50
NT-4 20
NGF 1000
NGF 500
NGF 100
NGF 50
PDB 50
Agents (ng/ml)

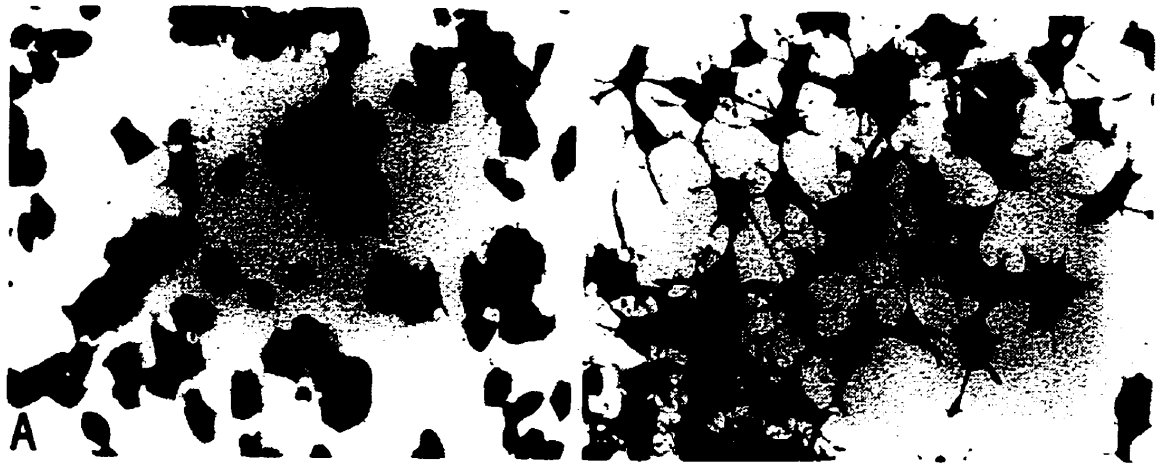

A

Figure II-6: Even at 100 ng/ml, the effect of bFGF on process extension does not match that of 50 ng/ml PDB. bFGF was renewed every 2 days when feeding medium was changed. Each value is the mean of 3 coverslips of cells; for clarity, SEM are not shown but these are less than 10% of the mean for the majority of points. On each coverslip, an average of 370 $\pm$ 5 O1+ cells were counted. All analyses were performed blind.

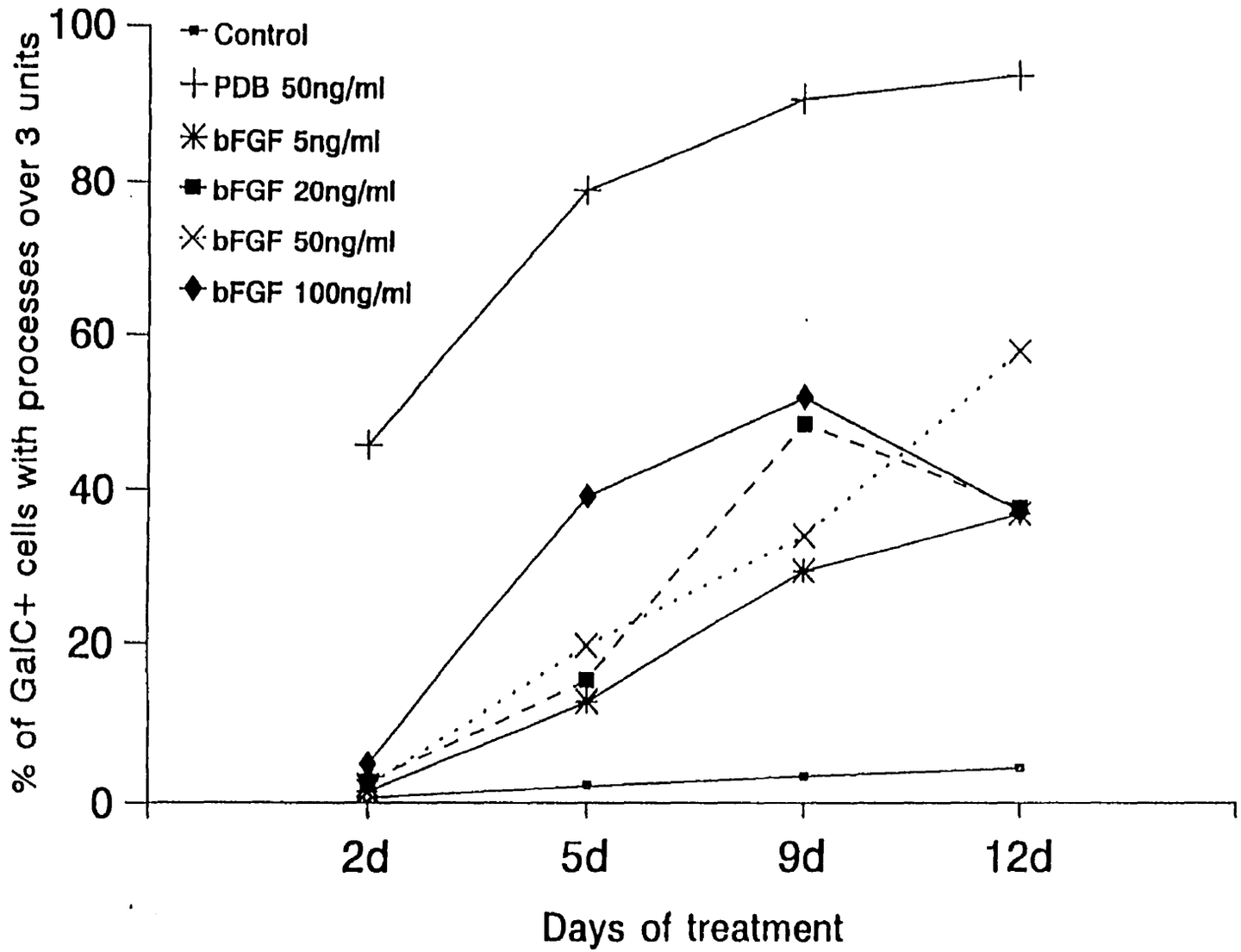
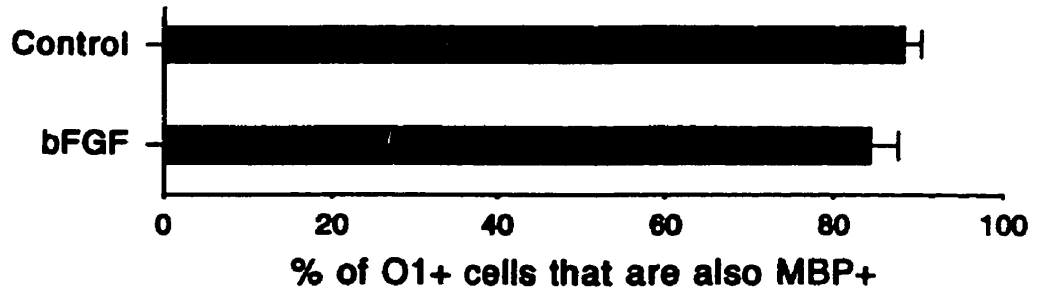

Control
PDB 50ng/ml
bFGF 5ng/ml
bFGF 20ng/ml
bFGF 50ng/ml
bFGF 100ng/ml
% of GalC+ cells with processes over 3 units
100
80
60
40
20
0
2d
5d
9d
12d
Days of treatment

Figure II-7: The maturity of adult human OLs is not altered by bFGF. Cells were incubated with 20 ng/ml bFGF as described in Figure II-4 and then double labeled for O1 and myelin basic protein (MBP). In the top panel, the % of O1+ cells that were also MBP immunoreactive was not statistically different between control and bFGF treatment (Student's t-test). In the lower panel, representative O1 (A) and MBP (B) double labeling is demonstrated, with panel C being the phase contrast view. In D-F, no MBP (E) could be found on O1+ cells (D) when the MBP antibody step was omitted from the staining protocol, indicating that the double labeling shown in A and B was not due to artifactual cross-over of fluorescence.


Control
bFGF
0
20
40
60
80
100
% of O1+ cells that are also MBP+

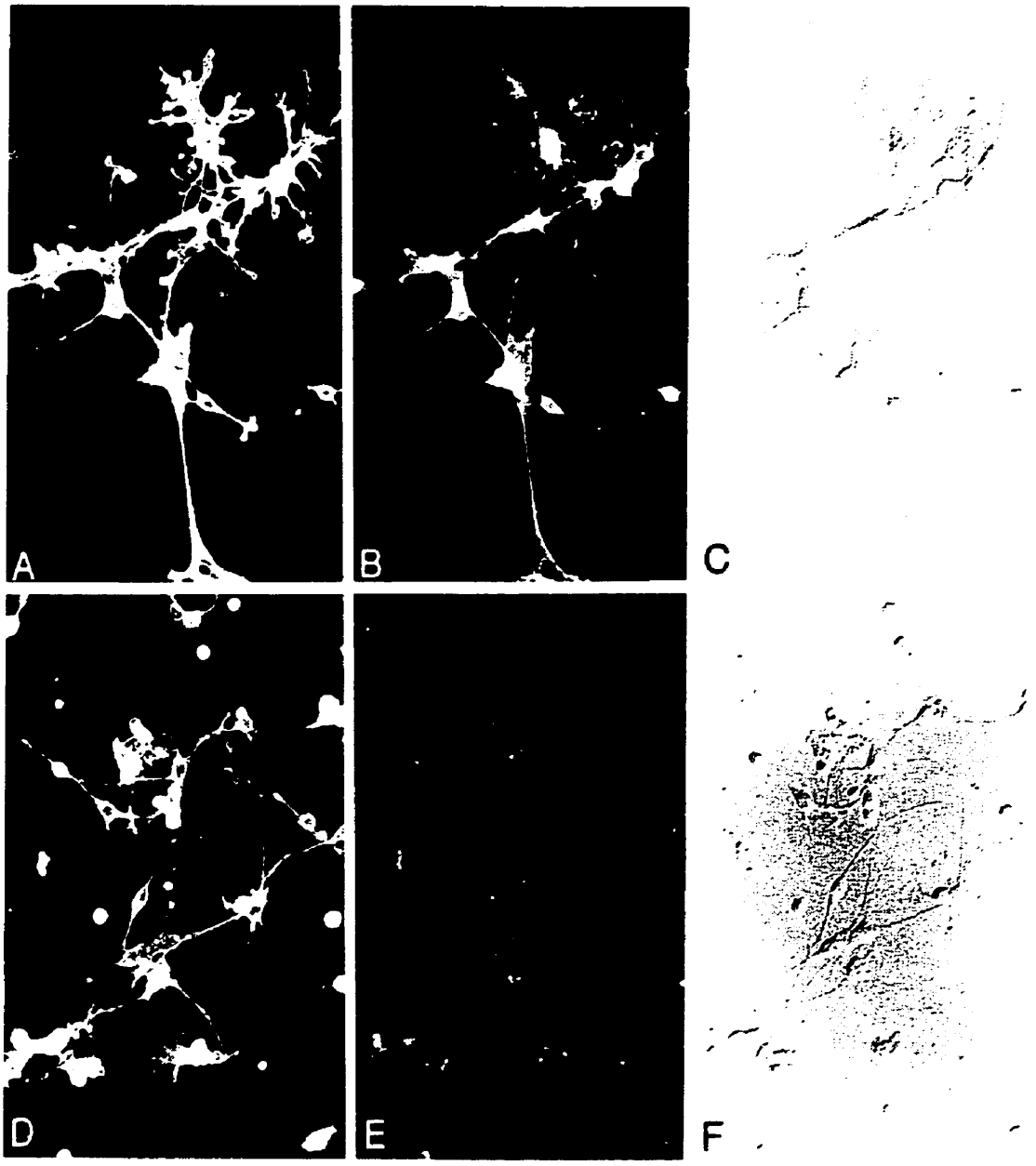

A
B
C
D
E
F

Figure II-8: The co-administration of bFGF with other growth factors did not result in synergy and did not cause process extension to approximate that induced by PDB. Values are mean $\pm$ SEM of 4 coverslips of cells on PL; on each coverslip, 167 $\pm$ 3 O1+ cells were counted on average. All concentrations are of 20 ng/ml except for NGF (500 ng/ml). All specimens were coded for analyses. All values have been normalized to that of bFGF, which caused 23.3% of O1+ cells to have processes over 3 soma unit, compared to 5.3% for untreated controls. $^{*}p<0.05$ compared to bFGF alone (1 way ANOVA with Duncan's multiple comparisons).

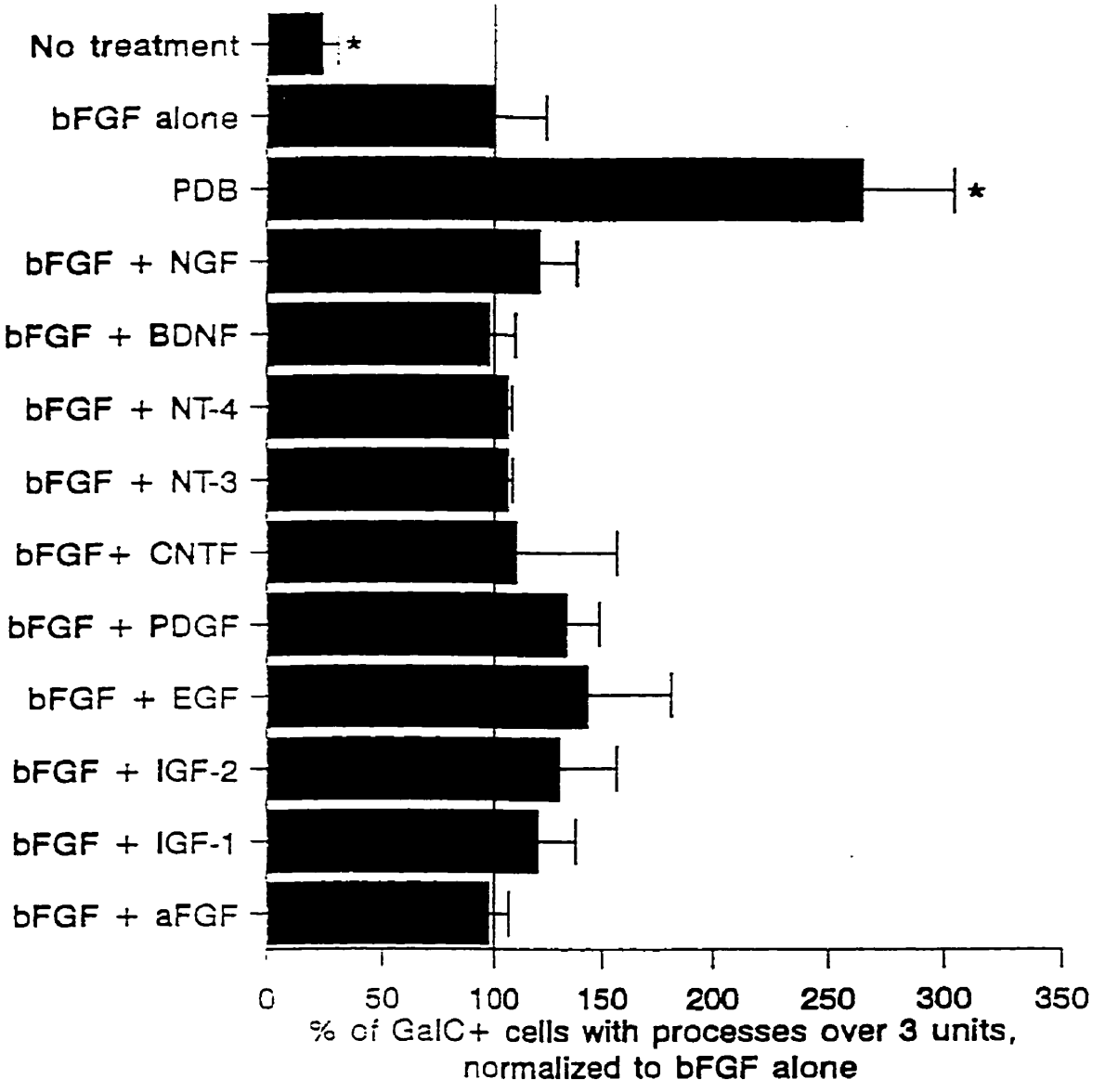

No treatment
bFGF alone
PDB
bFGF + NGF
bFGF + BDNF
bFGF + NT-4
bFGF + NT-3
bFGF+ CNTF
bFGF + PDGF
bFGF + EGF
bFGF + IGF-2
bFGF + IGF-1
bFGF + aFGF
*
*
0
50
100
150
200
250
300
350
% of GalC+ cells with processes over 3 units,
normalized to bFGF alone

Figure II-9: A neutralising antibody to bFGF (αbFGFI) dose-dependently reduced the process-promoting effects of live astrocytes, when compared to a non-neutralising bFGF antibody (αbFGFII) or an isotype control (IgG1). All concentrations of antibodies are in μg/ml. $^{*}p<0.05$ compared to control OLs on astrocytes not given antibody treatment (1 way ANOVA with Duncan's multiple comparisons, p at 0.05).

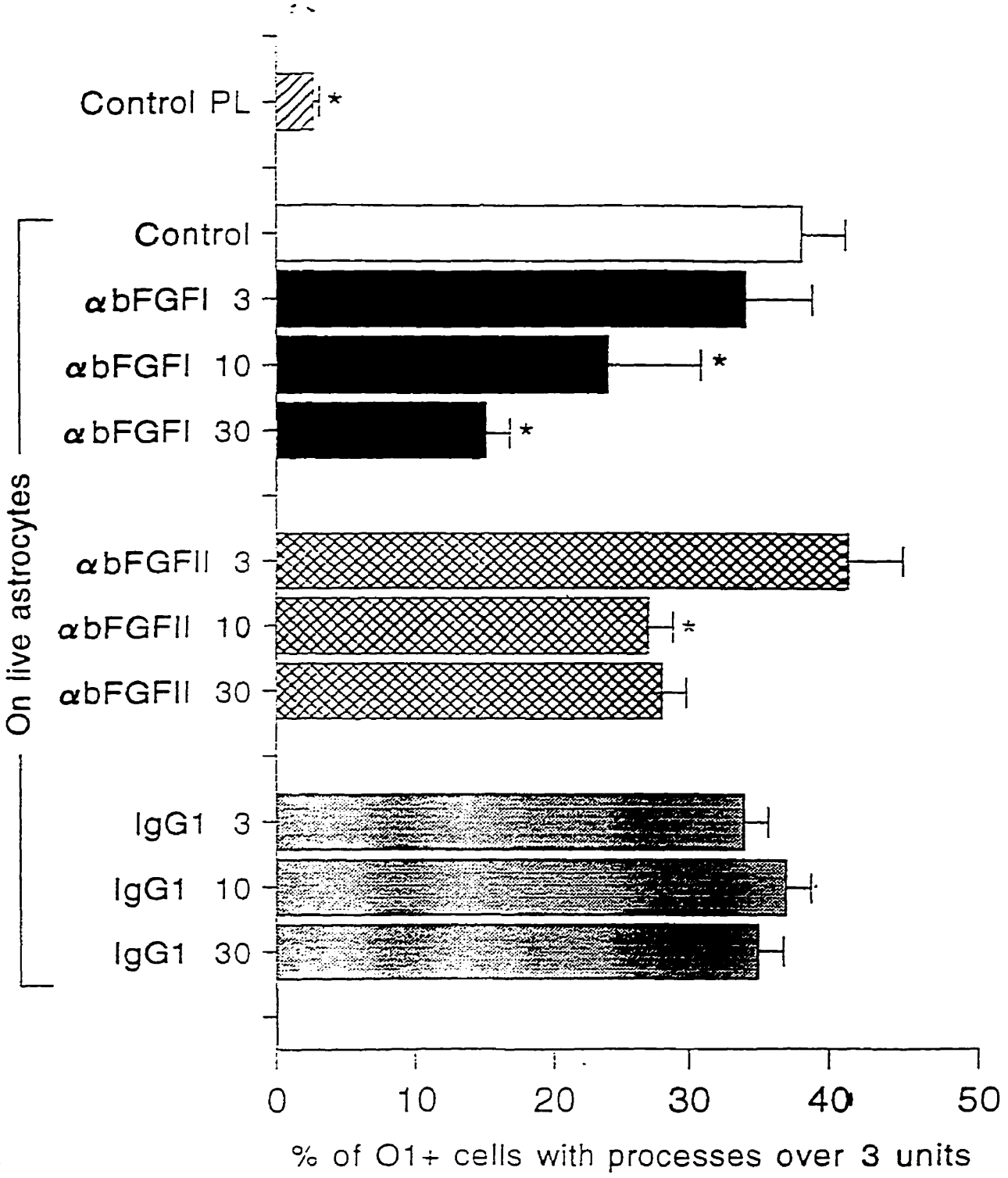

Control PL
*
Control
αbFGFI 3
αbFGFI 10
*
αbFGFI 30
*
αbFGFII 3
αbFGFII 10
*
αbFGFII 30
IgG1 3
IgG1 10
IgG1 30
On live astrocytes
0
10
20
30
40
50
% of O1+ cells with processes over 3 units

Figure II-10: The medium conditioned by astrocytes did not promote oligodendroglial process extension. Cells were incubated with astrocyte conditioned medium (CM) for a total of 4 days, with resupplementation of CM after 2 days. CM used was either unfractionated, or was subfractionated by Amicon filters with 10 or 30 kDa molecular size cutoff. In some cases, the CM was used at 50 or 100 x concentration. bFGF, with molecular weight of about 16 kDa, is expected, if present, to be contained within the 10 - 30 kDa fraction.

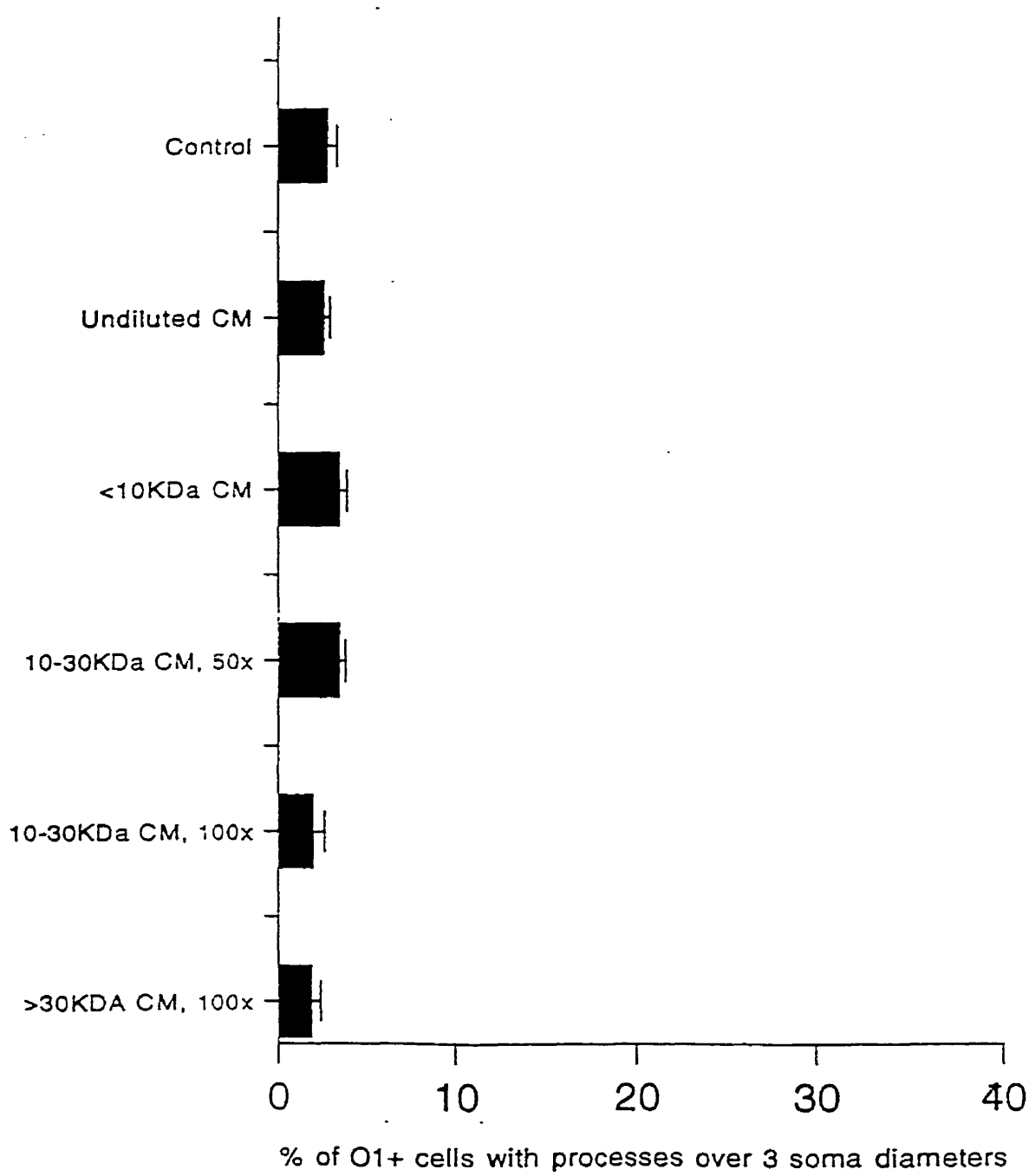

Control
Undiluted CM
<10KDa CM
10-30KDa CM, 50x
10-30KDa CM, 100x
>30KDA CM, 100x
0
10
20
30
40
% of O1+ cells with processes over 3 soma diameters

Figure II-11: The potency of live astrocytes in promoting oligodendroglial process outgrowth is matched by a combination of bFGF and astrocyte ECM (ASM). This figure shows 2 different batches of adult human OLs on 2 different series of fetal human astrocytes. In each case, bFGF (20 ng/ml, added twice every 2 days) and ASM produced sub-optimal process extension, but these synergized to attain the efficacy of live astrocytes.

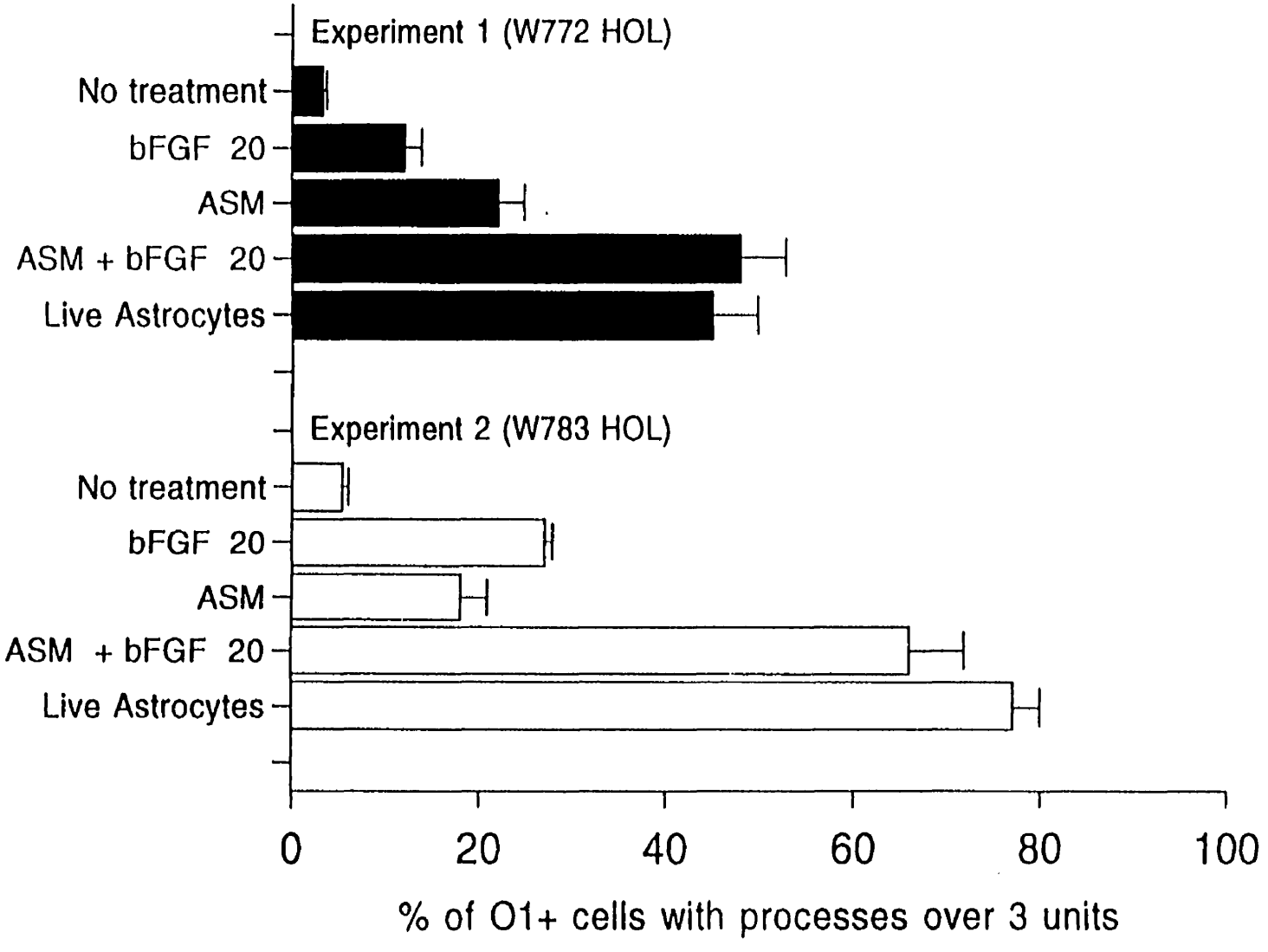

Experiment 1 (W772 HOL)
No treatment
bFGF 20
ASM
ASM + bFGF 20
Live Astrocytes
Experiment 2 (W783 HOL)
No treatment
bFGF 20
ASM
ASM + bFGF 20
Live Astrocytes
0
20
40
60
80
100
% of O1+ cells with processes over 3 units

Figure II-12: The astrocyte ECM (ASM) contains fibronectin (FN), vitronectin (VN) and laminin (LN). 30 μg protein of ASM is used in each case. The approximate molecular weights were, respectively, 220 kDa for fibronectin, 66 kDa for vitronectin and 200 kDa for laminin.

FN Standards
0.05 µg
0.02 µg
0.01 µg
ASM 30 µg
VN Standards
5 µg
2.5 µg
1 µg
0.5 µg
ASM 30 µg
LN standards
5 µg
2.5 µg
1 µg
0.5 µg
ASM 30 µg

Figure II-13: Purified fibronectin (FN), vitronectin (VN), laminin (LN) and heparan sulfate proteoglycan (HSPG) by themselves do not enhance oligodendroglial process extension; however, laminin and fibronectin, but not HSPG or vitronectin, potentiated the effects of 20 ng/ml bFGF. The purified ECM components were incubated onto 10 μg/ml PL coverslips for 2 h at 37°C as per manufacturer's instructions. The concentrations of ECM components tested (in μg/ml) are in the range that is recommended by their respective manufacturers. Purified ECM components by themselves did not elicit process extension that is statistically different from that of control PL substrate (1 way ANOVA with Duncan's multiple comparisons, $p<0.05$). The effect of ECM molecule + bFGF was compared to that of bFGF alone, with $^{*}p<0.05$. The morphology of processes in all cases was that of live astrocytes, i.e. non-membranous sheets.

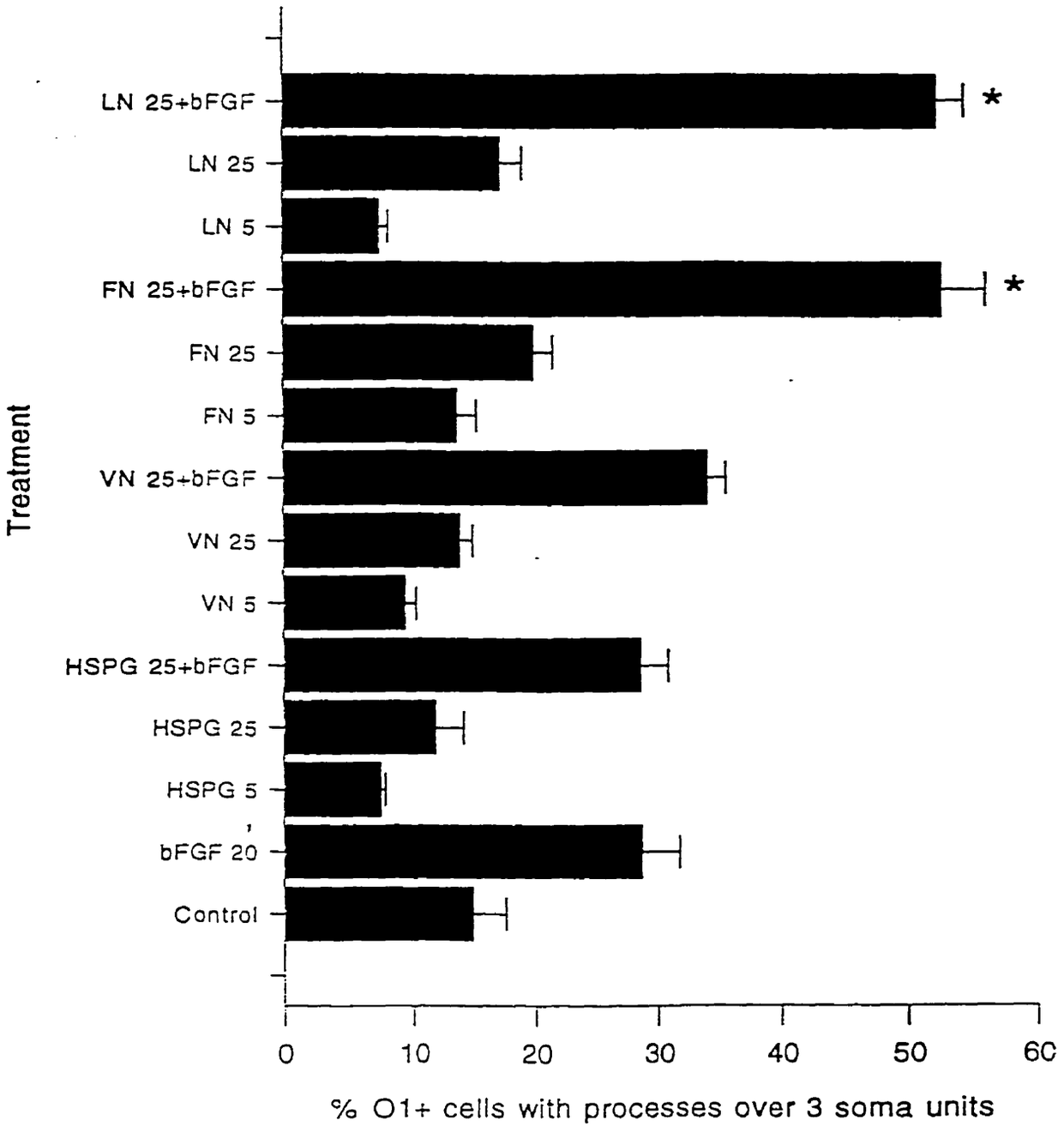

LN 25+bFGF
LN 25
LN 5
FN 25+bFGF
FN 25
FN 5
VN 25+bFGF
VN 25
VN 5
HSPG 25+bFGF
HSPG 25
HSPG 5
bFGF 20
Control
*
*
Treatment
0
10
20
30
40
50
60
% O1+ cells with processes over 3 soma units

Figure II-14: While astrocytes promote oligodendroglial process outgrowth, live astrocytes do not allow morphological maturation (membranous sheets in H) while the astrocyte ECM does. Cells were plated either on poly-l-lysine (A), live astrocytes (D) or astrocyte ECM (H) for 4 days. A, D and H are O1 immunoreactivity and their corresponding phase contrast and nuclear yellow frames are, respectively, B,E,I and C,F and J. Frame G demonstrates the corresponding GFAP astrocyte layer that the O1 cells in D were on.

A
B
C
D
E
F
G
H
I
J
K
L

In chapter II, I demonstrated that astrocytes promote process outgrowth by OL through the interaction of bFGF and astrocyte ECM. Previous studies showed that process extension by OLs is mediated by PKC (Yong et al., 1994). If PKC is central to the signal transduction cascade leading to process formation by OLs, then the effect of bFGF and astrocyte ECM should also involve PKC. In chapter III, the involvement of PKC in the bFGF- and astrocytic ECM- enhanced process formation by OLs is addressed by using selective inhibitors of PKC.

CHAPTER III

The Promoting effects of bFGF and Astrocyte Extracellular Matrix on Process Outgrowth by Adult Human Oligodendrocytes is mediated by Protein Kinase C

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Published in *Brain Research* 757:236-244 (1997)

Abstract

Process extension by oligodendrocytes (OLs) is a critical early step in myelin formation. We have previously reported that the basal- or phorbol ester- enhanced process outgrowth by adult human OLs is mediated by oligodendroglial protein kinase C (PKC). Recently, we demonstrated that astrocytes facilitated process outgrowth by adult human OLs through the interaction between astrocyte-derived basic fibroblast growth factor (bFGF) and astrocyte extracellular matrix (ECM). If PKC is central to the signal transduction cascade that leads to process formation by OLs, then the effects of bFGF and astrocyte ECM should also involve PKC. In the current study, we have addressed the involvement of PKC in the bFGF- and astrocyte ECM- enhanced process formation by adult human OLs by using a selective inhibitor of PKC, calphostin C. The results show that calphostin C dose-dependently reduced process extension elicited by bFGF and astrocyte ECM, at IC_{50} concentrations of 24.5 and 26.6 nM, respectively. At the concentrations of calphostin C that inhibited process extension by adult human OLs, necrosis (measured by lactate dehydrogenase release) and apoptosis (determined by using a fluorescent terminal deoxynucleotidyl transferase assay) of OLs did not occur. Finally, we demonstrate that another specific inhibitor of PKC, CGP 41 251, also reduced process formation that is elicited by bFGF and astrocyte ECM. Thus, all process-extending agents for adult human OLs identified to date signal through PKC, further implicating PKC of OLs as being central to the production of process extension, an early event in myelinogenesis.

Introduction

In the central nervous system, oligodendrocytes (OLs) wrap and compact around axons with their processes, forming myelin which allows for saltatory conduction of nervous impulses. Impairment of OLs and/or myelin causes a spectrum of conduction irregularities with eventual symptoms of neurological dysfunction. The human demyelinating disease, multiple sclerosis (MS), is characterized neuropathologically by a considerable loss of myelin with relative sparing of axons. However, substantial number of OLs are found at the edge of lesions, suggestive of replenishment of OL numbers (Raine et al., 1981; Prineas et al., 1989; 1993; Raine and Wu, 1993). The presence of such OLs, together with the relative sparing of axons in the disease, indicates the potential for remyelination, and indeed, remyelination does occur in MS although incomplete in its extent (reviewed in McLaurin and Yong, 1995). To enhance the extent of remyelination in MS or other demyelinating disorders, appropriate stimuli may be necessary; we have suggested that one such approach would be to promote process extension by surviving or newly-formed OLs, since these processes are the precursors of myelin. Understanding the mechanisms by which the OL extends its processes also has relevance to developmental myelination, since process extension is a critical and early event in normal myelin formation.

Using OLs in culture, we and others have suggested that process outgrowth by OLs requires the stimulation of oligodendroglial protein kinase C (PKC) activity

(Althaus et al., 1990; 1991; Yong et al., 1991; 1994). By determining the isozymes of PKC that are expressed in OLs, and by using phorbol ester derivatives that have preferential actions on specific isoforms of PKC, PKC α appears to be important in mediating the signal transduction that leads to process formation by adult human OLs (Yong et al., 1994). Furthermore, measurements of PKC enzyme activity of adult human OLs demonstrate that, similar to many cell types, PKC translocates from the cytosol to the particulate fraction of OLs following treatment with phorbol esters. However, unlike other cell types, the elevated PKC enzyme activity is maintained for long periods (up to 12 days analyzed) and does not undergo downregulation (Yong et al., 1994). Collectively, the evidence implicates PKC of OLs as an important pathway for process formation, at least when phorbol esters are used as pharmacological activators of PKC.

As phorbol esters are exogenous compounds, and in order to identify physiological activators of oligodendroglial PKC, we have screened other CNS cell types, and the large number of growth factors that they produce, for their ability to stimulate process extension by adult human OLs. We have found that astrocytes promote process outgrowth by adult human OLs and that the mechanism involves the interaction between astrocyte-derived basic fibroblast growth factor (bFGF, FGF-2) and astrocyte extracellular matrix (ECM) (Oh and Yong, 1996). The finding of efficacy of bFGF and astrocyte ECM places these, along with phorbol esters, as the only three agents thus far identified that can stimulate process extension by adult human OLs. If

PKC is vital to the signal transduction cascade that leads to process formation by OLs, then, as with phorbol esters, the effects of bFGF and astrocyte ECM should also involve PKC of OLs.

In the current study, we have used calphostin C, a selective inhibitor of PKC activity (Kobayashi et al., 1989; Basu, 1993), to address whether the process extension by adult human OLs treated with bFGF and astrocyte ECM is mediated by PKC. The results implicate PKC signal transduction as being central to process outgrowth by adult human OLs.

Abbreviations: OLs, oligodendrocytes; PDB, 4β -phorbol-12,13-dibutyrate; bFGF, basic fibroblast growth factor; ECM, extracellular matrix; PL, poly-l-lysine; PKC, protein kinase C; LDH, lactate dehydrogenase; TdT, terminal deoxynucleotidyl transferase; CP, calphostin C.

Materials and Methods

Cell culture

Adult human glial cells were derived from brain biopsy specimens from patients who underwent surgical resection to ameliorate drug intractable epilepsy. Samples were of areas adjacent to, but not containing, the epileptic focus. Dissociated cells were obtained using trypsin digestion and percoll gradient centrifugation and this protocol is described in detail elsewhere (Yong and Antel, 1992).

The initial isolate of dissociated cells, in our experience with over 400 adult human samples, contains on average, approximately 40% OLs, 40% microgliaand 20% astrocytes. To purify for particular cell types, dissociated cells were plated onto uncoated T-25 flasks. Since adult human OLs were poorly adherent in contrast to astrocytes and microglia, the floating cells were collected the next day and, when subjected to another round of differential adhesion, resulted in enriched OLs of over 90% purity (Yong and Antel, 1992). These purified OLs were plated at a density of 10^4 cells per well on poly-l-lysine (PL) coated 16 well chambers (Lab-Tek 16 well chamber slides, Nunc, Inc., Naperville, IL), or onto astrocyte ECM prepared from fetal human astrocytes. Although it would have been ideal to plate OLs onto matrix prepared by adult human astrocytes, the low yield and purity (70% at best) of adult human astrocytes (Yong and Antel, 1992) precluded such an experiment; in previous work (Oh and Yong, 1996), we have observed that oligodendroglial process formation was promoted both in mixed culture containing adult human astrocytes and in fetal human

astrocyte culture. Thus, to obtain astrocyte ECM, fetal human astrocyte cultures were utilized in this study.

Fetal human neural cells were cultured from the brains of fetuses (14 to 20 weeks fetal age) which were obtained at therapeutic abortions. The use of these specimens are approved by local institutional ethnic committees. The procedure to purify fetal human astrocytes has been described (Yong et al., 1992), and, to obtain an astrocyte ECM, the protocol of Cardwell and Rome (1988) was modified as described (Oh and Yong, 1996). In brief, fetal human astrocytes were seeded at a high density of 25,000 cells per well of PL-coated chamber slide in order to achieve near confluency by 2 days post-plating. Four days after, cells were lysed with water for 2 hours at 37°C, with 3 changes of water during this period. Using a light microscope, the removal of the cell layer was verified before OLs were seeded. We have determined by western blot analyses that the astrocyte ECM contains laminin, fibronectin, and vitronectin, among others (Oh and Yong, 1996).

Feeding medium in all cases was Eagle's minimum essential medium supplemented with 5% fetal bovine serum, 20 μg/ml gentamicin, and 0.1% dextrose. All culture reagents were purchased from Gibco BRL.

Administration of testing agents

Unless otherwise stated, all test agents were introduced to OLs at 2 days post-plating onto PL or astrocyte ECM. Treatment was followed every 2 days with

replenishment of medium and cells were harvested 2 days following the second or third treatment. In experiments to address whether PKC inhibitors could affect the process-extending action of bFGF, PKC inhibitors were given to OLs 1 hour prior to bFGF treatment. In experiments that addressed whether the effects of astrocyte ECM was PKC-mediated, OLs were preincubated with PKC inhibitors for 1 hour at room temperature prior to seeding onto astrocyte ECM; the plating efficiency of OLs was not affected by this prior treatment with PKC inhibitors. These treatment schedules were necessited by the observation that while OLs seeded onto PL would require several days to extend processes, unless bFGF or phorbol esters were given, OLs seeded onto astrocyte ECM extended processes soon after their seeding onto astrocyte ECM (unpublished observation). Basic FGF was used at 20 ng/ml to stimulate process extension since we have previously determined that this is an optimal concentration (Oh and Yong, 1996). 4β-phorbol-12,13-dibutyrate (PDB) was used as a representative phorbol ester to stimulate process extension by OLs (Yong et al., 1994).

Inhibitors of PKC that were utilized for the current study were calphostin C and the staurosporine derivative, CGP 41 251. Calphostin C has high selectivity for PKC; the reported IC_{50} is 50 nM for PKC while >50 μM is its IC_{50} for other enzymes including protein kinase A and other tyrosine kinases (Kobayashi et al., 1989). CGP 41 251 is less potent than staurosporine but has a much higher selectivity for PKC than staurosporine itself; the IC_{50} of CGP 41 251 for PKC is reported to be in the nM range

while its IC_{50} for protein kinase A and the EGFR-linked tyrosine kinase is over 2 μM (Meyer et al., 1989).

Immunocytochemistry and assessment of extent of process formation

Live cells in the chamber slides were fixed with 4% paraformaldehyde for 15 min and incubated with hybridoma supernatant containing a mouse monoclonal antibody designated O1 (Sommer and Schacher, 1981), which recognizes galactocerebroside (GalC) and related lipids (monogalactosyldiglyceride and psychosine) which are specific to OLs (Bansal et al., 1989). Following 45 min incubation at room temperature, goat anti-mouse immunoglobulin conjugated to rhodamine (1:150 dilution, Cappell, Lexington, MA) was added for another 45 min. Cells were then incubated with Hoescht dye to label nuclei. The whole chamber slide was coverglassed with gelvatol. Using an immunofluorescence microscope, the percentage of GalC + (i.e. O1+) cells with processes longer than 3 soma diameters in length was tabulated as previously described (Yong et al., 1991; 1994).

LDH assay

To determine whether the observed effect of calphostin C on process formation was due or not to necrotic injury to cells, the colorimetirc/spectrophotometric method for the determination of lactate dehydrogenase (LDH) released into the culture medium from injured cells was performed as previously described (McLaurin and Yong, 1995).

In brief, the culture medium is incubated with pyruvic acid for 30 min at 37°C. LDH in the culture medium converts the substrate pyruvic acid to lactic acid. The amount of pyruvic acid remaining after the incubation is inversely proportional to the amount of LDH activity in the medium collected. Hydrazone reagent is then used to interact with the pyruvic acid resulting in a colored hydrazone compound. This colored reagent is measured by colorimeter/spectrophotometer and LDB activity is calculated in B-B U/ml (Berger-Broida) according to instruction by the manufacturer (Sigma).

OLs were treated with calphostin C and medium was collected after 24 h for LDH analyses.

TdTassay

To determine whether calphostin C in the concentrations used was reducing process extension because of the induction of apoptosis, a fluorescent method was employed to detect fragmented DNA characteristic of apoptosis. Two days after the 3rd treatment with calphostin C in concentrations of 10 to 50 nM, OLs were fixed in acetone: methanol (1:1) for 20 minutes at -20°C and rehydrated with PBS at 4°C. Cells were then incubated at 37°C for 30 minutes in the following: 0.3 µl of terminal deoxynucleotidyl transferase (TdT) enzyme (Promega), 0.3 µl of biotin-dUTP (Boehringer Mannheim), 0.6 µl of 5X TdT reaction buffer and ddH_2O to make up 30 µl total volume per chamber. Cells were washed and incubated in streptavidin - FITC (1:100) for 30 minutes. Subsequently, incubation with propidium iodide (100 ng/ml) for

10 minutes followed. Chamber slide was mounted using Gelvatol and analyzed under fluorescence microscopy. Apoptotic cells containing 3' hydroxyl termini on fragmented DNA acted as substrates for TdT. Normal cells with a low number of 3' hydroxyl DNA ends were not fluorescent by this technique.

Statistical analyses

Because multiple treatments, or multiple concentrations of each treatment, were used in all experiments, a one way analysis of variance (ANOVA), and Bonferroni method were used for statistical comparison.

Results

Calphostin C inhibits the effects of bFGF, astrocyte ECM, and PDB on process formation by adult human OLs.

In previous work (Yong et al., 1991), we have determined that the process-promoting action of PDB could be blocked by two relatively non-selective inhibitors of PKC, staurosporine and chelerythrine, and by the relatively selective PKC inhibitor, CGP 41 251. In the present study, we demonstrate that calphostin C, a selective PKC inhibitor, attenuated the promoting effects of bFGF, PDB and astrocyte ECM on process formation by adult human OLs (Fig. III-1,-2 and -3). Figure III-1A shows process extension by adult human OLs when they were treated with bFGF or PDB, or when cultured on astrocyte ECM; the administration of calphostin C inhibited this response in a dose-dependent manner (Fig. III-2 and -3). The calculated IC_{50} for calphostin C on attenuating the action of PDB and bFGF is 20.5 nM and 24.5 nM respectively, and this corresponds well to the reported IC_{50} of calphostin C for PKC of 50 nM (Kobayashi et al., 1989).

Astrocyte ECM could also promote process extension by OLs when compared to cells seeded on PL. Calphostin C inhibited the effect of astrocyte ECM (Fig. III-3) with a calculated IC_{50} of 26.6 nM.

Inhibitory effects of calphostin C on process outgrowth is not due to necrosis of adult human OLs.

In order to determine whether calphostin C was producing necrotic death to OLs, which would thus account for its inhibitory effect on process extension, LDH assays were carried out. Figure III-4 shows that, following the incubation of OLs with 10 to 50 nM calphostin C, LDH levels in the culture medium did not differ from that of untreated OLs.

Calphostin C - treated OLs were also examined using light microscopy. There was no apparent observable disruption of membranes of calphostin C - treated OLs from that of untreated OLs; cells remained phase bright and the cell soma was round shaped. In contrast, OLs treated with the membrane detergent digitonin (50 μg/ml), used as a positive control for LDH release, had disrupted and fragmented membranes using light microscopy, and LDH release into the culture medium was almost 2-fold higher than that of controls (Fig. III-4).

Inhibitory effects of calphostin C on process outgrowth is not due to apoptosis of adult human OLs.

The possible apoptotic effect of calphostin C on adult human OLs was also examined, by the TdT assay, to determine whether this gave rise to the observed decrease in process formation by OLs. Figure III-6 show that, within the concentration range used to inhibit process extension by the OLs, there was no induction of apoptosis of OLs by calphostin C. However, higher concentrations of

calphostin C (>100nM) was able to induce a significant number of cells to undergo apoptosis (Fig. III-5 and -6).

CGP 41 251 decreases the effects of bFGF and astrocyte ECM on process extension by adult human OLs.

To support the calphostin C - derived results that the promoting effects of bFGF and astrocyte ECM were mediated by PKC, another selective PKC inhibitor, CGP 41 251, a staurosporine derivative, was also tested. Figure III-7 demonstrates that the promoting effect of bFGF on process extension by OLs was significantly reduced by CGP 41 251 treatment at 500 nM concentration, while the effect of astrocyte ECM was significantly inhibited at 1000 nM concentration of CGP 41 251.

Discussion

Previous work from this laboratory (Yong et al., 1991; 1994), using phorbol esters as pharmacological ligands, has suggested that oligodendroglial process extension is mediated by PKC. Recently, focusing on endogenous compounds in order to identify physiological stimulators of oligodendroglial process outgrowth, we determined that astrocytes promoted process extension by adult human OLs in vitro; this astroglial effect involved the interaction between astroglial-derived bFGF and astrocyte ECM (Oh and Yong, 1996). In the current study, the central importance of oligodendroglial PKC in mediating process outgrowth was examined by addressing whether the effect of all agents known to date to enhance process outgrowth (i.e. PDB, bFGF and astrocyte ECM) could be affected by a selective inhibitor of PKC, calphostin C. Calphostin C has been shown to act at the diacylglycerol-binding site of the C1 domains of PKC (Basu, 1993); since this site is unique to PKC among kinases, calphostin C has high selectivity for PKC (Kobayashi et al., 1989).

The results here reveal that the biological action of bFGF and astrocyte ECM in evoking process formation by OLs involves PKC, since process extension caused by these growth factors was blocked by calphostin C at IC_{50} concentrations that correspond to the reported IC_{50} of calphostin C for PKC. Furthermore, process outgrowth induced by bFGF and astrocyte ECM was blocked by another selective inhibitor of PKC, CGP 41 251.

Basic FGF is known to bind at the cell surface to its receptor, resulting in receptor dimerization and activation of the receptor tyrosine kinase. While this stimulation of tyrosine kinase activity is one of the early effects of bFGF, the subsequent production of diacylglycerol (DAG), through hydrolysis of phosphatidyl inositol bisphosphate, can stimulate PKC activity. Oury et al., (1992) reported that bFGF enhanced the production of DAG in rat granulosa cells. That bFGF treatment can stimulate PKC activity has been shown for endothelial cells, where PKC is thought to mediate the mitogenic and angiogeniceffects of bFGF (Presta et al., 1989; Hu and Fan, 1995; Kent et al., 1995). Again in endothelial cells, PKC is thought to mediate the bFGF protection against radiation-induced apoptosis (Haimovitz-Friedman et al., 1994). Neurite outgrowth that is enhanced by growth factors including bFGF and insulin-like growth factor-1 is thought to be mediated through a PKC-dependent pathway (Roivainen et al., 1995; Fagerstrom et al., 1996); a body of evidence suggests that PKC plays a major role in neurite outgrowth (Leli et al, 1992; Aigner et al., 1995; Shea et al., 1995). For OLs, the application of bFGF is thought to increase PKC activity which leads to the proliferation or differentiation of OLs (Bhat et al., 1992; Deloulme et al., 1992; Radhakrishna and Almazan, 1994).

In order to determine the possible toxicity of calphostin C on OL, which may thus account for its effect on oligodendroglialprocess extension, necrotic and apoptotic effects of calphostin C were analyzed by LDH and TdT assays, respectively. In Figures III-4 and -6, there was no significant toxicity of calphostin C in the range of concentrations used to inhibit PKC activity and oligodendroglialprocess extension. In

addition, using light microscopy, no apparent membrane disruption of cells was observed with the treatment of calphostin C. With higher concentrations of calphostin C (>100nM), however, significant numbers of OLs underwent apoptotic death. In this regard, a large literature exists which indicates that increasing PKC activity, using phorbol esters for example, suppresses apoptosis while inhibitors of PKC induce apoptotic death to cells (Lotem et al., 1991; Haimovitz-Friedman et al., 1994; Javis et al., 1994). We have observed that antisense oligonucleotides to PKC α induce apoptosis to glioma cells (Dooley et al., submitted). Thus, the results of high concentrations of calphostin C (>100nM) inducing apoptosis of oligodendrocytes support the findings that interference with PKC will eventually lead to apoptosis. Nonetheless, at the concentrations of calphostin C that inhibited oligodendroglial process extension, apoptosis was not observed.

The role of PKC in regulating myelin formation has been suggested from other lines of evidence. Firstly, PKC activity is known to be developmentally regulated where PKC enzyme levels increase gradually at birth and then peak between 14 and 28 days postnatally in the rodent brain (Shoyab et al, 1976; Hashimoto et al., 1988), a period which correlates that with of in vivo developmental myelination of rodent brain. Furthermore, Yoshimura et al., (1992) demonstrated that CNS PKC enzyme levels increase from postnatal 14 to 42 days in parallel with the deposition of myelin proteins. The activation of PKC in cultured OLs has been also reported to result in phosphorylation of myelin basic protein (MBP) (Vartanian et al., 1986) and 2',3'-cyclic

nucleotide 3'-phosphodiesterase (Vartanian et al., 1992), which are considered to be essential myelinogenic events (Sternberger et al., 1978; Roach et al., 1983; Shine et al., 1992). Anderson et al. (1994) have shown that PKC is involved in the cAMP regulation of MBP gene expression. Our previous reports, that phorbol esters increase process extension by the OL, an early step in myelin formation, also support the involvement of PKC in myelination (Yong et al., 1988; 1991; 1994). Finally, the current results, demonstrating that selective PKC inhibitors block the oligodendroglialprocess extension that is elicited by all identified growth factors with this property, provide strong evidence for the involvement of PKC in regulating myelin development.

Acknowledgements

We thank the Multiple Sclerosis Society of Canada for support of operating funds. Luke Y.S. Oh was a recipient of a studentship from the Multiple Sclerosis Society of Canada. We thank Ciba Geigy Basel, Switzerland, for the generous gift of CGP 41 251.

Figure III-1: Calphostin C inhibits process extension by adult human OLs. Figure A shows the representative morphology of OLs when treated with either bFGF or PDB or when grown on astrocyte ECM, while frame B is of sister cultures treated with these factors in the presence of 30 nM calphostin C. Cells were OLs as determined by O1 immunoreactivity. X800 magnification.

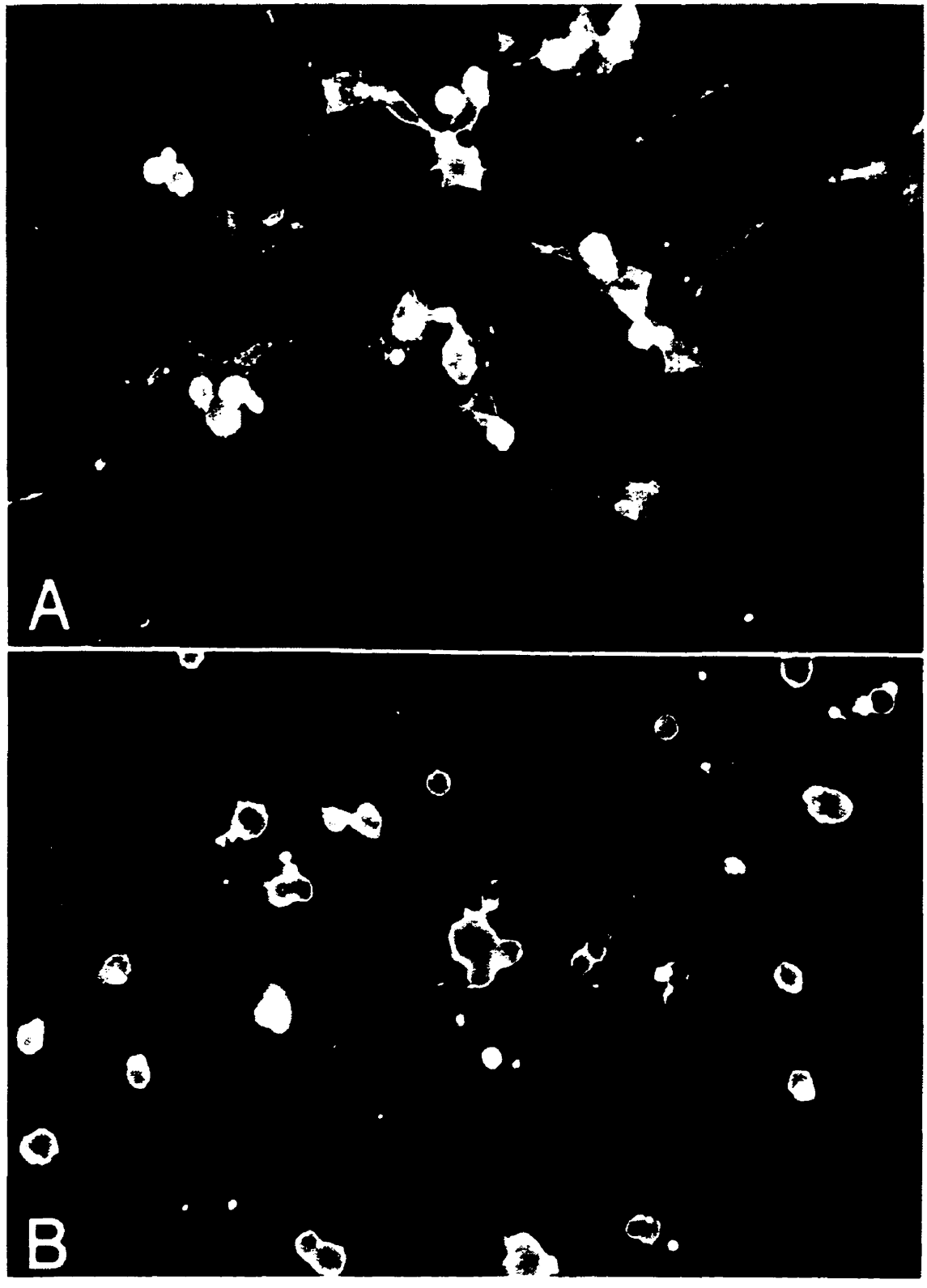

A
B

Figure III-2: Calphostin C (CP) inhibits the promoting effect of PDB on process outgrowth by adult human OLs. The concentrations of test agents were 50 ng/ml for PDB and 10-50 nM for calphostin C. Controls were OLs on poly-l-lysine (PL)-coated chambers not given any treatment. Each value is the mean $\pm$ SEM of 4 replicate chambers of cells, which is representative of multiple experiments. On average 159 $\pm$ 2 O1+ cells/chamber were analyzed. *, $p<0.05$; **, $p<0.01$ compared to No CP.

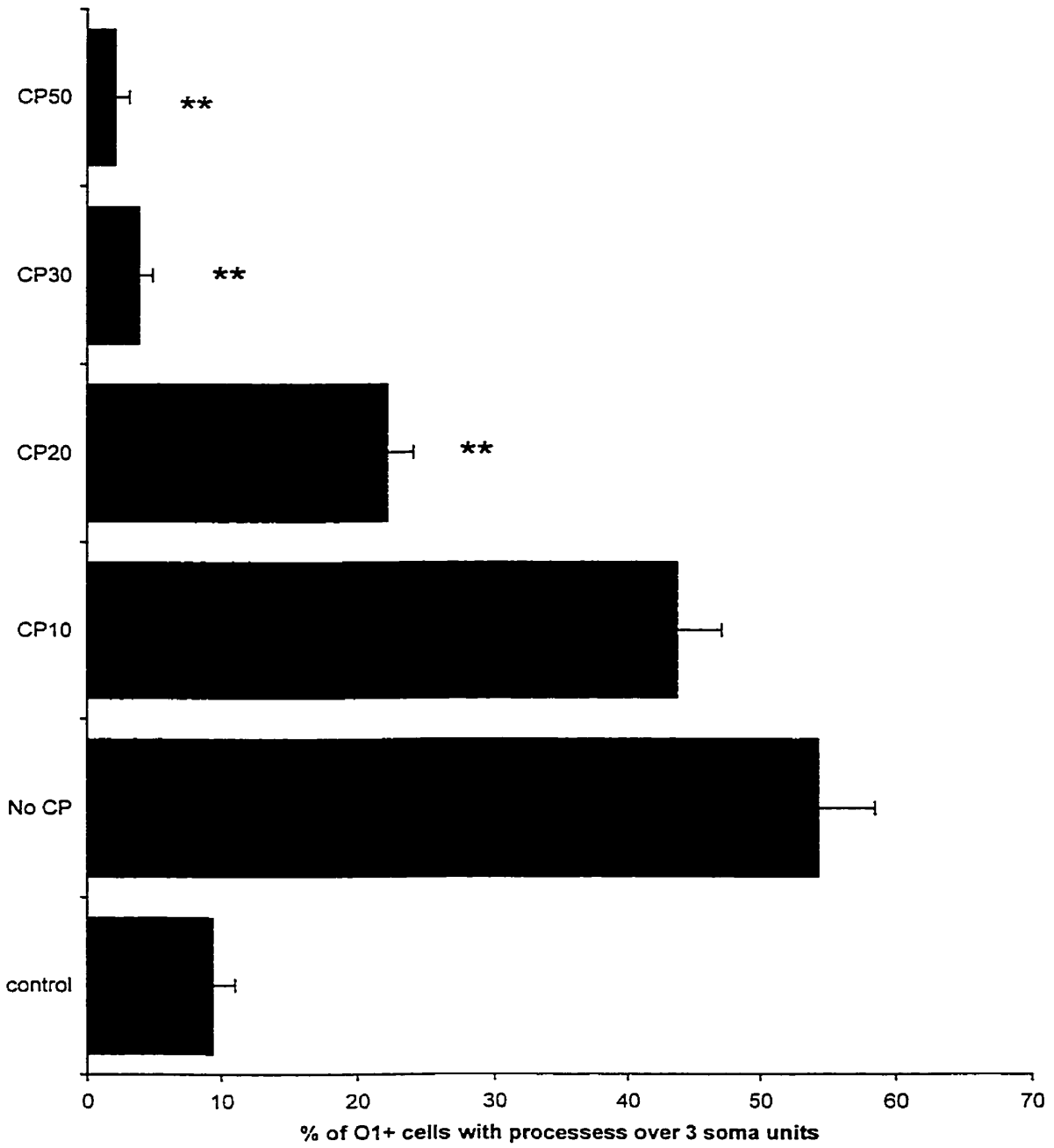

CP50
**
CP30
**
CP20
**
CP10
No CP
control
0
10
20
30
40
50
60
70
% of O1+ cells with processess over 3 soma units

Figure III-3: Calphostin C (CP) inhibits the promoting effects of bFGF and astrocyte ECM on process formation by OLs. The concentration of bFGF was 20 ng/ml. Concentration of calphostin C is given in nM. Controls were OLs on PL-coated chambers not given any treatment. Each value is the mean $\pm$ SEM of 4 replicate chambers of cells, which is representative of several experiments. On each chamber, an average of 154 $\pm$ 2 O1+ cells were counted. *, $p<0.05$; **, $p<0.01$ compared to No CP of each group.

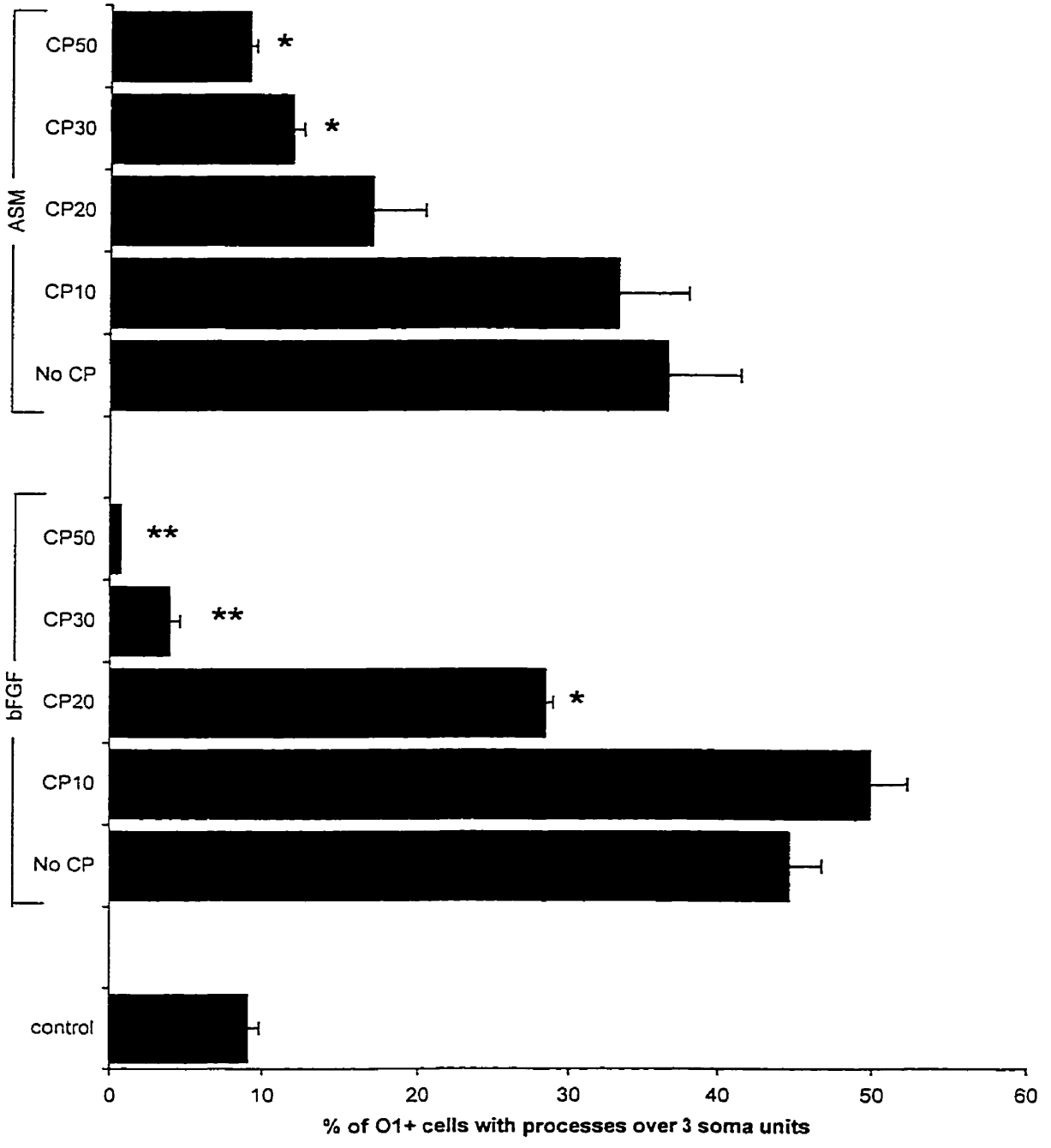

ASM
CP50
CP30
CP20
CP10
No CP
bFGF
CP50
CP30
CP20
CP10
No CP
control
*
*
**
**
*
0
10
20
30
40
50
60
% of O1+ cells with processes over 3 soma units

Figure III-4: The concentrations of calphostin C used to inhibit the process formation of OLs do not induce necrosis of OLs. The cell conditioned medium was collected 24 h after treatment and analyzed for LDH release. Calphostin C was used at 10-50 nM concentrations and digitonin, the positive control, at 50 μg/ml. Each value is the mean ± SEM of triplicate chambers of cells. **, $p<0.01$ compared to control.

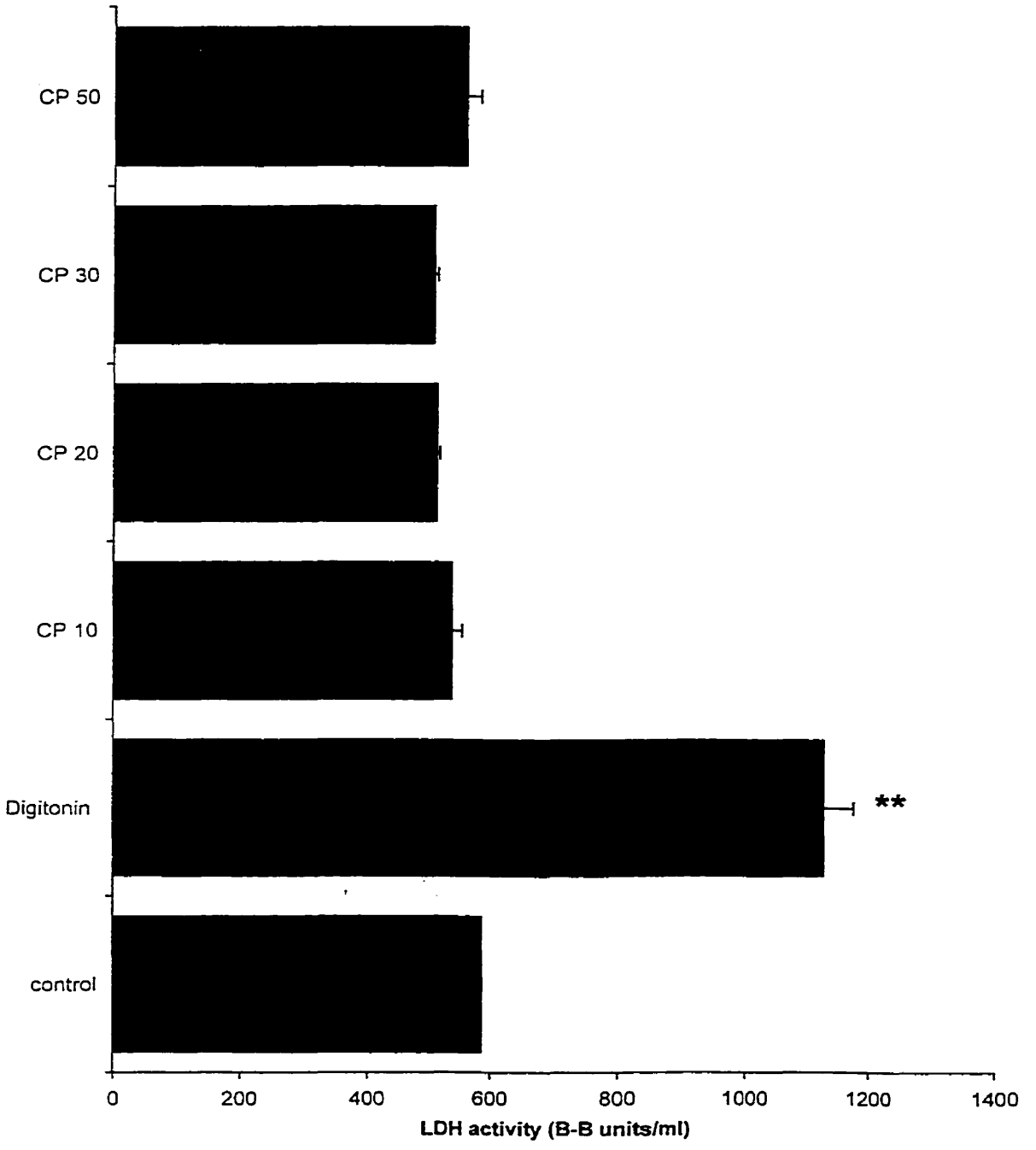

CP 50
CP 30
CP 20
CP 10
Digitonin
control
**
0
200
400
600
800
1000
1200
1400
LDH activity (B-B units/ml)

Figure III-5: TdT assay to detect apoptotic OLs. Control OLs are in frames A to C while panels D to F are corresponding figures of OLs treated with 100 nM calphostin C to demonstrate apoptosis. Frames A and D are of propidium iodide labelling to demonstrate all cell nuclei, B and E are of TdT positivity, while C and F are the corresponding Nomaski optics micrographs. As demonstrated in B, there are no apoptotic cells in control cultures.

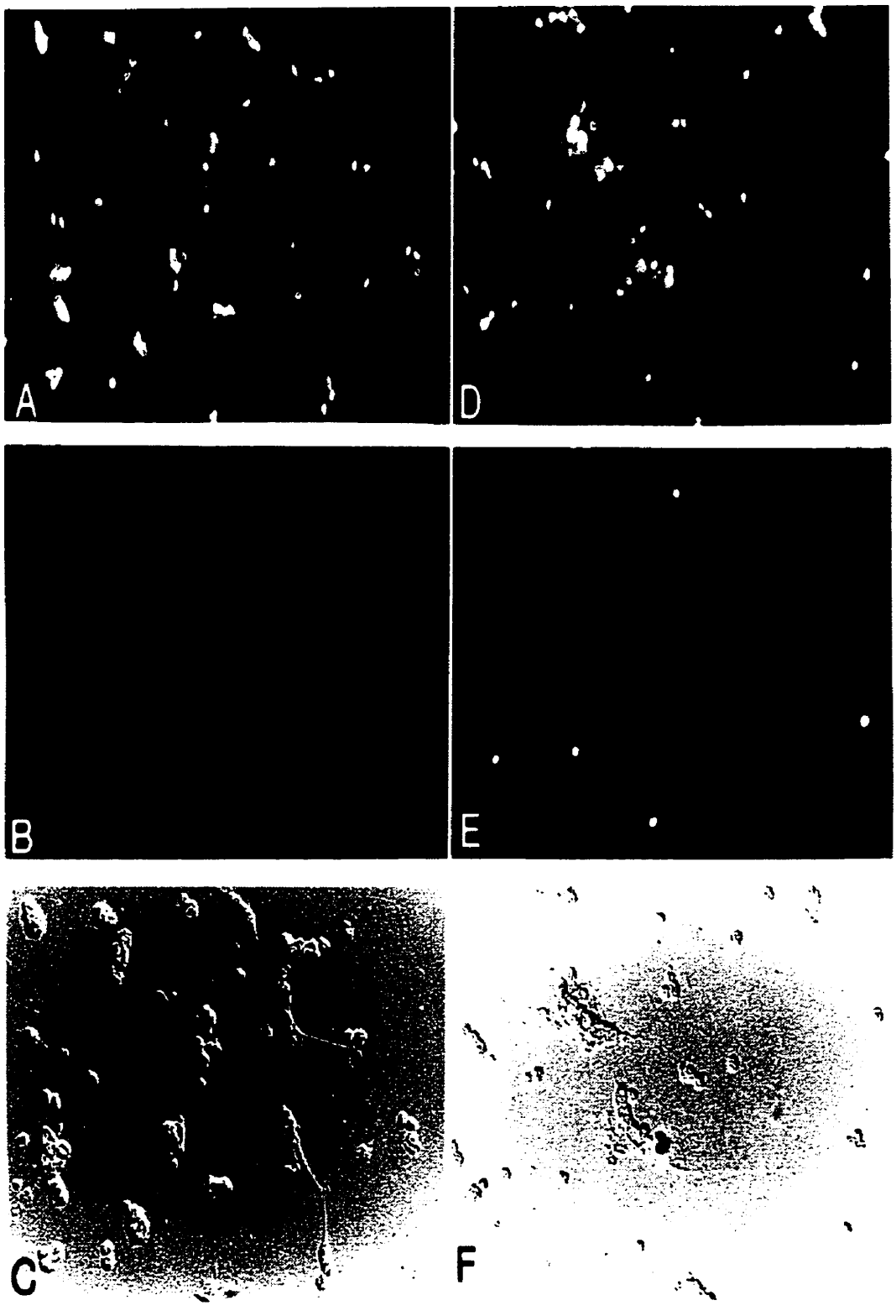

A
D
B
E
C
F

Figure III-6: Apoptosis of OLs is not induced by the concentrations of calphostin C (10-50 nM) used for inhibition of oligodendroglial process extension but was induced by 100 nM calphostin C, the positive control. All cells were on PL-coated chambers with controls being cells not exposed to calphostin C. Apoptosis was analyzed by the fluorescent TdT assay as described in the text. Each value is the mean $\pm$ SEM of 4 chambers of cells; on average, 145 $\pm$ 3 O1+ cells/chamber were counted. **, $p<0.01$ compared to control.

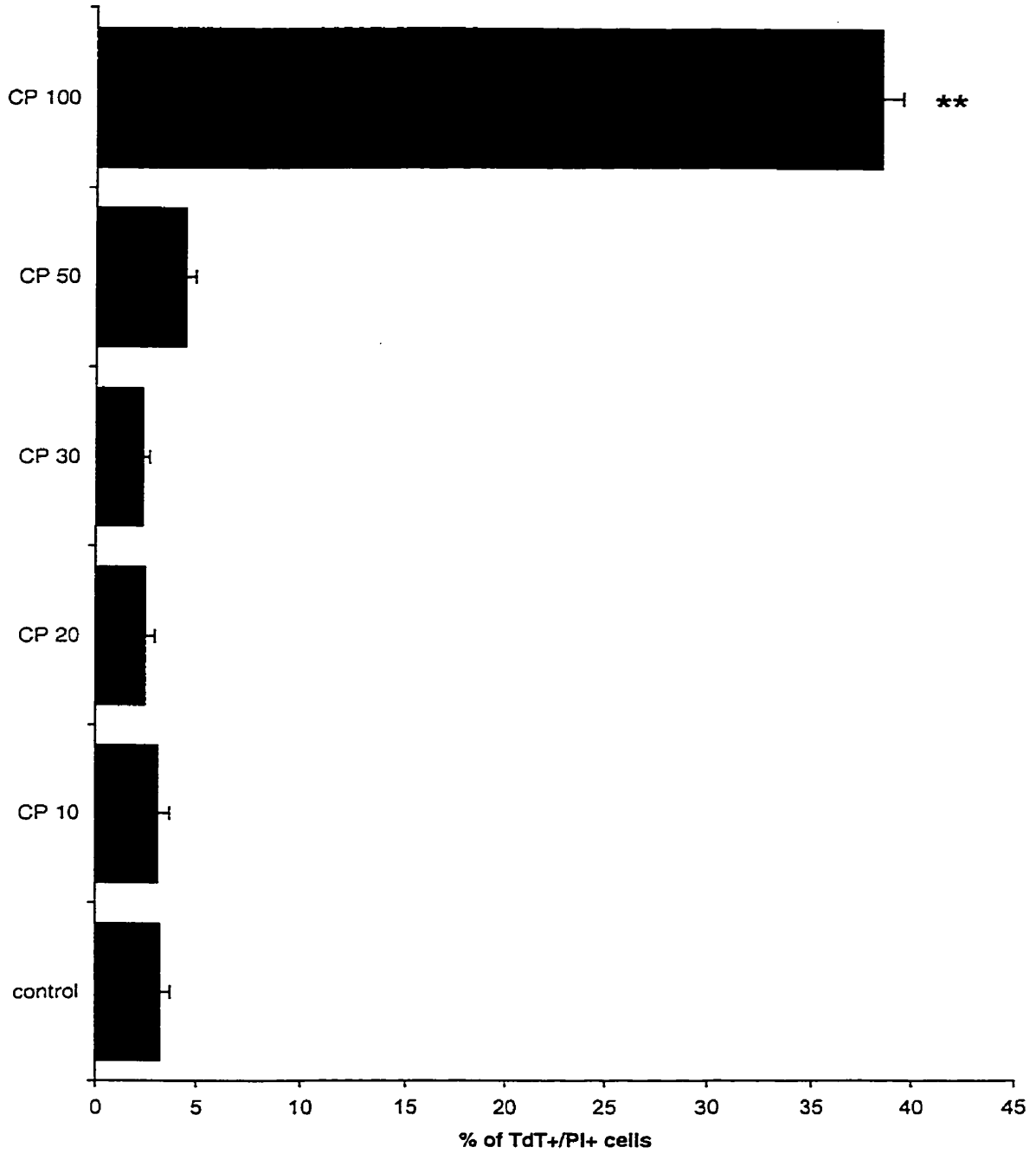
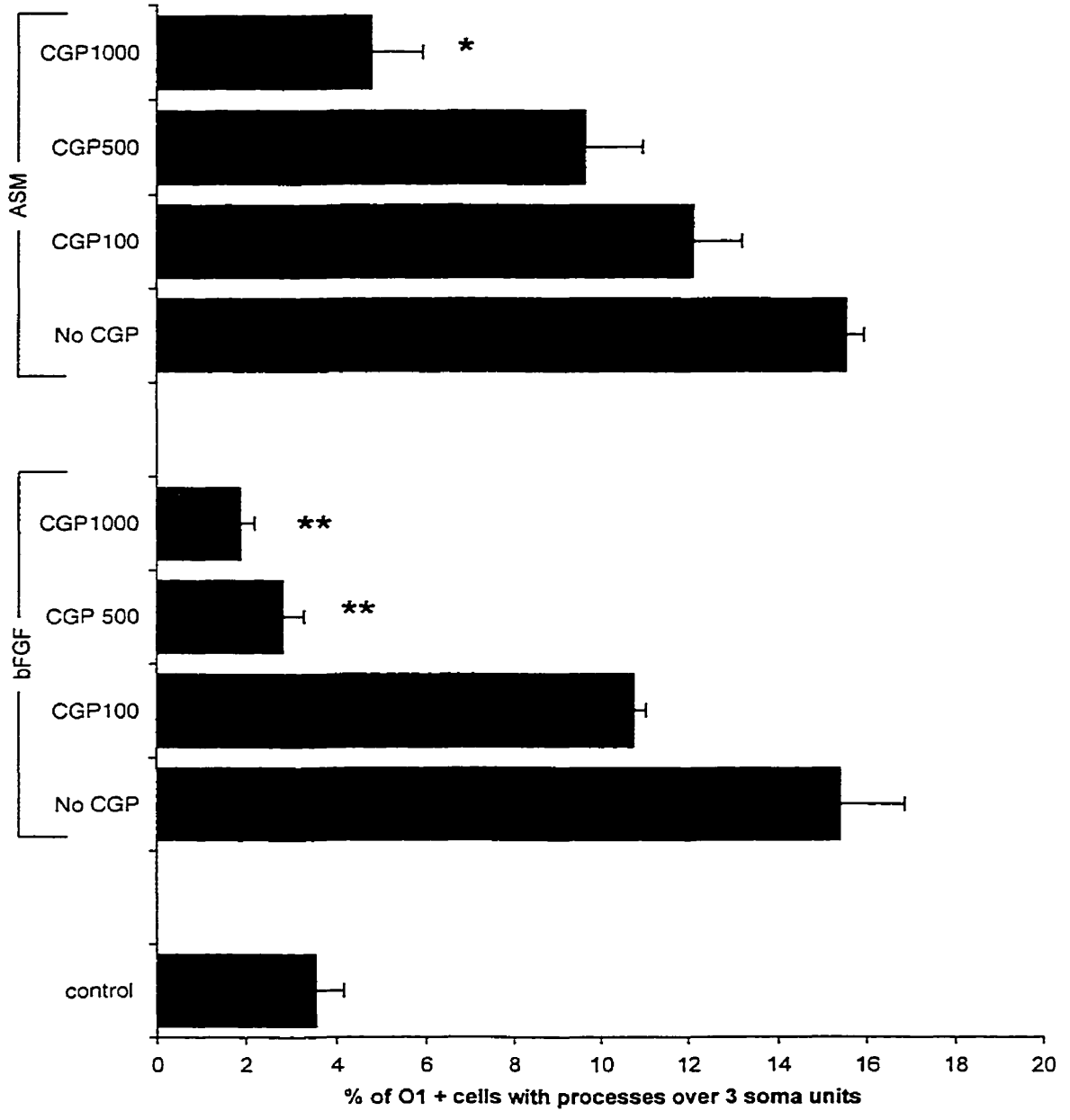

CP 100
CP 50
CP 30
CP 20
CP 10
control
**
0
5
10
15
20
25
30
35
40
45
% of TdT+/PI+ cells

Figure III-7: CGP 41 251, a selective inhibitor of PKC activity, also inhibits the promoting effects of bFGF and ASM on process extension by OLs. Basic FGF was used at 20 ng/ml and CGP 41 251 in 100-1000 nM concentrations. Controls were OLs on PL-coated chambers not given any treatment. Each value is the mean $\pm$ SEM of 4 chambers of cells; an average of 152 $\pm$ 2 O1+ cells were analyzed. *, $p<0.05$; **, $p<0.01$ compared to No CGP of each group.


ASM
CGP1000
CGP500
CGP100
No CGP
bFGF
CGP1000
CGP 500
CGP100
No CGP
control
*
**
**
0
2
4
6
8
10
12
14
16
18
20
% of O1 + cells with processes over 3 soma units

In chapter III, PKC was demonstrated as an important mediator in bFGF- and astrocyte ECM-induced OL process outgrowth. In chapter IV, I tested the hypothesis that MMPs are involved in process outgrowth by OLs since these proteinases are involved in the physiological remodeling of the ECM, which may be necessary for the OL processes to advance. With identification of MMP-9 up-regulation during myelinating period in the developing mouse optic nerve, the functional role of MMP-9 in OL process outgrowth is addressed in vitro.

CHAPTER IV

Matrix Metalloproteinase-9/Gelatinase B is Required for Process Outgrowth by Oligodendrocytes

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(In press 1999)

Abstract

Oligodendrocytes (OLs) extend processes to contact axons as a prerequisite step in myelin formation. As the OL processes migrate towards their axonal targets, they modify adhesion to their substrate, an event that may be regulated by matrix metalloproteinases (MMPs). In the mouse optic nerve, MMP-9/gelatinase B increases during myelin formation. Although tissue inhibitor of metalloproteinase (TIMP)-3 also increases in parallel, the developing optic nerve has focally active MMPs demonstrable by in situ zymography. The distribution of proteolytic activity is similar to that of myelin basic protein, a marker of myelin formation. OLs in culture secrete MMP-9, and express active cell-associated metalloproteinases at the growing tips of their processes. TIMP-1 and a function perturbing anti-MMP-9 antibody attenuate outgrowth of processes by OLs, indicating a requirement for MMP-9 in process outgrowth. Process reformation is retarded significantly in OLs cultured from MMP-9 null mice, as compared to controls, providing genetic evidence that MMP-9 is necessary for process outgrowth. These data show that MMP-9 facilitates process outgrowth by OLs in vivo and in culture.

Introduction

Myelin is a critical regulator of neuronal function in the central nervous system (CNS). Myelin formation and remyelination by oligodendrocytes (OLs) require a number of interacting factors including the availability and maturation of OLs, an appropriate microenvironment containing growth factors and extracellular matrix (ECM) molecules, and an adequate cross-talk with axons to form myelin (Ludwin, 1989; McLaurin and Yong, 1995). In human demyelinating diseases, such as multiple sclerosis (MS), significant loss of myelin and OLs occurs, eventually causing devastating neurological dysfunction. However, surviving and/or newly formed OLs, which are present around the edges of lesions in the MS brain (Ghatak, 1992; Wu et al., 1992), attempt remyelination, although the extent is often incomplete (Ghatak et al., 1989; Prineas et al., 1989; Wu et al., 1992; Raine and Wu, 1993). OL process outgrowth is an early critical step for myelination/remyelination. Understanding the mechanisms by which OLs extend their processes may lead to strategies that can enhance remyelination.

In culture, OLs form processes as they mature (Gard and Pfeiffer, 1990). We have found that outgrowth of OL processes is facilitated by the astrocyte ECM (Oh and Yong, 1996). Furthermore, phorbol esters and basic fibroblast growth factor (bFGF) promote process outgrowth by OLs (Yong et al., 1991; Oh and Yong; 1996; Bansal and Pfeiffer, 1997), by activating protein kinase C (PKC) (Althaus et al., 1992; Yong et al., 1994; Oh et al., 1997). The downstream effectors of PKC activation in OL that promote process outgrowth, however, are poorly understood.

For OL processes to advance from the soma, it is likely that the pericellular environment will be subjected to significant remodeling by proteinases. The family of matrix metalloproteinases (MMPs) is known to degrade most, if not all, proteinaceous ECM molecules including collagen, fibronectin, laminin, and a variety of proteoglycans (Yong et al., 1998). There are at least 18 members of MMPs, which are categorized into four groups depending on substrate preference and structural differences: collagenases, gelatinases, stromelysins, and membrane-type MMPs. MMPs have been shown to be involved in physiologic processes (e.g. embryogenesis, ovulation, bone growth /remodeling, and angiogenesis) and also in pathology (e.g. tumor metastasis, rheumatoid arthritis, and periodontal disease) (Woessner, 1994). In the CNS, MMPs have been studied in several conditions such as glioma invasiveness, leukocyte infiltration, and neurite outgrowth (reviewed in Yong et al., 1998). Growth factors including nerve growth factor and FGFs appear to stimulate neurons to up-regulate MMPs in correspondence with neurite outgrowth (Machida et al., 1989; Muir, 1994). We have reported that phorbol esters increase MMP-9 (gelatinase B) secretion by OL in parallel with increased process extension (Uhm et al., 1998), whereas, calphostin C, a selective inhibitor of PKC activation, attenuates MMP-9 production by OLs in parallel with decreased process formation. In this study, we have asked whether MMP-9 is required for process outgrowth by OLs in vitro and during myelination in vivo.

Materials and Methods

Cell culture and drug treatment

Adult human OLs were derived from brain biopsy specimens from patients who underwent surgical resection to ameliorate drug-intractable epilepsy. Samples containing white and grey matter were of areas adjacent to, but not containing the epileptic focus. Bovine cells were derived from the corpus callosum of adult animals obtained from a local meat-processing center. Adult, three-month-old mice were used to obtain murine cells; unless otherwise stated, the strain was the CD1 outbred.

Cells were dissociated by trypsin digestion and isolated by Percoll gradient centrifugation according to a protocol described elsewhere (Yong and Antel, 1997). The initial cell isolates, consisting of OLs, astrocytes and microglia, were plated onto uncoated T-25 cm^2 flasks. In contrast to astrocytes and microglia, adult OLs are poorly adherent on uncoated substrate; floating cells were collected the following day and, when subjected to another round of differential adhesion, resulted in OL cultures of over 95% purity. Purified OLs were then plated onto Lab-Tek 16-well chamber slide (Nunc, Inc., Naperville, IL) or glass cover slips coated with 10 μg/ml poly-L-ornithine (Sigma, St. Louis, MO).

For MMP inhibition, 1,10-phenanthroline (Sigma, St. Louis, MO), recombinant human TIMP-1 (compliments of Dr. Andy Docherty, Cell-tech Therapeutics Ltd., Slough, UK), and sheep anti-porcine MMP-9 antibody (gift of Dr. G. Murphy, University of East Anglia, Norwich, UK) were added to OL cultures 1 hour prior to adding 10 nM 4β-phorbol-12,13-dibutyrate (PDB, LC Laboratories, Woburn, MA).

OLs were cultured in Minimum Essential Medium supplemented with 10% fetal bovine serum, 20 μg/ml gentamycin and 0.1% dextrose. To collect medium conditioned by OLs for zymography, serum-free medium supplemented with hormones, as described elsewhere, was added (Boutros et al., 1997).

Immunocytochemistry and analyses of process formation in vitro

Oligodendrocytes derived from adult brains were stained with an O1 monoclonal antibody that recognizes galactocerebroside, a marker for mature OLs (Bansal et al. 1989). Human and bovine OLs extended several thread-like processes from soma, which facilitated the counting of the percentage of O1+ cells with processes over 3-soma diameters, as previously described (Yong et al., 1994; Oh and Yong, 1996). However, the processes of mouse OLs were in the form of membranous-like sheets, which did not allow the documentation of OLs with processes over a given length criterion. Thus, we employed a computer-assisted technique to measure the area of processes extended out from murine OL soma. In brief, using a digital camera to acquire images and an Image pro image analysis program, we measured the total area covered by OLs (O1+ staining); this area measurement also included their nuclei. The area covered by nuclei (Hoescht-stained) was also measured. To obtain the extent of processes generated by OLs, the total measured area of O1+ immunoreactivity was subtracted with that of Hoescht dye staining.

Gelatin zymography and reverse gelatin zymography

Gelatin-substrate gel electrophoresis was used to detect gelatinases (Stuve et al., 1996; Uhm et al., 1998). In brief, serum-free medium conditioned by OL was collected and mixed (1:3) with 4X gel loading buffer. The samples were separated at 4°C on a 12 % SDS-gel containing 1mg/ml gelatin. The gel was then washed twice and incubated overnight on a shaker at room temperature with rinse buffer containing 2.5% Triton X-100, 50 mM Tris-HCl, pH 7.5, and 5mM $CaCl_2$. The Triton X-100 wash extracted SDS, allowing gelatinases to renature within the gel. The following day, each gel was incubated with reaction buffer containing 50 mM Tris-HCl, pH 7.5 and 5mM $CaCl_2$ for 16 –18 hours at 37°C, to allow proteinases to degrade gelatin in their immediate vicinity. After rinsing with water, each gel was stained with Coomassie Blue for four hours. Incubation of the gel with destaining solution (1:3:6 of acetic acid: methanol: water) revealed the expression of gelatinases as clear bands (zone of gelatin degradation) against a dark background. The identity of MMPs was based on their molecular weight and was confirmed by Western blot and immuno-depletion experiments (Uhm et al., 1998).

In some experiments, the whole cell lysate of OLs was used for zymography. To obtain lysates, the medium of OLs was removed and cells were washed once with PBS. Extraction buffer, containing 1% Triton X-100, 500 mM Tris-HCl, pH 7.6, 200 mM NaCl, and 10 mM $CaCl_2$, was then added. The lysate was collected, and 20 μg of total protein was loaded per lane.

For gelatin zymography of the developing mouse optic nerves, homogenates of early postnatal CD1 mouse optic nerves were used. The optic nerves from mice were

carefully dissected and quick-frozen at –80°C. At least 5 mice per age-group were combined to prepare an optic nerve sample. The optic nerves were homogenized in extraction buffer (described above), and total protein content was analyzed by Bradford protein assays. Thirty μg total protein of each sample was mixed with 4X gel loading buffer and separated on SDS-polyacrylamide gels as described above.

Reverse gelatin zymography was employed to reveal physiological inhibitors of MMPs (i.e. TIMP-1 to -4). The gel was prepared in the same manner as gelatin zymography with the exception that gelatinases were also added to the SDS/gelatin gel. Following electrophoresis and during incubation of the gel with the reaction buffer described above, the impregnated gelatinases degrade gelatin throughout the gel except at areas where the inhibitory TIMPs are located; these areas remain as dark blue bands following Coomassie Blue staining of gels and destaining (Leco et al., 1994; Kossakowska et al., 1998).

In situ zymography in vivo and in vitro

To localize net gelatinolytic activity of MMPs by in situ zymography, FITC-labeled DQ gelatin that is intramolecularly quenched {available in a gelatinase/collagenase assay kit (EnzChek, Molecular Probes, Inc., Eugene, Oregon)} was used as a substrate for degradation by gelatinases. Proteolysis by gelatinases yields cleaved gelatin-FITC peptides that are fluorescent. The localization of fluorescence indicates the sites of net gelatinolytic activity. Optic nerves of postnatal 7 and 9 days mice were dissected and rinsed in cold PBS to remove blood vessels and debris. The optic nerves were then immersed in OCT compound (Tissue-Tek, Torrance, CA) and

quick-frozen into a block on dry ice. The optic nerves in OCT block was cut into 8 μm sections using a cryostat (Leica, Wetzler, Germany) and collected sequentially. The optic nerve sections were stored at -80°C until used for in situ zymography and immunohistochemistry. For in situ zymography, sections were thawed and incubated with reaction buffer (0.05 M Tris-HCl, 0.15 M NaCl, 5 mM $CaCl_2$, 0.2 mM NaN_3, pH 7.6) containing 40 μg/ml DQ gelatin overnight. At the end of the incubation period, and without fixation or washes gelatinolytic activity of MMPs was localized and photographed by fluorescence microscopy. Images were acquired by a Spot digital camera with computer imaging program (Image-Pro Plus). Adjacent cryostat sections were fixed with 4% paraformaldehyde, followed by immunohistochemistry for MBP (Serotec, Oxford, UK) or GFAP (Dako Corp., Scarborough, Ontario) as described previously (Balasingam and Yong, 1996).

To determine in situ gelatinolytic activity of OLs in culture, cells were plated onto coverslips and treated with 10 nM PDB to promote their process extension. Live cells were stained with the O1 antibody as described above, then washed with PBS, and incubated with the in situ zymography reaction buffer (described above) overnight at 37°C. We noted that if cells were pre-fixed, this substantially reduced their capacity to degrade gelatin-FITC, likely because cells needed to be alive to perform their proteolytic function. However, if cells were fully functional, the intensity of gelatinolytic signal was also reduced, presumably because cells phagocytose the gelatin substrate and cleave it intercellularly. As a compromise, 0.2 mM sodium azide was incorporated into the reaction buffer and incubated with live cells. Under these

conditions the cells shut down metabolism, retain O1 staining, but still enable cell surface-associated gelatinolysis to occur.

As negative controls for in situ zymography, 50 μM 1,10-phenanthroline or 500 ng/ml TIMP-1 was added to the reaction buffer prior to applying to the cells or to the frozen cryostat optic nerve sections to inhibit metalloproteinases.

Statistical analyses

Because multiple treatments, or multiple concentrations of each treatment, were used in all experiments, a one way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons were employed for statistical analyses.

Results

MMP-9 and TIMPs are expressed in mouse optic nerve during myelin formation

During myelination oligodendrocytes extend processes, a function similar to axonal outgrowth which has been shown to involve altered interactions with extracellular matrix (ECM) regulated by proteolysis (Monard, 1988; McGuire and Seeds, 1990; Muir, 1994). In the mouse optic nerve, myelin formation takes place between postnatal day (P) P5 to P12 (Foran and Peterson, 1992). We observed that myelin formation in the CD1 outbred mouse optic nerve increases dramatically between P7 and P9. Myelin basic protein (MBP) immunoreactivity, a marker for myelin formation, was low on P7, and abundant on P9 (Fig. IV-1A).

MMP-9 levels paralleled the progressive developmental myelination in the mouse optic nerve. By gelatin zymography assays we found that there was a gradual increase in MMP-9 levels from P3 to P11 mouse optic nerves, whereas expression of MMP-2 decreased (Fig. IV-1B). MMP activity is regulated not only by expression and activation of the enzymes, but also by endogenous inhibitors. During developmental myelination (Fig. IV-1C), we observed no significant changes in expression of TIMP-1 (28 kDa) or TIMP-2/-4 (21 kDa and 22 kDa, respectively) by reverse gelatin zymography. In contrast, TIMP-3 (24 kDa) increased from P3 to P11.

A novel in situ zymography method reveals that active MMPs are present during myelin formation in vivo

The observation that both MMP-9 and TIMP-3 increased during the period of myelin formation led us to ask whether there was net proteolytic activity. We developed a new in situ zymography method to demonstrate net proteolytic activity in mouse optic nerve. When frozen sections were incubated with intramolecularly fluorescein-quenched gelatin, the digestion of gelatin yielded cleaved fluorescent peptides that enabled us to locate the gelatinolytic activity. The majority of the gelatinolytic activity appeared to be associated with cells. TIMP-1 or 1,10-phenanthroline significantly reduced the gelatinolytic activity (Fig. IV-2), indicating that the proteolytic activity was due to MMPs. Both MMP-2 (gelatinase A) and MMP-9 (gelatinase B), which are present at this time in development, cleave gelatin. While the assay does not discriminate between MMP-2 and MMP-9, it is likely that proteolytic activity largely represents that of the latter, given the increase of MMP-9 during this period. Importantly, there was significant gelatinolytic activity in the P9 mouse optic nerve, at a time point when dramatic myelin formation was occurring.

Mouse optic nerve contains both OL and astrocytes. To identify the cellular source of the gelatinolytic activity we subjected sequential cryostat frozen sections to immunohistochemistry specific for OL (MBP) or astrocytes {glial fibrillary acidic protein (GFAP)}, or to in situ zymography; it was not possible to perform both manipulations on the same section because the processing used in immunohistochemistry often interfered with the in situ zymography signals or destroyed the gelatinolytic activity. The distribution of OLs, as shown by MBP immunoreactivity, was similar to that of gelatinolytic activity, longitudinally along the mouse optic nerve (Fig. IV-3). In contrast, astrocytes, visualized with GFAP labeling, are distributed

perpendicular to the gelatinolytic signal. These data indicate that much of the gelatinolytic activity is expressed on OLs and their processes.

Oligodendrocytes in culture express active MMPs

The regeneration of processes by adult brain-derived OLs in vitro recapitulates the myelinating program since myelin is stripped off during the isolation of cells. We have investigated whether MMP expression found during developmental myelin formation in vivo is recapitulated during process outgrowth in OLs isolated from bovine, human, and mouse brains (Fig. IV-4). MMP-9 production, process extension, and inhibition of process extension by MMP inhibitors took place when OLs were cultured on a poly-L-lysine- or poly-L-ornithine-coated surfaces (unpublished observation) or on astrocyte-derived ECM (Uhm et al., 1998). Therefore, all subsequent experiments were performed using poly-L-ornithine-coated surface as the cell substrate.

Although mouse OLs had a more mature (membranous sheets) morphology compared to their (thread-like processes) bovine (Fig. IV-4) or human counterparts, OLs from all species increased process formation when treated with 4β-phorbol-12,13-dibutyrate (PDB), an activator of protein kinase C (Yong et al., 1994). MMP-9 secretion accompanied process outgrowth by OLs (Fig. IV-4A to 4E). Based on molecular weight determination (approximately 92 kDa for human or bovine, and 105 kDa for murine samples), the MMP-9 detected in the conditioned media of OL cultures was of the zymogen. Incubation of OL conditioned medium with 1mM of 4-aminophenyl mercuric acetate (APMA) at 37 °C for 15 min converted the zymogen

form of MMPs to active forms of MMPs detectable at lower molecular weight in zymograms (approximately 85 kDa for active MMP-9) (Fig. IV-4F). The absence of detectable active MMP-9 in the conditioned medium is likely because cells confine the activation of MMPs to the pericellular environment, thereby preventing widespread proteolysis (Murphy and Knauper, 1997). Nevertheless OLs produced cell-associated active MMP-9, which was seen in the cell lysate of OLs by gelatin zymography (Fig. IV-4F).

To address where in the OL active proteinases are produced, we next used in situ zymography, coupled with counter-staining OLs with an O1 antibody that recognizes galactocerebroside, a marker for mature OL. We found net gelatinolytic activity of MMPs on the processes and on the advancing tip of OL processes (Fig. IV-5), in keeping with the postulate that these areas advance the OL boundaries. A surprising observation is that net gelatinolytic activity was also manifest in a uniform manner around the OL soma. That the distribution of uniform gelatinolytic activity is peculiar to OLs is indicated by localized and punctate distribution of gelatinolytic activity in another cell type, BHK (baby hamster kidney) cells (Fig. IV-5D1). Thus, depending on the cell type, MMPs are utilized at different locations.

In summary, the results of gelatin and in situ zymographies indicate that activation and utilization of MMPs occur in the pericellular environment of the OL.

MMP-9 facilitates process outgrowth by oligodendrocytes

We next asked whether the gelatinolytic activity and process extension are mechanistically related, by blocking MMP function. TIMP-1, a physiological inhibitor

of MMP activity, reduced the process outgrowth stimulated by PDB treatment in human, bovine and mouse OLs (Fig. IV-6). Moreover, most of the MMP action could be attributed to MMP-9. Addition of a function-blocking antibody to MMP-9 (Librach et al., 1991) reduced process outgrowth of bovine OLs to the level where few OLs bore notable long processes (Fig. IV-7). However, the addition of soluble MMP-9 to OLs did not induce process outgrowth (data not shown). These results indicate that MMP-9 facilitates process outgrowth by OLs, and that OLs from human, bovine, and mouse share this common mechanism for process outgrowth.

Process outgrowth is deficient in OLs derived from MMP-9 null mice

If MMP-9 plays a key role in OL process outgrowth, OLs derived from MMP-9 null mice (Vu et al., 1998) should be defective in process formation. Indeed, OLs derived from MMP-9 null mice had reduced capability to form processes spontaneously, when compared to OLs from wild-type mice (Fig. IV-8). Furthermore, while wild-type OLs promoted process outgrowth in response to PDB, it was noted that OLs from MMP-9 null mice did not respond to stimulation. The results indicate that MMP-9 is required for both basal and PDB-induced process formation by OLs.

Discussion

Proteases are recognized to be of increasing importance in the regulation of development and physiology of the nervous system (Woessner, 1994; Yong et al., 1998). Migration of neural progenitor cells appears to be an MMP-mediated process (Amberger et al., 1997). Angiogenesis during CNS development is also associated with MMPs (Canete-Soler et al., 1995). MMPs are implicated in promoting neurite outgrowth and growth cone extension. Growth factors such as NGF and FGFs induced MMP expression in correspondence with promoting neurite outgrowth (Machida et al., 1989; Muir, 1994), while MMP inhibitors attenuate growth cone activity (Sheffield et al., 1994). MMP-2 is localized to the growth cones of dorsal root ganglion neurons, where it is thought to degrade growth inhibitory chondroitin sulfate proteoglycans, thus facilitating neurite extension (Zuo et al., 1998). Proteinases have also been implicated in long-term potentiation and synaptic growth (Baranes et al., 1998).

In this study, we show that MMPs facilitate process extension by the OL in the CNS. We first found that myelin formation in the developing mouse optic nerve is accompanied by increased MMP-9 and net gelatinolytic activity. In this regard, there was not a complete overlap of in situ zymography signal with MBP immunoreactivity in the mouse optic nerve, likely because MBP immunoreactivity does not discriminate the different individual stages of myelin formation. OL processes that have completely ensheathed axons, but before compaction to extrude their cytoplasm (reviewed in McLaurin and Yong, 1995), could still be MBP immunoreactive but may no longer require MMPs to remodel the microenvironment. To analyze the function of MMP-9

we used cultured OLs. We found that the enhancement of process outgrowth by PDB in adult brain-derived OLs was accompanied by increased MMP-9 expression and was blocked by TIMP-1 or a function-blocking antibody to MMP-9. Gelatinolytic activity was present at the tip of the advancing OL processes in vitro, consistent with a function for MMP-9 in mediating remodeling interactions at the advancing tip. Furthermore, OLs cultured from mice with a genetically targeted null mutation in MMP-9 exhibited a reduced capacity to form processes. Collectively, these results implicate MMP-9 in mediating process outgrowth by OLs.

It is noteworthy that the active gelatinolytic signals seen by in situ zymography are localized to the surface of OL processes. This is consistent with observations that, although MMPs are secreted molecules, they are localized to the pericellular areas where they are required, rather than being diffusely distributed to effect widespread destruction. Although little is known about receptors for MMP-9, others have shown that MMPs can be restricted to the pericellular areas by binding to cell surface integrin receptors (Brooks et al., 1996) or to transmembrane forms of MMPs (reviewed in Murphy and Knauper, 1997); interaction with CD44 may be another mechanism to localize MMP activity to the cell surface (Yu and Stamenkovic, 1999). A requirement of this cellular localization of active MMP-9 may explain the lack of facilitation of OL process outgrowth when active MMP-9 was added directly to the culture medium of OLs. In support of this interpretation, Deryugina et al. (1998) have reported that cell-associated, rather than soluble, MMP-2 mediates the contraction of polymerized collagen gels by glioma cells.

Given the cell surface localization, it is of further interest that gelatinolytic activity was uniformly distributed along the OL soma as well as the processes. While the latter distribution is likely used to extend processes, the reason for the cell soma distribution is unclear. In contrast, gelatinolytic activity in another cell type, BHK cells, was punctate and at specific sites reminiscent of "invadopodia", which are sites of interface of cells with substrate (Monsky et al., 1993; 1994). These results are in keeping with immunocytochemical data showing that MMPs are distributed both on neurites and on neuronal cell bodies (Zuo et al., 1998). A plausible role for these cell surface MMPs may be the release of growth factors that are anchored on the ECM. In this way the MMPs in the vicinity of OL somata could promote OL survival.

What regulates MMP-9 production by OLs? In vitro, MMP-9 transcription is PKC-dependent (Uhm et al., 1998). The MMP-9 promoter contains several TRE (phorbol ester responsive element) regions, where the AP-1 family of transcription factors, stimulated through PKC, bind (Karin et al., 1997). In other cell types, bFGF or adhesion to specific ECM components can enhance MMP-9 expression (Larjava et al., 1993; Tremble et al., 1994; Weston and Weeks, 1996). Because bFGF and astrocyte ECM activate PKC of OLs (Oh et al., 1997), and facilitate OL process outgrowth (Oh and Yong, 1996), we suggest that these are possible candidates for regulating MMP-9 in vivo.

While our results show a beneficial role for MMPs in CNS development and regeneration, MMPs are highly expressed in pathological processes of the nervous system, including Alzheimer's disease, stroke, malignant gliomas and multiple sclerosis (MS) (reviewed in Yong et al., 1998). When MMP-2 is administered by intracerebral

injection, severe blood brain barrier breach results with accompanying edema and hemorrhage (Rosenberg et al., 1992). In MS, MMPs are up-regulated in the CNS during inflammation, and are expressed predominantly by perivascular leukocytes or parenchymal microglia (Cuzner et al., 1996; Maeda and Sobel, 1996; Anthony et al., 1997; Cossins et al., 1997). Indeed, several synthetic inhibitors of MMPs alleviate inflammation and disease in animals afflicted with experimental allergic encephalomyelitis (EAE), a model of MS (Gijbel et al., 1994; Hewson et al., 1995; Matyszak and Perry, 1996; Liedtke et al., 1998). Moreover, MMP-7 and MMP-9 appear to be utilized by inflammatory cells to infiltrate into the CNS (Stuve et al., 1996; Kieseier et al., 1998; Liedtke et al., 1998). Recently, Belien et al. (1999) reported that the expression of membrane-type 1 MMP confers upon glioma cells the ability to degrade myelin inhibitory proteins and to invade along CNS myelin. Thus, whether or not MMPs are beneficial or detrimental may depend on several factors, including the cellular sources, the pericellular environment, the specific MMP in question, and the stage of lesion development in the disease. Therefore, it is essential to determine the specific roles of MMPs and their participation at different stages of disease development or recovery. The indiscriminate use of MMP inhibitors may control detrimental activity of MMPs (e.g. in inflammation), but may, in the longer term, also inhibit repair.

Acknowledgements

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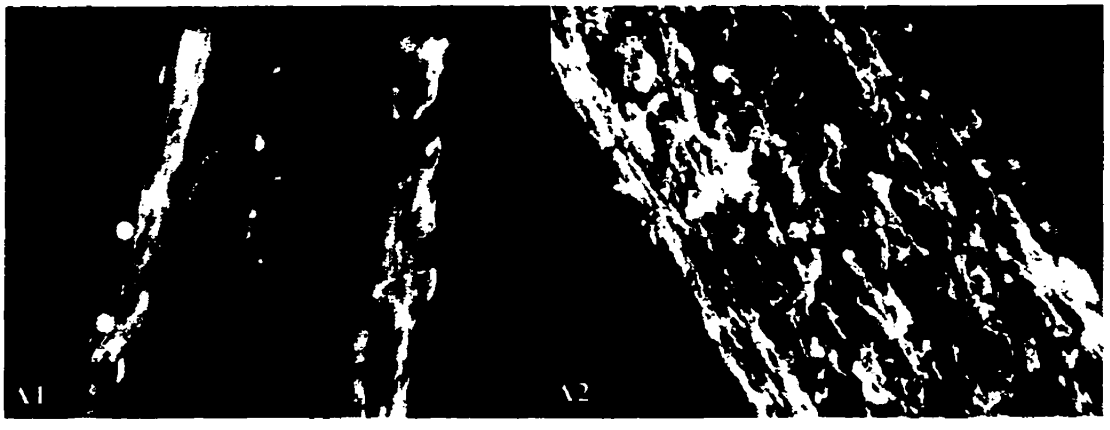
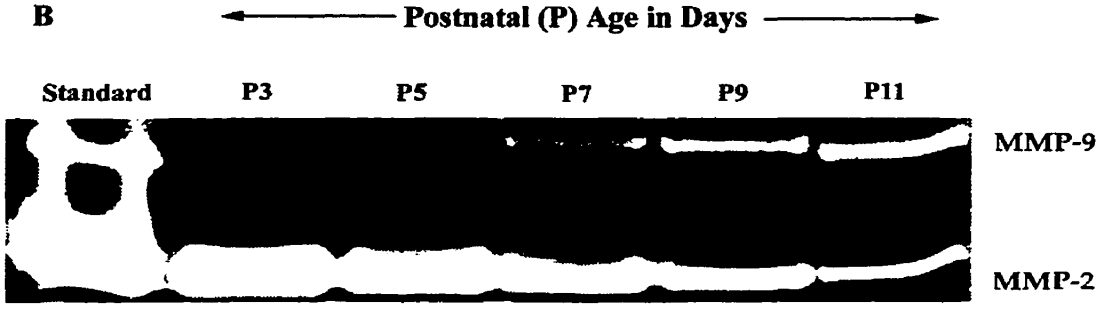
Figure IV-1: Expression of MMP-2 and MMP-9 during myelination in developing optic nerve. The period of myelin formation in the CD1 mouse optic nerve is shown by MBP immunofluorescence. Panel (A1) shows weak immunoreactivity of MBP at P7 while strong MBP staining indicate active myelination at P9 (A2). Panel (B), gelatin zymography of optic nerve from P3 to P11 CD1 mice, shows gradual increase in MMP-9 in contrast to the decrease in MMP-2 during this period. Panel (C) shows reverse gelatin zymography for TIMPs. TIMP-3 increased in mouse optic nerve during the period of myelination (P3 to P11), while no significant changes in TIMP-1 or TIMP-2/-4 levels were detected.



B


Postnatal (P) Age in Days
Standard
P3
P5
P7
P9
P11
MMP-9
MMP-2

C

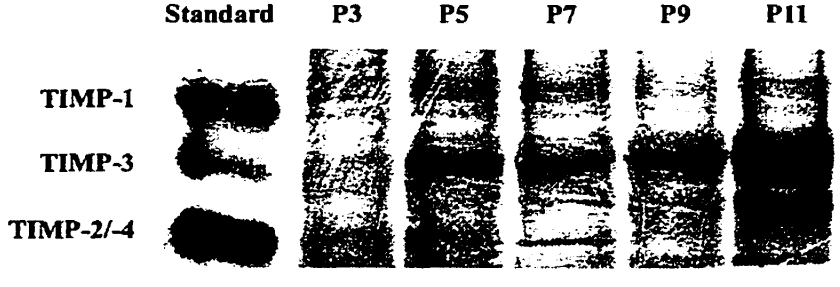

Standard
P3
P5
P7
P9
P11
TIMP-1
TIMP-3
TIMP-2/-4

Figure IV-2: Localization of MMP activity in developing optic nerve in vivo by in situ zymography. Cryostat section of P9 mouse optic nerve shows proteolytic activity of MMPs by in situ zymography (A). Localization of gelatinolytic activity (A) was reduced in the presence of (B) TIMP-1 (500 ng/ml) or (C) 1,10-phenanthroline (50 μM). The bar scale is 50 μm.

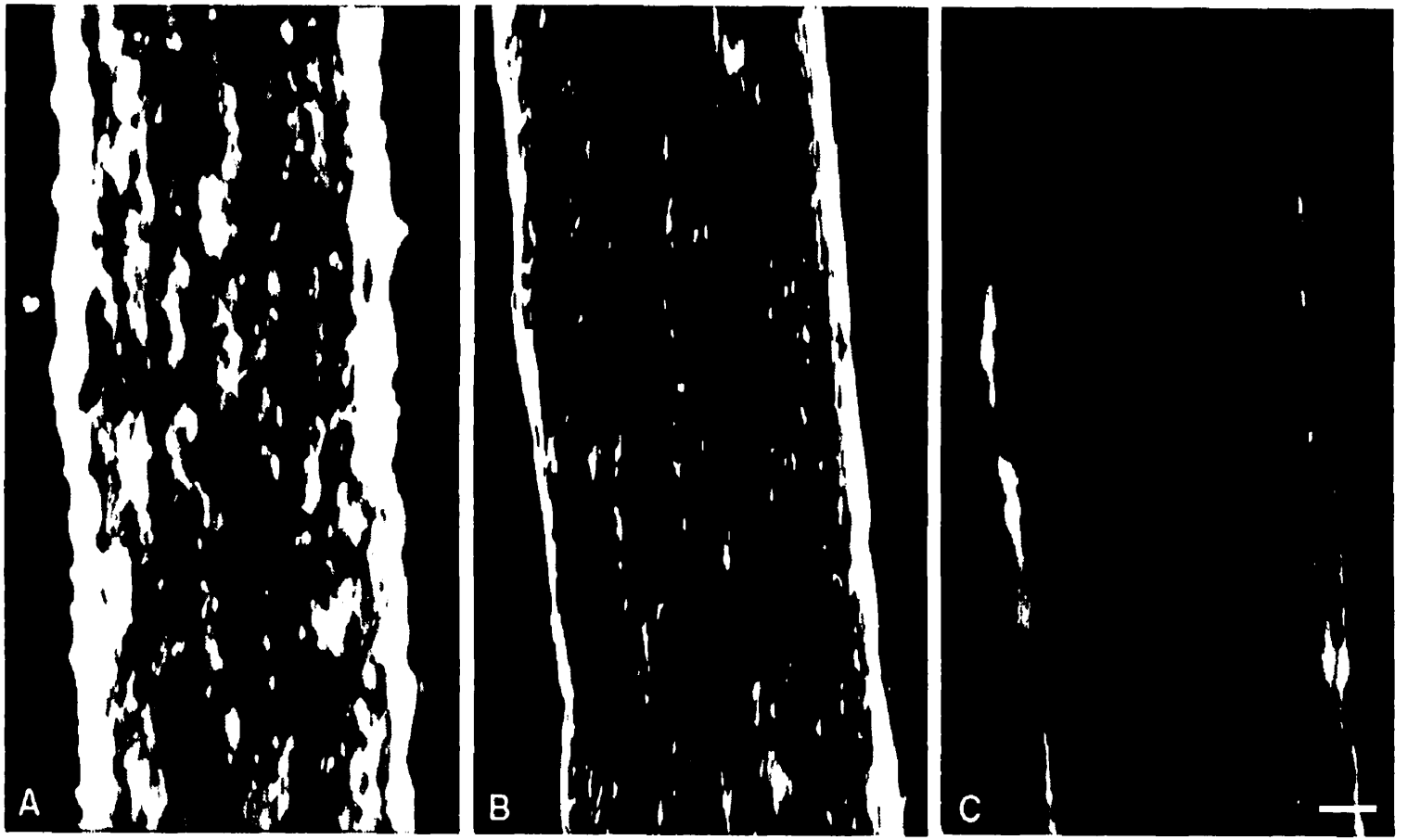

A
B
C

Figure IV-3: Comparison of localization of gelatinase activity with MBP or GFAP immunoreactivity in developing optic nerve. Immunohistochemistry of MBP (A and D) and GFAP (C and F), and in situ zymography (B and E) of P9 mouse optic nerve show that the pattern of proteolytic activity shown by in situ zymography appeared to be similar to that of longitudinal MBP immunoreactivity. In contrast, GFAP immunoreactivity was mainly perpendicular to that of MBP and in situ zymography (C and F). The bar scales are 100 μm (A-C), and 50 μm (D-E).

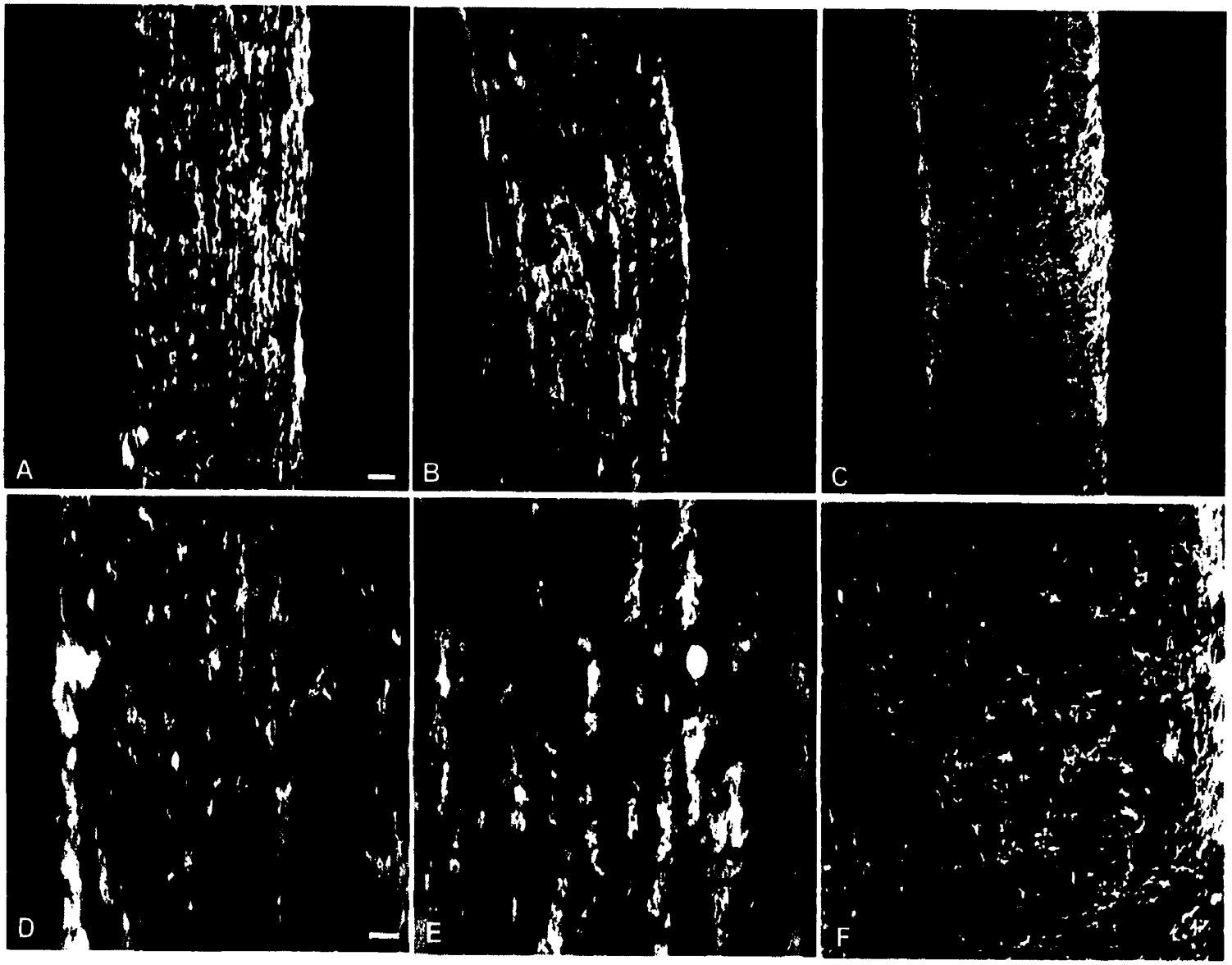
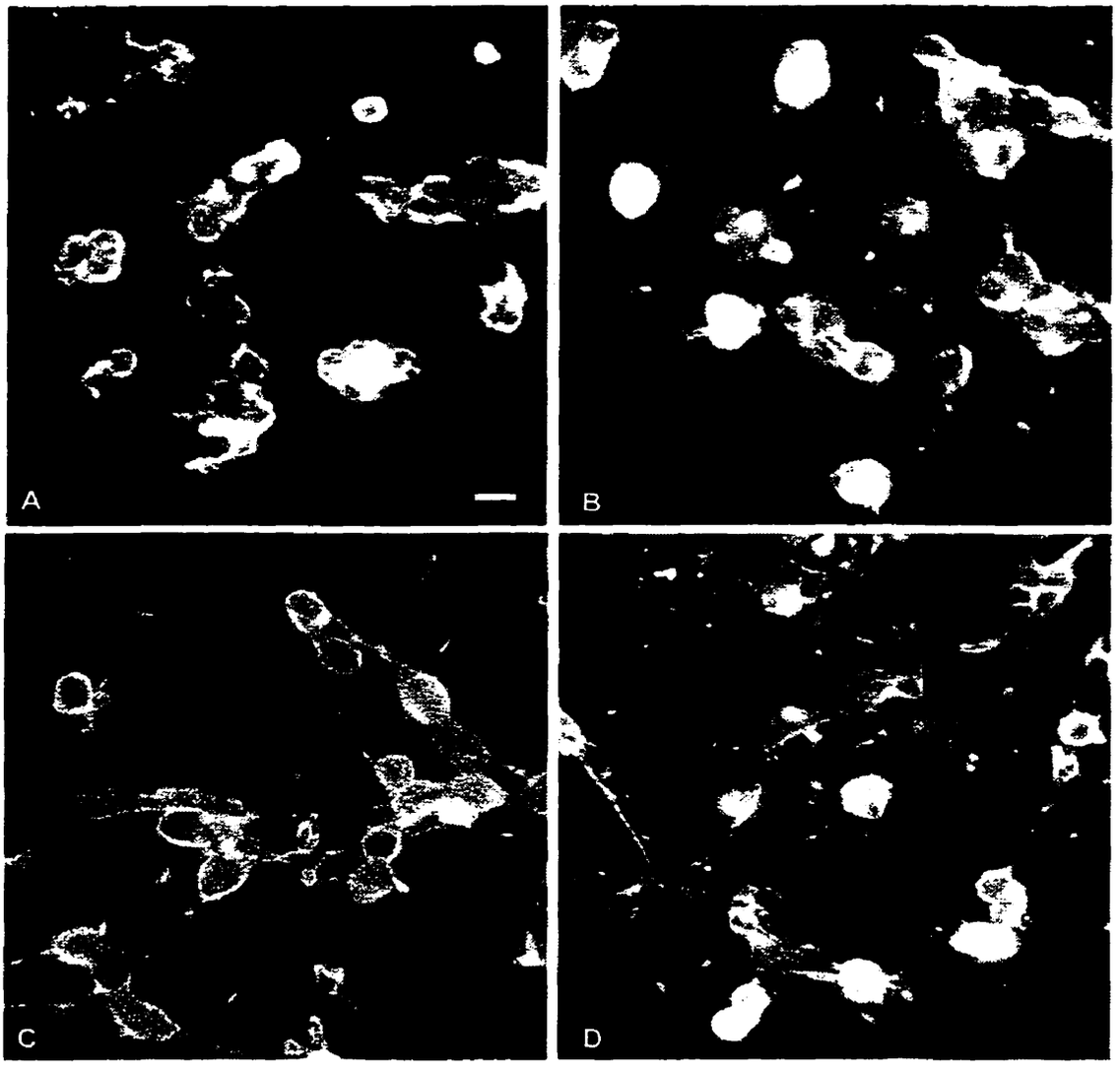

A
B
C
D
E
F

Figure IV-4: Morphology and MMP-9 expression by OLs. Bovine or murine OLs express MMP-9 and form processes upon PDB treatment. Panels (A and B) are control untreated bovine (A) and murine (B) OLs. In response to PDB treatment, both bovine (C) and murine (D) OLs up-regulate the extent of their process formation. In terms of morphology, human OLs resemble bovine OLs under basal and PDB-stimulated conditions. The bar scale in Panel (A) represents 10 μm. The expression of MMP-9 correlates with the process outgrowth induced by PDB (E) (shown for bovine OLs only). (F) Conditioned medium (CM) of OL cultures from mouse contain mainly pro-MMP-9, since APMA treatment of the conditioned medium (CM+APMA) converts pro-MMP-9 to the lower molecular weight active MMP-9. However, active MMP-9 was detected in OL cell lysate (Lysate).


A
B
C
D

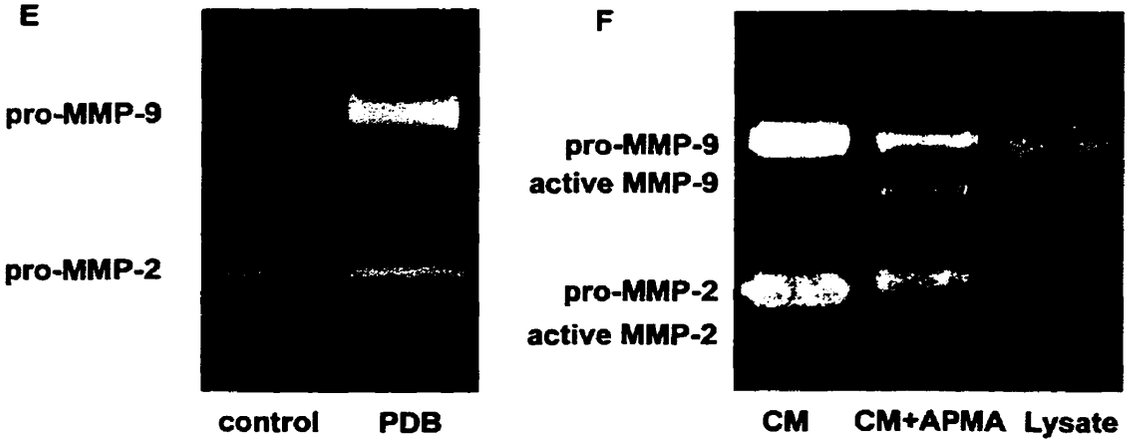

E
pro-MMP-9
pro-MMP-2
control
PDB
F
pro-MMP-9
active MMP-9
pro-MMP-2
active MMP-2
CM
CM+APMA
Lysate

Figure IV-5: Localization of MMP activity in OLs in culture by in situ zymography. Panels (A and B) are O1+ bovine OLs treated with PDB to induce process formation. Net proteolytic activity of MMP (C) was localized on OL soma, processes, and the tip of process (insert of C), while in the presence of 1,10-phenanthroline (50 μM), the MMP activity by OLs was inhibited (D). The uniform distribution of MMP-9 in OLs (C) contrasts the punctate gelatinolytic activity in BHK cells (D1); (D2) indicates MMP activity of BHK cells in the presence of 1,10-phenanthroline. Panel (E) is a superimposed image overlapping signal from O1 immunoreactivity (A), and in situ zymography (C), while Panel (F) is the corresponding superimposed image of (B) and (D). The bar scales in Panel (A and D1) represent 10 μm.

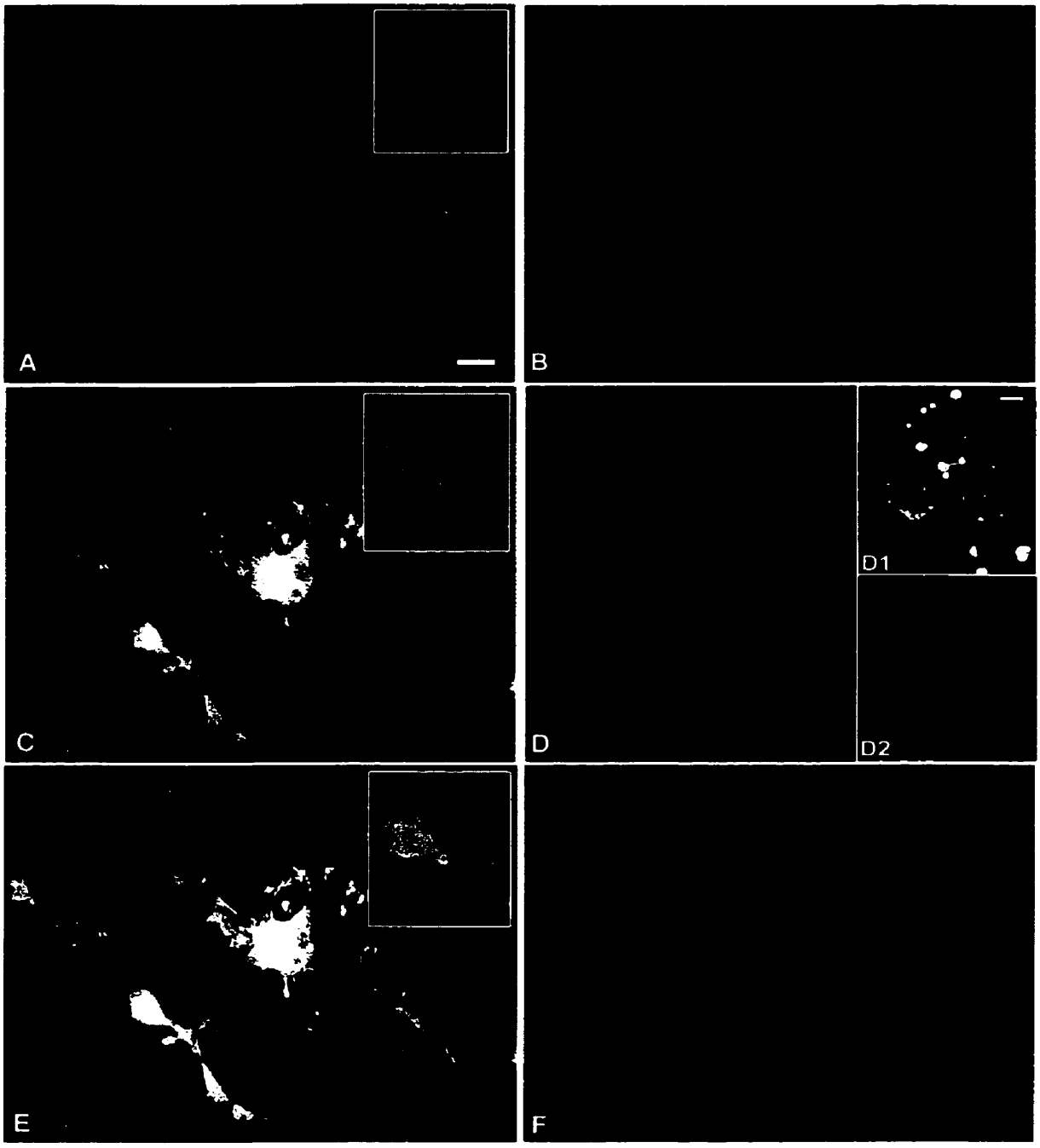

A
B
C
D
D1
D2
E
F

Figure IV-6: Effects of TIMP-1 on process outgrowth by OLs. The process outgrowth induced by 10 nM PDB in (A) human and bovine OLs and (B) mouse OLs was inhibited by recombinant human TIMP-1. TIMP-1 concentrations are given as ng/ml. Each bar is the mean $\pm$ SEM of four coverslips of cells. * $p<0.01$; ** $p<0.001$ compared to PDB treatment alone.

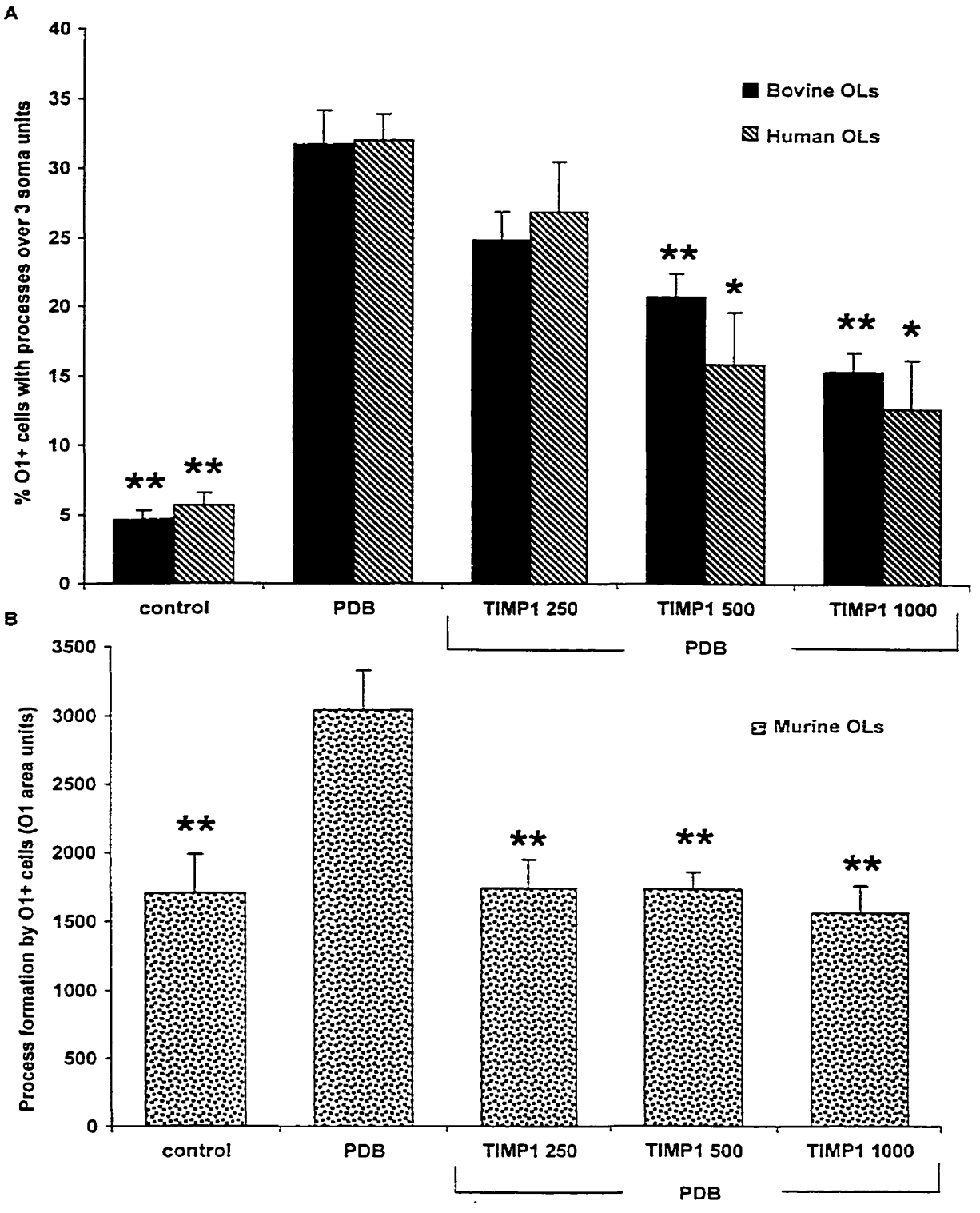
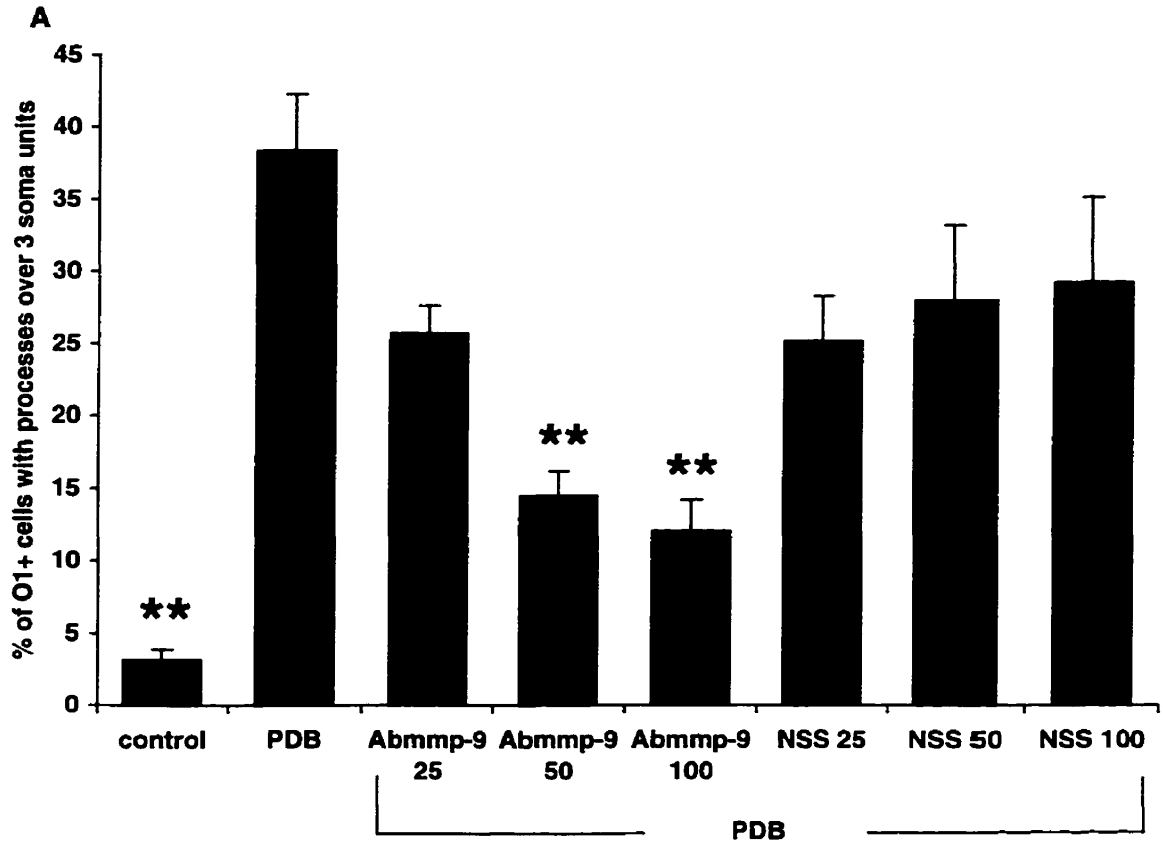

A
% O1+ cells with processes over 3 soma units
40
35
30
25
20
15
10
5
0
Bovine OLs
Human OLs
**
**
**
*
**
*
control
PDB
TIMP1 250
TIMP1 500
TIMP1 1000
PDB
B
Process formation by O1+ cells (O1 area units)
3500
3000
2500
2000
1500
1000
500
0
Murine OLs
**
**
**
**
control
PDB
TIMP1 250
TIMP1 500
TIMP1 1000
PDB

Figure IV-7: Effect of a neutralizing antibody to MMP-9 on process outgrowth by OLs. Panel (A) shows that a neutralizing MMP-9 antibody (Abmmp-9) inhibits the PDB-induced process outgrowth by bovine OLs. The concentrations of sheep anti-porcine MMP-9 and pre-immune normal sheep serum (NSS) were used are in μg/ml. Panel (B) shows the inhibitory effect of Abmmp-9 in OL process outgrowth induced by PDB, while in Panel (C), in the absence of Abmmp-9, OLs extend significant processes in response to PDB. ** $p<0.001$ compared to PDB treatment alone.


A
% of O1+ cells with processes over 3 soma units
45
40
35
30
25
20
15
10
5
0
**
**
**
control
PDB
Abmmp-9 25
Abmmp-9 50
Abmmp-9 100
NSS 25
NSS 50
NSS 100
PDB

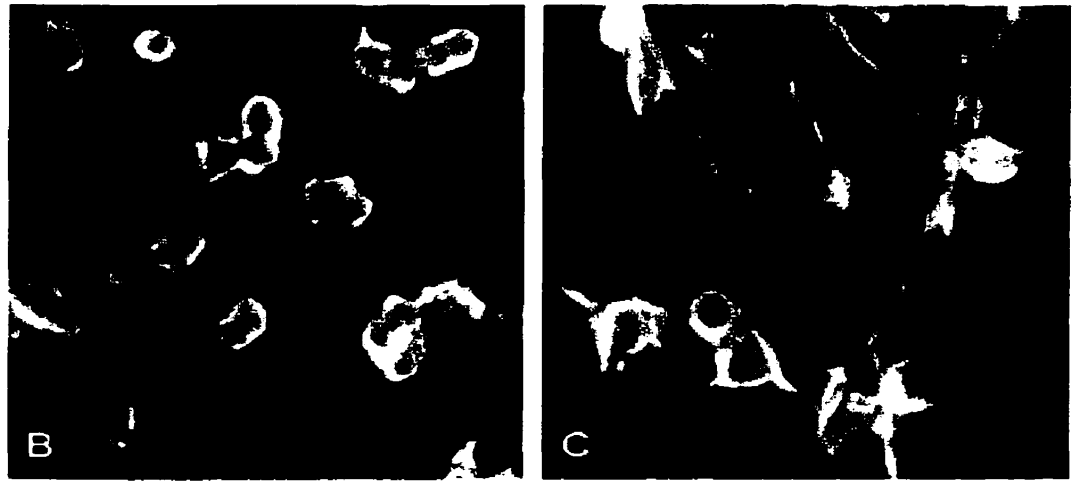
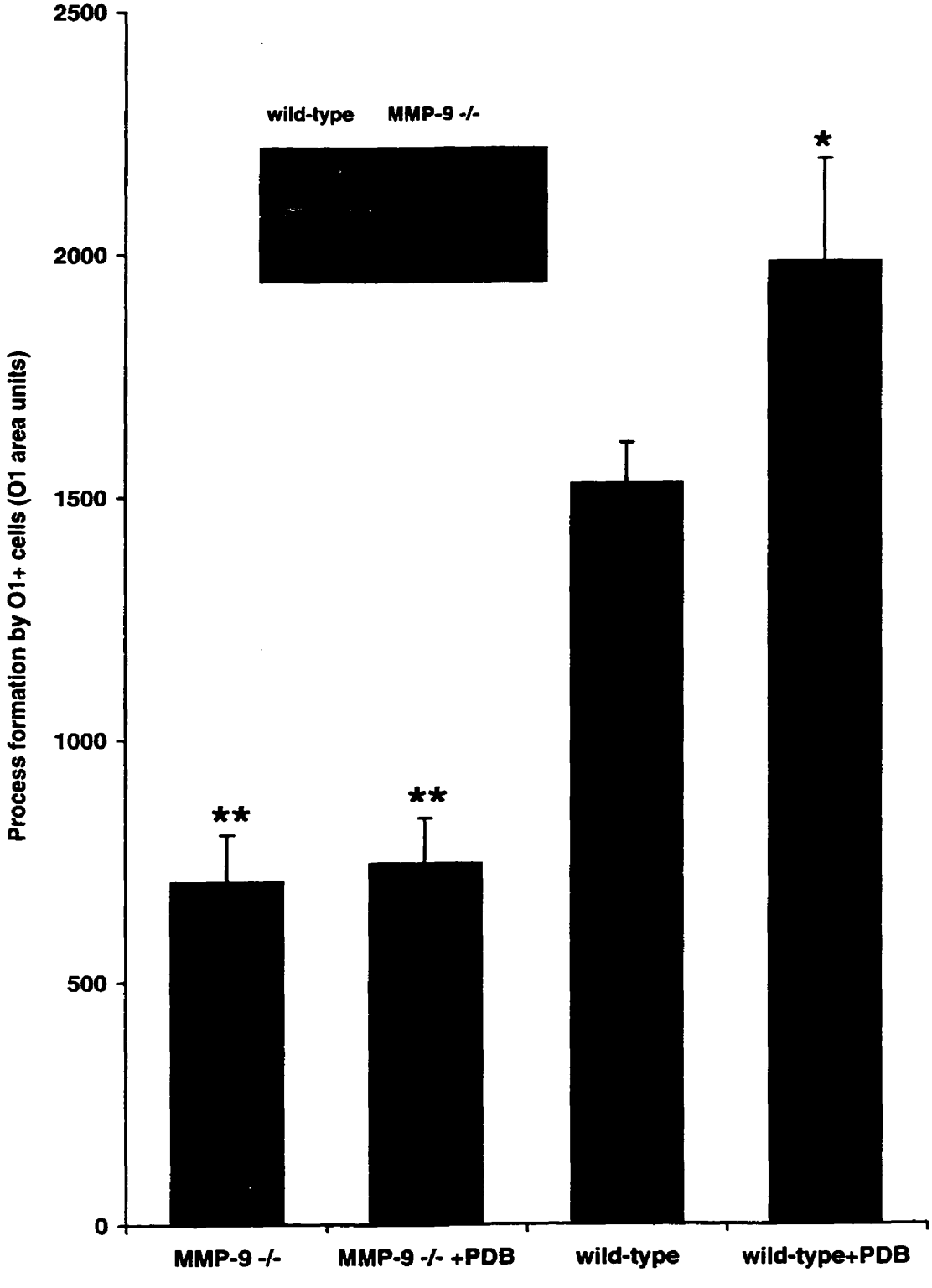

B
C

Figure IV-8: Effect of a targeted mutation in MMP-9 on process extension by OLs in culture. OLs derived from MMP-9 null mice exhibit reduced capacity to extend processes compared to OLs from wild-type mice, either under basal culture conditions or in response to 10 nM PDB. * $p<0.05$, ** $p<0.001$ compared to wild-type. A zymogram demonstrating the lack of MMP-9 in MMP-9 null mice, compared to wild-type, is inserted on the upper left.


2500
2000
1500
1000
500
0
Process formation by O1+ cells (O1 area units)
wild-type
MMP-9 -/-
**
**
*
MMP-9 -/-
MMP-9 -/- +PDB
wild-type
wild-type+PDB

CHAPTER V

Discussion of overall thesis and future direction

GENERAL DISCUSSION

Since the observation of CNS regeneration by Ramon y Cajal in 1928, numerous careful examinations have shown that CNS regeneration and remyelination are possible and in fact occurs in response to injuries (Ghatak et al., 1989; Prineas et al., 1993; Raine and Wu, 1993). However, unlike the peripheral nervous system, repair in the CNS is incomplete or fails. With respect to demyelinating diseases such as MS, one of the many challenges is to improve the extent of remyelination. Since morphological, biochemical and immunohistochemical analyses of the myelin sheath during remyelination (Ludwin and Sternberger, 1984; Ludwin, 1988; Jordan et al., 1990) support the concept that remyelination recapitulates developmental myelination, extensive studies have been carried to understand the mechanisms of developmental myelination in order to enhance the extent of remyelination in the CNS (Chapter I).

In this thesis, the mechanism of process outgrowth by OLs, an early event in myelinogenesis, is elucidated with respect to physiological activators and a role for PKC and MMPs. While much of the experiments were performed in vitro, inviting the comment that process extension in vitro may not mimic myelinogenesis in vivo in certain aspects, it should be noted that the in vitro observations are tested for similar roles in vivo (e.g. experiments of Chapter IV). Thus, in Chapter II, a role for astrocytes in promoting process outgrowth by OLs is demonstrated. The mechanism of the astrocyte effects appears to be through the interaction of bFGF and astrocyte-derived ECM. That astrocytes play an important role in OL process extension makes intrinsic sense, since astrocytes develop temporally in the CNS before OLs (Raff et al., 1983;

Raff, 1989), and because these two cell types are in close contact (Butt and Ransom, 1993; Butt et al., 1994) in the CNS. In contrast to astrocytes, purified microglia were toxic to OLs. The toxicity of microglia was negated if the microglia cultures contained in excess of 10% astrocytes (unpublished observation), highlighting the importance and fine balance of cell-cell interactions in the CNS.

At present, the identity of the astrocyte ECM component(s) that is responsible for inducing process outgrowth by OLs is unknown, but I will discuss several directions that would resolve this in the sections below on ECM.

My identification of bFGF and astrocyte ECM as promoters of OL process outgrowth place these factors, and phorbol esters, as the only known agents to date that have the capacity to enhance process extension by OLs cultured from the adult human brain. In Chapter III, by using relatively selective PKC antagonists, calphostin C and CGP41 251, my studies have led to the conclusion that PKC in OLs is central to OL process extension, since PKC mediates the promoting effects of bFGF and astrocyte-derived ECM in a range of concentrations that does not elicit cell death.

In Chapter IV, as downstream effectors of PKC activation, my results implicate a role for MMPs, particularly MMP-9, in facilitating process outgrowth by OLs during myelination in the mouse optic nerve. In vitro, the inhibition of MMP activity, specifically that of MMP-9, decreases process outgrowth by OLs. Furthermore, OLs derived from adult MMP-9 null mice have reduced ability to extend processes.

Collectively, my thesis has addressed the mechanism of process outgrowth by OLs which can be summarized in three aspects (Fig V-1): 1) physiological stimulator, where astrocytes are shown to promote OL process outgrowth through interaction

between bFGF and unknown ECM molecules, 2) signal transduction or mediators of process outgrowth, where I demonstrate that PKC is central to the promoting action of bFGF and astrocyte-derived ECM on process outgrowth by OLs, and 3) effector molecules, where my results suggest that MMP-9, downstream of PKC activation, facilitates OL process formation. In the sections below, I shall discuss my specific results and suggest future directions.

FGF FAMILY

In Chapter II, basic FGF (and aFGF to lesser extent) was shown to significantly promote process outgrowth by adult human OLs. However, bFGF (FGF-2) and aFGF (FGF-1) are members of a larger family which comprises at least 20 members to date with significant homology; for instance, aFGF (FGF-1) and bFGF share about 50 % sequence identity (Eckenstein, 1994; Klint and Claesson-Welsh, 1999). With the identification and characterization of more FGF family members, it would be of interest to address effects of other FGF family members on OL biology. Although FGF-1, -2, and -9 do not have "classical" leader sequence for secretion, they have been shown to be secreted possibly by novel mechanisms (Mason, 1994; Friesel and Maciag, 1995; Bikfalvi et al., 1997). FGFs are potent mitogens for cells from mesodermal and neuroectodermal origins and influence cell motility, differentiation, survival, and neurite extension (Galzie et al., 1997; Klint and Claesson-Welsh, 1999). Some FGFs such as FGF-4 (hst-1) have been identified as oncogenes indicating a possible role in pathological processes (Mason, 1994). While FGF-7 was identified as a mitogen for

keratinocytes (Finch et al, 1989), FGF-9 was shown to promote the proliferation of astrocytes (Miyamoto et al., 1993).

FGFs bind to receptors and there are four members of the FGF receptor family (FGFR1-4) (Galzie et al., 1997; Klint and Claesson-Welsh, 1999). FGFR contains three extracellular immunoglobulin (Ig) -like domains, a single transmembrane domain, and two intracellular kinase domains. The extracellular Ig-like domains of FGFR are responsible for specificity and affinity to different FGF members (Mason, 1994; Friesel and Maciag, 1995; Klint and Claesson-Welsh, 1999). The presence of isoforms of each FGFR by alternative splicing increases the complexity of FGF-FGFR interactions. Several studies employing in situ hybridization and immunohistochemistry have demonstrated distinct patterns of FGFR expression in the CNS; FGFR-2 mRNA expression was concentrated in the fiber tracts (Asai et al., 1993) while OL expressed significant levels of FGFR-1 mRNA in vivo (Gonzalez et al., 1995). Redwine et al. (1997) reported that PDGFR-α + OL progenitor cells express significant levels of FGFRs in adult mouse brain. In vitro, OLs were shown to express FGFR-2 mRNA whereas neurons express FGFR-1 and -4 mRNA (Miyake et al., 1996). Bansal et al. (1996) demonstrated that FGFRs are expressed in a specific manner during OL development; the level of FGFR-1 increases gradually as OLs differentiate, whereas FGFR-2 is predominantly expressed by differentiated OLs. Pre-OLs express high level of FGFR-3 while FGFR-4 is not detectable in OLs. OLs expressing a mutated FGFR-1 transgene are not able to migrate when transplanted into the neonatal rat brain suggesting a role of FGF in OL migration (Osterhout et al., 1997).

A number of studies implicated the importance of proteoglycans, specifically heparin sulfate proteoglycan (HSPG), in FGF binding to the receptor (Galzie et al., 1997; Klint and Claesson-Welsh, 1999). Although it is unclear whether HSPG is necessary for FGF signaling, it is generally accepted that HSPG stabilize or enhance FGF-FGFR interactions. In vivo HSPG is abundant in a variety of ECM molecules; they include perlecan, transmembrane molecules such as syndecans, betaglycan and certain isoforms of CD44 (Mason, 1994). Perlecan and syndecans have been demonstrated to promote bFGF binding to the receptors effecting mitogenesis and angiogenesis (Aviezer et al., 1994; Steinfeld et al., 1996). In vitro, heparin has been shown to form oligomerization of aFGF resulting in dimerization and activation of FGFRs which in turn induce c-fos mRNA transcription and cell proliferation (Spivak-Kroizman et al., 1994). As noted in Chapter II, the addition of HSPG did not potentiate the OL process forming capacity of bFGF. The reason for this is not immediately apparent but can be attributable to soluble serum factors, possibly HSPG, already present in the medium.

Cell adhesion molecule (CAM) domain has been identified in FGFR, and adhesion molecules including N-CAM, L1, and N-cadherin bind FGFRs and stimulate neurite outgrowth in vitro (Williams et al., 1994; Doherty et al., 1995). These stimulatory effects of CAMs appear to be independent of bFGF signaling implicating the multifunctional role of FGFRs which is supported by the diversity of molecules that bind to cytoplasmic region of FGFRs. The cytoplasmic tail of FGFRs can bind to several signal transduction molecules including FAK, PI 3-K (phosphoinositol 3-kinase)

and PLC γ, and adapter proteins including Grb2 which involves MAP kinase signaling pathway (Galzie et al., 1997; Klint and Claesson-Welsh, 1999).

With respect to future direction, it would be of interest to examine other members of the FGF family to determine if these were more effective than bFGF in promoting OL process extension. In this regard, the direct administration of individual FGF member to OL cultures can be conducted. Indeed, recently, FGF-16 has been demonstrated to induce oligodendrocyte proliferation (Danilenko et al., 1999). With certain ECM constituents, one can also study the possible combinational effects on OL biology. Furthermore, in vivo function perturbation experiments, using neutralizing antibodies to bFGF (or other FGF members) could be performed to establish a role for FGFs in the mouse optic nerve during myelin formation. FGFR knockout models would be of interest to evaluate not only a role for FGFs but also that of the interacting adhesion molecules which may influence myelin formation and remyelination. Finally, while isolated studies have shown FGFR-2 mRNA in fiber tracts (Asai et al., 1993), a systematic study of FGFR (1-4) expression in the developing OL during the period of myelin formation, correspondent with studies of FGF expression by interacting astrocytes, would be of interest to test the hypothesis that astrocytes provide the stimulus for OLs to extend their processes during myelin formation through a mechanism involving bFGF.

EXTRACELLULAR MATRIX

In Chapter II, it is demonstrated that ECM deposited by astrocytes contains laminin, fibronectin, and vitronectin among others. The inability of purified ECM components to promote OL process outgrowth by themselves has left unresolved the identity of the astrocyte-derived ECM component. While there is accumulating evidence that certain ECM constituents such as laminin and tenascin have stimulatory activity for neurite outgrowth (Varnum-Finney et al., 1995; Condic and Letourneau, 1997), a role for ECM in OL biology is poorly appreciated. Bartsch et al. (1993) reported that during myelination, tenascin-R (i.e. janusin) is transiently expressed on myelin sheath in the mouse optic nerve. Recently, the same group has shown that tenascin-R is expressed by OLs in vitro and promote OL adhesion and differentiation in an autocrine manner (Pesheva et al., 1997).

The term "integrin" was originally coined to reflect a role of receptors in integrating the intracellular cytoskeleton with ECM. The integrin receptor family is composed of non-covalently linked α and β subunits. There are at least 12 α subunits and 9 β subunits, which can form various heterodimers. In the extracellular space, integrin receptors on cell surfaces bind to ECM molecules. However, in addition to the interaction with ECM, integrins are well recognized for their interaction with a variety of cell adhesion molecules, growth factor receptors (Yamada and Miyamoto, 1995) and even MMPs. Insulin-like growth factor receptors interact with $\alpha v\beta 5$ integrin modulating metastasis of tumors (Brooks et al., 1997). In glioma cells, $\alpha v\beta 3$ integrin receptor has been shown to interact with the C-terminal of MMP-2 (Brooks et al., 1996; Deryugina et al., 1997). Integrin receptors bind to uPAR (urokinase plasminogen activator receptor) which also has binding site for vitronectin and uPA (Stewart et al.,

1995; Blasi, 1997; Chapman, 1997). These interactions suggest potential mechanisms to localize MMP activity to specific site since PA (plasminogen activator) is an activator of broad range of MMPs.

Although integrin itself does not have any kinase activity, many kinases can bind to the cytoplasmic tail of integrins either directly or indirectly; these include FAK, PLC γ, PI 3-K, Ras, Raf, MEK, and ILK (integrin-linked kinase) (Yamada and Miyamoto, 1995; Giancotti, 1997). FAK is important in recruiting and activating several signaling cascades including MAPK (mitogen activated protein kinase) and PKC to affect cell proliferation, migration, differentiation as well as apoptosis (Guan, 1997). Cells from FAK-deficient mice demonstrated reduced motility without any significant disruption in focal adhesion (Ilic et al., 1995).

Another important aspect of integrin signaling is the rearrangement of cytoskeletal proteins. It has been reported that ligation of integrin receptors induces direct or indirect binding and localization to focal contacts of PKC, focal adhesion kinase (FAK), and cytoskeletal proteins including talin, α-actinin, tensin, actin, vinculin, and paxillin (Yamada and Miyamoto, 1995; Lewis et al., 1996). Intracellular signal transduction through PKC or PI 3-K regulates the activation of integrin receptors to bind ligands such as laminin and fibronectin (Kolanus and Seed, 1997). Upon ligation, redistribution of integrin receptors by cytoskeleton reorganization has been demonstrated (Felsenfeld et al., 1996).

For many years, investigators have focused on either effect of growth factors or that of ECM constituents separately in cellular responses. In a more physiological context, however, growth factors and ECM constituents are present together and

influence cells simultaneously. In this regard, it is noteworthy (Chapter II) that certain ECM components augment the effect of bFGF in promoting OL process outgrowth. It is unclear how bFGF and the ECM substrates (i.e. laminin and fibronectin) synergize to promote process formation by OL while no effects are observed by ECM substrates alone. One speculation is that bFGF may influence integrin receptor expression or activation to mediate ECM signaling in OLs. A number of growth factors modulate the expression and activation of specific integrin receptors and affect their interaction with ECM molecules. For instance, bFGF and TGF-β have been shown to change the expression of integrin receptors resulting in differential adhesion of endothelial cells and vascularization (Enenstein et al., 1992; Klein et al., 1993; Brooks et al., 1994). Angiogenesis induced by bFGF or TNF-α is regulated in an αvβ3-dependent manner whereas that induced by TGF-α is regulated in an αvβ5-dependent manner (Friedlander et al., 1995). Neurite outgrowth is promoted by laminin or collagen I by interacting with integrin receptors that are up-regulated by NGF or aFGF (Zhang et al., 1993; Weaver et al., 1995). In addition, in mouse endothelial cells, the mRNA level encoding the αv integrin subunit is up-regulated upon PKC activation (Tang et al., 1995), implicating PKC activity in the regulation of integrin receptors (Kolanus and Seed, 1997).

There is accumulating evidence that ECM molecules induce specific MMP expression. Administration of RGD peptides induces the expression of MMP-3,-2 and -9 in chondrocytes (Arner and Tortorella, 1995), while the levels of MMP-1,-3 and -9 are up-regulated in fibroblasts (Werb et al., 1989). Fibronectin and vitronectin contain an RGD domain, which interact with a range of integrin receptors including α5β1,

αvβ3, and αvβ4. Thus, the same ECM molecule can elicit different cellular responses depending on integrin receptors. Fibronectin, for example, can interact with α4β1 and α5β1 in fibroblast; ligation of the CS-1 domain of fibronectin to α4β1 inhibits the expression of MMPs while RGD-domain binding to α5β1 up-regulates the levels of MMPs (Werb et al., 1989; Huhtala et al., 1995). Therefore, cellular response to ECM is dependent on what and how ECM molecules are presented and what integrin receptors are expressed. Collagen provided in 3D gel induces α2 integrin and MMP-1 mRNA in fibroblasts; this effect appears to be mediated by PKC ζ (Xu and Clark, 1997).

In summary, the results of Chapter II indicate that the astrocyte ECM is a promoter of OL process outgrowth, although the identity of the ECM component(s) is not known. However, the evidence of an intimate interaction between ECM and integrin suggests several approaches to identify the astrocyte ECM constituents that promote OL process extension. First, the integrin receptor profile present on OLs can be addressed. In this regard, given that integrins are a large family, I would focus first on the β1 integrins for the following reasons. Many of the ECM molecules that are present in the astrocyte matrix are known to interact with β1 integrins. In addition, the β1 subunit can form complexes with a variety of α chains. Furthermore, an anti-β1 antibody inhibited myelination by Schwann cells (Fernandez-Valle et al., 1994) and OLs are known to express β1 integrins (Malek-Hedayat and Rome, 1994; Milner et al., 1996). Indeed, preliminary experiments using a β1 integrin immunoprecipitation approach have revealed an increase in αvβ1, α5β1 and α3β1 integrins associated with increased process extension (unpublished observation). Thus, the profile of integrin receptors expressed by OLs during myelinogenesis is of interest.

A second approach to identify interacting ECM components that are involved in OL process outgrowth is to administer function perturbing antibodies against specific integrin receptors on OLs. Thirdly, astrocyte ECM treated with antibodies against specific ECM components (guided by the integrin results), or with blocking peptides, can be examined for their efficacy in promoting OL process outgrowth. Furthermore, in vivo, the examination and identification of ECM components that are expressed transiently during the period of developmental myelination or remyelination in demyelinated lesion will suggest which ECM constituents to target. Finally, using the mouse optic nerve where the myelinating period can be examined systematically, the injection of function perturbing antibody directed against specific ECM constituents will help to reveal the identity of important ECM molecules that facilitate OL process extension and myelin formation. Overall, these experiments should provide more insights of the complex interactions of FGF, ECM, MMP and integrin receptors in OL biology. In the long term, it may lead to the administration of several factors (i.e. growth factor and ECM molecule) in combination to enhance the extent of remyelination in demyelinated lesion.

PROTEIN KINASE C

In Chapter III, a role for PKC in OL process outgrowth is demonstrated by using relatively selective PKC antagonists. However, whether specific PKC isozymes are involved was not addressed. Previous work suggested that PKC α is an important isoform in OL process outgrowth; OLs in vitro formed significant processes in response

to thymeleatoxin which activate cPKCs (i.e. PKC α,β and γ), whereas no OL process formation was observed in response to resiniferatoxin which activates PKC βI and γ (Yong et al., 1994). With the recent improvement of specificity of PKC antibody and PKC activity assay, it is feasible to address a role of individual PKC isoforms in OL biology.

In Chapter IV, MMPs have been investigated as downstream effectors of PKC activation in OLs. It should be noted that the expression of MMP alone is not sufficient for OL process outgrowth as demonstrated by the lack of process formation when recombinant MMP-9 was added to the culture medium. Rather the simultaneous orchestration of many other effectors would be required to achieve process formation by OLs; one of these aspects is cytoskeletal reorganization, and process formation by OL probably requires significant redistribution and up-regulation of cytoskeletal proteins. Accumulating evidence shows that the modulation of cytoskeletal structure significantly influences OL development including morphology and distribution of myelin proteins (Wilson and Brophy, 1989; Benjamins and Nedelkoska, 1994; Lunn et al., 1997). Although early OL progenitors contain vimentin, an intermediate filament, this has not been observed in mature OLs. Instead, extensive networks of actin filaments and microtubules are found in OLs; while fine processes of OLs are rich in actin filaments, mature OL processes contain mainly microtubules (Gonatas et al., 1982; Wilson and Brophy, 1989). Myelin proteins are closely associated with cytoskeletal proteins; actin filaments are associated with CNP whereas microtubules interact with MBP and PLP (Bizzozero et al., 1982; Wilson and Brophy, 1989). Transport of MBP mRNA to process may involve microtubules and their motor proteins such as kinesin and dynein

(Ainger et al., 1993; Hirokawa, 1998). In malignant glioma cells, inhibition of actin polymerization by cytochalasine down-regulates MMP-9 and alters cell morphology (MacDougall and Kerbel, 1995; Shravan et al., 1998) whereas cytochalasine treatment increases the expression of MMPs in fibroblasts (Unemori and Werb, 1986; Tomasek et al., 1997) suggesting that actin polymerization is involved in the regulation of MMP expression.

There is growing evidence that PKC phosphorylates cytoskeletal proteins and that PKC activation induces cytoskeletal rearrangement (Toker, 1998). A number of PKC substrates including RACK (receptor for activated C kinase) and MARCKS (myristoylated alanine-rich C kinase substrate) are implicated to interact with actin filaments (Hartwig et al., 1992; Battaini et al., 1997). PKC ε has a unique actin-binding motif, which localized at the nerve terminals (Prekeris et al., 1996). In addition, PKC βII and ζ also have distinct actin-binding motifs and interact with actin (Blobe et al., 1996; Gomez et al., 1996). PKC α and ε are translocated to focal adhesions following integrin activation in smooth muscle cells (Haller et al., 1998). PKC activation in fibroblasts cultured on a fibronectin substrate induces focal adhesion formation with localization of various cytoskeletal proteins such as vinculin, talin as well as integrin β1 subunit (Woods and Couchman, 1992).

Several reports have demonstrated a role for PKC in phosphorylation of FAK (Vuori and Ruoslahti, 1993; Mogi et al., 1995; Lewis et al., 1996; Zhang et al., 1996). Although at present it is unknown whether or how FAK may influence OL process outgrowth, it would be worthwhile to examine this in regard to process outgrowth.

In summary, the overall picture of how PKC affects OL process outgrowth is incomplete. While MMP-9 production is a consequence of PKC activation in OLs that likely facilitates the advancement of OL process through the ECM, other effectors downstream of PKC activation remain to be investigated. Since cell morphology and MMP production is closely related to actin polymerization, it will be intriguing to examine PKC activation and related actin polymerization in OLs. For instance, administration of inhibitors of actin polymerization such as cytochalasine followed by PKC activation will reveal the necessity for actin polymerization in process outgrowth by OLs. Furthermore, colocalization of specific PKC isoforms with FAK or the actin cytoskeleton by immunohistochemistry and co-immunoprecipitation may lead to the identification of specific PKC isoforms responsible for process outgrowth in OLs.

MATRIX METALLOPROTEINASES

There are over 30 different zinc dependent metallopeptidase families, which are categorized into five groups depending on the mode of zinc binding. The metzincins are those that bind the active zinc using three histidine residues within the sequence motif HExxHxxGxxH. There are four subgroups of metzincins; astacins, serralysins, adamalysins and the MMPs. Within the MMP family or matrixins, at least 18 members are known and are divided into four groups (Chapter I). In Chapter IV, two MMPs (i.e. MMP-2 and -9) have been examined with respect to process outgrowth by OLs; MMP-9 is inducible by PKC activation in OLs and appears to be required for process outgrowth

by OLs whereas MMP-2 is not. However, it should be noted that other MMP members cannot be excluded from participating in OL biology including myelination.

To evaluate whether other MMPs are involved in myelin formation, the profile of MMP expression may be determined during developmental myelination by using approaches such as multi probe RNAse protection assay. Once the profile of MMPs that is expressed is determined, a number of approaches to elucidate their functions may be conceived; first, inhibition of the particular MMP activity can be analyzed in vivo by administering function perturbing antibody during myelin formation in murine optic nerve. Specific ribozyme, or other antisense approaches may be used. Ribozyme provides high sensitivity and catalytic efficacy, and one ribozyme can cleave many target mRNAs. Using MMP-9 ribozymes it has been demonstrated that MMP-9 is required in metastasis of tumor cells (Hua and Muschel, 1996; Sehgal et al., 1998). Second, OLs expressing specific MMPs can be studied in vitro for process forming capacity or in vivo for remyelinating capacity by transplanting these into demyelinated lesions of rodent brain. It has been shown that using adenovirus infection, MMP-9, TIMP-1 and -2 can be effectively over-expressed in smooth muscle cells (Baker et al., 1996). Third, specific MMP knockout models can be employed to address the role of particular MMP member in myelin formation.

Several MMP gene knockout mice have become available (Khokha et al., 1995; Shapiro, 1998). In this thesis, OLs from adult MMP-9 null mice exert reduced ability to extend processes compared to that of wild-type OLs (Chapter IV). Generally, however, MMP knockout mice do not show severe defects during development or in adulthood. Impaired angiogenesis and suppression of tumorigenesis have been reported in MMP-2,

-9, or -7 knockout models (Wilson et al., 1997; Itoh et al., 1998; Vu et al., 1998). TIMP-1 knockout mice also exists and an initial study of metastasis of lung tumor cells did not show significant changes from wild-type controls (Soloway et al., 1996); it is possible that other TIMPs may compensate for the lack of TIMP-1 in the knockout mice. Gene-knockout models provide valuable tool to investigate roles of individual MMPs and TIMPs in numerous physiological events including developmental myelination and repair in the demyelinated lesion. Normal myelin formation and up-regulation of MMP-12 during myelination in the optic nerve of MMP-9 null mice (unpublished observation) implicate redundancy of these proteinases. Determination of whether remyelination occurs and if so what MMPs are involved in that process in demyelinated lesion in MMP-9 null mice will provide valuable insight of the role of MMPs in vivo. Although several reports have demonstrated localization of MMPs in infiltrated monocytes, microglia, and astrocytes in demyelinated lesions, certain controversy remains to be clarified. In addition to acute and chronically active lesion, studies of MMPs in remyelinated sites have to be pursued where OLs are likely to utilize MMP for remyelination.

Gene-knockout experiments and over-expression experiments must both be interpreted with some caution as the ablation of one gene may lead to compensation by another gene during development, and non-physiological high concentrations of a particular protein may lead to effects that can be either instructive or misleading. Overall, with this caution in mind, future investigation focusing on 1) inhibition of MMP activity in vivo, 2) MMP over-expressing OLs and 3) MMP knockout models, should lead to a better appreciation of the role of MMPs during OL myelin formation.

CONCLUDING PERSPECTIVES

In summary, I have demonstrated that astrocytes promote OL process outgrowth, a prerequisite event for myelin formation, through the interaction between bFGF and astrocyte ECM and that PKC mediates these promoting effects using MMP production as an effector molecule.

I would like to provide a provocative scheme of how OL process outgrowth occurs during myelin formation. As adjacent astrocytes secrete bFGF and deposit stimulatory ECM molecules, FGF and integrin receptors on OLs interact with these to result in PKC activation in OLs. Cytoskeletal rearrangement within OLs occurs to allow for process to form and the advancement of OL processes through ECM is enabled by MMPs (i.e. MMP-9). The stimulus for MMP production by OLs possibly stems from the signals initiated by bFGF and ECM. The activity of MMP-9 is then controlled by TIMPs, particularly TIMP-3 bound on ECM. The presence of TIMPs may turn the tip of OL process towards permissive regions of ECM where TIMPs are absent or present at low level. Regions of high concentrations of stimulators (i.e. bFGF bound in ECM and permissive ECM molecules) may attract OL processes, further activating PKC and stimulating MMP-9 production. Thus, the precise balance between stimulators and inhibitors is likely to control and guide the advancement of OL processes towards their target axons. It is presently unknown if OL processes are destined for their target axons through a defined genetic program, or whether OL processes proceed haphazardly towards axons. However, the scheme that I have

proposed would suggest that OL process extension towards axons occurs in a defined and controlled manner, dictated by specific interactions between localized bFGF, permissive ECM, production of MMPs and inhibition by TIMPs.

Overall, in this thesis, the mechanism of process outgrowth by OLs is described. There are still many challenges to be overcome to understand the complexity of OL biology with particular respect to myelin formation. It is my hope that studies in this thesis will help to broaden our understanding of myelination and someday may lead to better treatment to improve devastating demyelinating diseases such as MS.

Figure V-1: A schematic representation of mechanism of process outgrowth by OLs. 1. Stimulators; astrocytes stimulate OLs through the interaction between bFGF and ECM molecules (Oh and Yong, 1996) which bind FGF and integrin receptors, respectively. 2. Mediators; OL PKC is involved in signal transduction pathways of bFGF and astrocyte ECM to promote process outgrowth by OLs (Oh et al., 1997). 3. Effectors; As downstream of PKC activation, MMPs, particularly MMP-9, is up-regulated and involved in process extension by OLs (Oh et al., 1999).

I. STIMULATORS

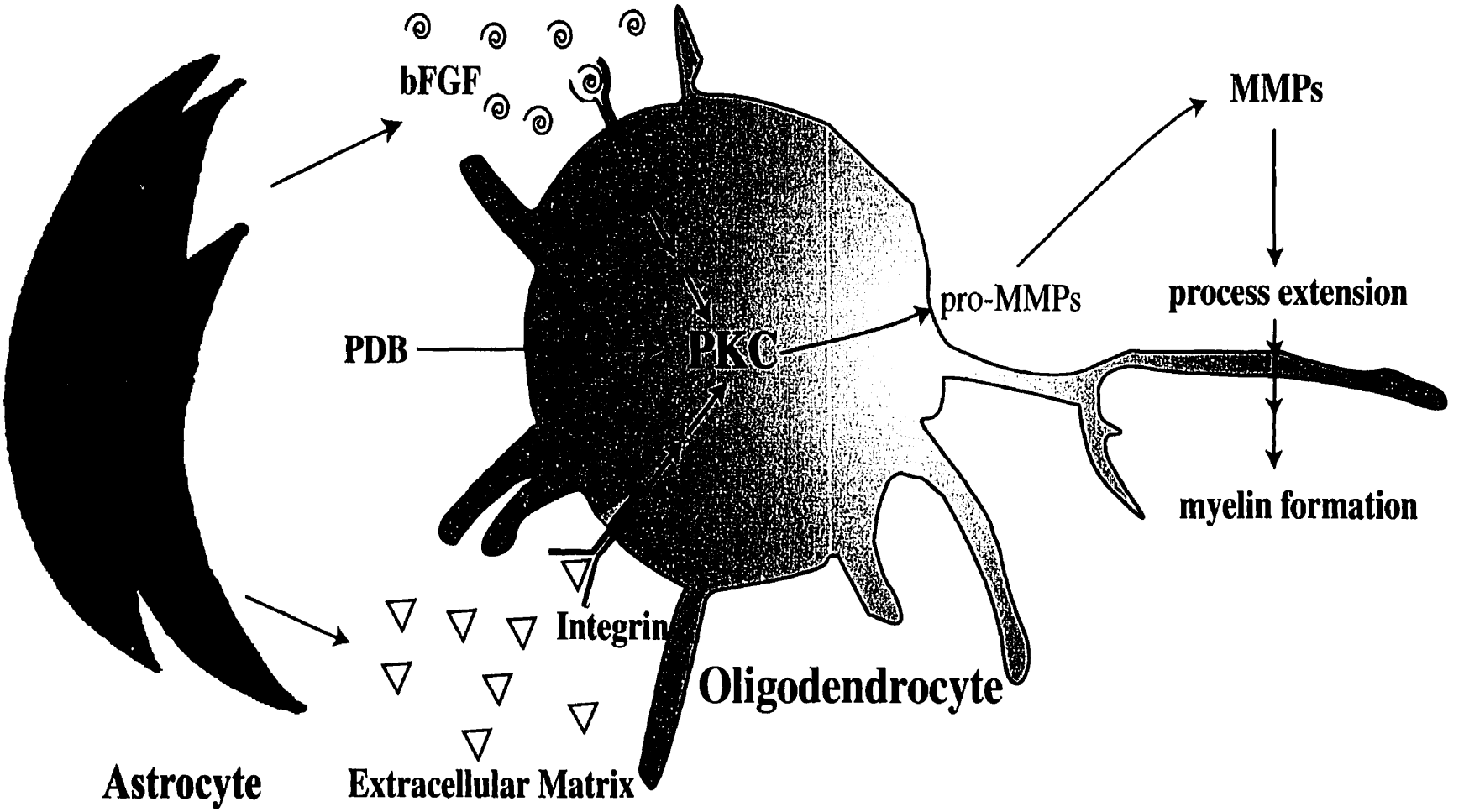
Oh, L.Y.S. and Yong, V.W. (1996) Astrocytes promote process outgrowth by adult human oligodendrocytes in vitro through interaction between bFGF and astrocyte extracellular matrix. GLIA, 17:237-253

II. MEDIATORS

Oh, L.Y.S., Goodyer, C.G., Olivier, A., and Yong, V.W. (1997) The promoting effects of bFGF and astrocyte extracellular matrix on process outgrowth by adult human oligodendrocytes are mediated by protein kinase C. Brain Res., 737:236-244

III. EFFECTORS

Oh, L.Y.S., Krekoski, C.A., Edwards, D.R., Donovan, F., Werb, Z., and Yong, V.W. (1999) Matrix Metallo-proteinase-9/Gelatinase B is required for process outgrowth by oligodendrocytes (manuscript submitted).



REFERENCES

Ahonen M, Baker AH, Kahari VM (1998) Adenovirus-mediated gene delivery of tissue inhibitor of metalloproteinases-3 inhibits invasion and induces apoptosis in melanoma cells. Cancer Res 58:2310-2315.

Aigner L, Arber S, Kalfhammer JP, Laux T, Schneider C, Botteri F, Brenner HR, Caroni P (1995) Overexpression of the neural growth-associated protein GAP-43 induces nerve sprouting in the adult nervous sustem of transgenic mice. Cell 83 (2):269-278.

Ainger K, Avossa D, Morgan F, Hill SJ, Barry C, Barbarese E, Carson JH (1993) Transport and localization of exogenous myelin basic protein mRNA microinjected into oligodendrocytes. J Cell Biol 123:431-441.

Althaus HH, Klöppner S, Schmidt-Schultz T, Schwartz P (1992) Nerve growth factor induces proliferation and enhances fiber regeneration in oligodendrocytes isolated from adult pig brain. Neurosci Letts 135:219-223.

Althaus HH, Schroter J, Spoerri P, Schwartz P, Kloppner S, Rohmann A, Neuhoff V (1991) Protein kinase C stimulation enhances the process formation of adult oligodendrocytes and induces proliferation. J Neurosci Res 29:481-489.

Althaus HH, Schwartz P, Klöppner S (1990) Protein kinases A and C are involved in oligodendroglial process formation. In Cellular and Molecular Biology of Myelination. (Jeserich G, Althaus HH, Wachneldt TV, eds), pp 247-253. Berlin, Heidelberg: Springer-Verlag.

Amberger VR, Avellana-Adalid V, Hensel T, Baron-van EA, Schwab ME (1997) Oligodendrocyte-type 2 astrocyte progenitors use a metalloendoprotease to spread and migrate on CNS myelin. Eur J Neurosci 9:151-162.

Amor S, Groome N, Linington C, Morris MM, Dornmair K, Gardinier MV, Matthieu JM, Baker D (1994) Identification of epitopes of myelin oligodendrocyte

glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice. J Immunol 153:4349-4356.

Amur-Umarjee S, Phan T, Campagnoni AT (1993) Myelin basic protein mRNA translocation in oligodendrocytes is inhibited by astrocytes in vitro. J Neurosci Res 36:99-110.

Amur-Umarjee SG, Dasu RG, Campagnoni AT (1990) Temporal expression of myelin-specific components in neonatal mouse brain cultures: evidence that 2',3'-cyclic nucleotide 3'- phosphodiesterase appears prior to galactocerebroside. Dev Neurosci 12:251-262.

Anderson S, Miskimins R (1994) Involvement of protein kinase C in cAMP regulation of myelin basic protein gene expression. J Neurosci Res 37:604-611.

Ang LC, Bhaumick B, Jurrlink BH (1993) Neurite promoting activity of insulin, insulin-like growth I and nerve growth factor on spinal motoneurons is astrocyte dependent. Dev Brain Res 74:83-88.

Ang LC, Bhaumick B, Munoz DG, Sass J, Juurlink BH (1992) Effects of astrocytes, insulin and insulin-like growth factor I on the survival of motoneurons in vitro. J Neurol Sci 109:168-172.

Anthony DC, Ferguson B, Matyzak MK, Miller KM, Esiri MM, Perry VH (1997) Differential matrix metalloproteinase expression in cases of multiple sclerosis and stroke. Neuropathol Appl Neurobiol 23:406-415.

Araujo DM, Cotman CW (1992) Basic FGF in astroglial, microglial, and neuronal cultures: Characterization of binding sites and modulation of release by lymphokines and trophic factors. J Neurosci 12:1668-1678.

Archer DR, Cuddon PA, Lipsitz D, Duncan ID (1997) Myelination of the canine central nervous system by glial cell transplantation: a model for repair of human myelin disease. Nature Medicine 3:54-59.

Ard MD, Bunge RP (1988) Heparan sulfate proteoglycan and laminin immunoreactivity on cultured astrocytes: relationship to differentiation and neurite growth. J Neurosci 8:2844-2858.

Armstrong RC, Dorn HH, Kufta CV, Friedman E, Dubois-Dalcq ME (1992) Pre-oligodendrocytes from adult human CNS. J Neurosci 12:1538-1547.

Armstrong RC, Harvath L, Dubois-Dalcq ME (1990) Type 1 astrocytes and oligodendrocyte-type 2 astrocyte glial progenitors migrate toward distinct molecules. J Neurosci Res 27:400-407.

Arner EC, Tortorella MD (1995) Signal transduction through chondrocyte integrin receptors induces matrix metalloproteinase synthesis and synergizes with interleukin-1. Arthritis Rheum 38:1304-1314.

Arregui CO, Carbonetto S, McKerracher L (1994) Characterization of neural cell adhesion sites: point contacts are the sites of interaction between integrins and the cytoskeleton in PC12 cells. J Neurosci 14:6967-6977.

Asai T, Wanaka A, Kato H, Masana Y, Seo M, Tohyama M (1993) Differential expression of two members of FGF receptor gene family, FGFR-1 and FGFR-2 mRNA, in the adult rat central nervous system. Mol Brain Res 17:174-178.

Asch AS, Leung LLK, Shapiro J, Nachman RL (1986) Human brain glial cells synthesize thrombospondin. Proc Natl Acad Sci USA 83:2904-2908.

Asotra K, Macklin WB (1993) Protein kinase C activity modulates myelin gene expression in enriched oligodendrocytes. J Neurosci Res 34:571-588.

Asotra K, Macklin WB (1994) Developmental expression of protein kinase C isozymes in oligodendrocytes and their differential modulation by 4 beta- phorbol-12,13-dibutyrate. J Neurosci Res 39:273-289.

Aviezer D, Hecht D, Safran M, Eisinger M, David G, Yayon A (1994) Perlecan, basal lamina proteoglycan, promotes basic fibroblast growth factor-receptor binding, mitogenesis, and angiogenesis. Cell 79:1005-1013.

Avossa D, Pfeiffer SE (1993) Transient reversion of O4+ GalC- oligodendrocyte progenitor development in response to the phorbol ester TPA. J Neurosci Res 34:113-128.

Baird A, Böhlen P (1991) Fibroblast growth factors. In Peptide growth factors and their receptors (Sporn MB, Roberts AB, eds), pp 369-418. Heidelberg: Springler-Verlag.

Baker AH, Wilkinson GW, Hembry RM, Murphy G, Newby AC (1996) Development of recombinant adenoviruses that drive high level expression of the human metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 and -2 genes: characterization of their infection into rabbit smooth muscle cells and human MCF-7 adenocarcinoma cells. Matrix Biol 15:383-395.

Balasingam V, Tejada-Berges T, Wright E, Bouckova R, Yong VW (1994) Reactive astrogliosis in the neonatal mouse brain and its modulation by cytokines. J Neurosci 14:846-856.

Balasingam V, Yong VW (1996) Attenuation of astroglial reactivity by interleukin-10. J Neurosci 16:2945-2955.

Bansal R, Kumar M, Murray K, Morrison RS, Pfeiffer SE (1996) Regulation of FGF receptors in the oligodendrocyte lineage. Mol Cell Neurosci 7:263-275.

Bansal R, Pfeiffer SE (1992) Novel stage in the oligodendrocyte lineage defined by reactivity of progenitors with R-mAb prior to O1 anti-galactocerebroside. J Neurosci Res 32:309-316.

Bansal R, Pfeiffer SE (1994) Inhibition of protein and lipid sulfation in oligodendrocytes blocks biological responses to FGF-2 and retards

cytoarchitectural maturation, but not developmental lineage progression. Dev Biol 162:511-524.

Bansal R, Pfeiffer SE (1997) FGF-2 converts mature oligodendrocytes to a novel phenotype. J Neurosci Res 50:215-228.

Bansal R, Warrington AE, Gard A, Ranscht B, Pfeiffer SE (1989) Multiple and novel specificities of monoclonal antibodies O1, O4 and R-mAb used in the analysis of oligodendrocyte development. J Neurosci Res 24:548-557.

Baranes D, Lederfein D, Huang YY, Chen M, Bailey CH, Kandel ER (1998) Tissue plasminogen activator contributes to the late phase of LTP and to synaptic growth in the hippocampal mossy fiber pathway. Neuron 21:813-825.

Barlett WP, Knapp PE, Skoff RP (1900) Glial conditioned medium enables jimpy oligodendrocytes to express properties of normal oligodendrocytes: production of myelin antigens and membranes. Glia 1:253-259.

Barres BA, Hart IK, Coles HSR, Burne JF, Voyvodic JT, Richardson WD, Raff MC (1992) Cell death and control of cell survival in the oligodendrocyte lineage. Cell 70:31-46.

Barres BA, Raff MC (1993) Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. Nature 361:258-260.

Barres BA, Schmid R, Sendnter M (1993) Multiple extracellular signals are required for long-term oligodendrocyte survival. Development 118:283-295

Bartsch U, Pesheva P, Raff M, Schachner M (1993) Expression of janusin (J1-160/180) in the retina and optic nerve of the developing and adult mouse. Glia 9:57-69.

Basu A (1993) The potential of protein kinase C as a target for anticancer treatment. Pharmacol Ther 59:257-280

Basbaum CB, Werb Z (1996) Focalized proteolysis: spatial and temporal regulation of extracellular matrix degradation at the cell surface. Curr Opin Cell Biol 8:731-738.

Battaini F, Pascale A, Paoletti R, Govoni S (1997) The role of anchoring protein RACK1 in PKC activation in the ageing rat brain. Trends Neurosci 20:410-415.

Belien ATJ, Paganetti PA, Schwab ME (1999) Membrane-type 1 matrix metalloproteinase (MT1-MMP) enables invasive migration of glioma cells in central nervous system white matter. J Cell Biol 144:373-384.

Benjamins JA, Nedelkoska L (1994) Maintenance of membrane sheets by cultured oligodendrocytes requires continuous microtubule turnover and Golgi transport. Neurochem Res 19:631-639.

Bernier L, Braun PE (1991) Relationship of CNP to regulated events in myelinogenesis. Trans Amer Soc Neurochem 22:265.

Bertolotto A, Rocca G, Schiffer D (1990) Chondroitin 4-sulfate proteoglycan forms an extracellular network in human and rat central nervous system. J Neurol Sci 100:113-123.

Besnard F, Perraud F, Sensenbenner M, Labourdette G (1989) Effects of acidic and basic fibroblastic growth factors on proliferation and maturation of cultured rat oligodendrocytes. Int J Dev Neurosci 7:401-409.

Bhat NR, Hauser KF, Kindy MS (1992) Cell proliferation and protooncogene induction in oligodendroglial progenitors. J Neurosci Res 32:340-349.

Bhat S, Pfeiffer SE (1986) Stimulation of oligodendrocytes by extracts from astrocyte-enriched cultures. J Neurosci Res 15:19-27.

Bikfalvi A, Klein S, Pintucci G, Rifkin DB (1997) Biological roles of fibroblast growth factor-2. Endocr Rev 18:26-45.

Bizzozero OA, Pasquini JM, Soto EF (1982) Differential effect of colchicine upon the entry of proteins into myelin and myelin related membranes. Neurochem Res 7:1415-1425.

Bjartmar C, Hildebrand C, Loinder K (1994) Morphological heterogeneity of rat oligodendrocytes: electron microscopic studies on serial sections. Glia 11:235-244.

Blaschuk KL, ffrench-Constant C (1998) Developmental neurobiology: notch is tops in the developing brain. Curr Biol 8:R334-R337

Blasi F (1997) uPA, uPAR, PAI-1: key intersection of proteolytic, adhesive and chemotactic highways? Immunol Today 18:415-417.

Blobe GC, Stribling DS, Fabbro D, Stabel S, Hannun YA (1996) Protein kinase C betaII specifically binds to and is activated by F-actin. J Biol Chem 271:15823-15830.

Boutros T, Croze E, Yong VW (1997) Interferon-beta is a potent promoter of nerve growth factor production by astrocytes. J Neurochem 69:939-946.

Braun PE, De Angelis D, Shtybel WW, Bernier L (1991) Isoprenoid modification permits 2',3'-cyclic nucleotide 3'- phosphodiesterase to bind to membranes. J Neurosci Res 30:540-544.

Braun PE, Sandillon F, Edwards A, Matthieu JM, Privat A (1988) Immunocytochemical localization by electron microscopy of 2'3'- cyclic nucleotide 3'-phosphodiesterase in developing oligodendrocytes of normal and mutant brain. J Neurosci 8:3057-3066.

Brooks PC, Clark RA, Cheresh DA (1994) Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science 264:569-571.

Brooks PC, Klemke RL, Schon S, Lewis JM, Schwartz MA, Cheresh DA (1997) Insulin-like growth factor receptor cooperates with integrin alpha v beta 5 to promote tumor cell dissemination in vivo. J Clin Invest 99:1390-1398.

Brooks PC, Stromblad S, Sanders LC, von Schalscha TL, Aimes RT, Stetler-Stevenson WG, Quigley JP, Cheresh DA (1996) Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. Cell 85:683-693.

Brück W, Schmied M, Suchanek G, Brück Y, Breitschopf H, Poser S, Piddlesden S, Lassmann H (1994) Oligodendrocytes in the early course of multiple sclerosis. Ann Neurol 35:65-73.

Brunner C, Lassmann H, Waehneldt TV, Matthieu JM, Linington C (1989) Differential ultrastructural localization of myelin basic protein, myelin/oligodendroglial glycoprotein, and 2',3'-cyclic nucleotide 3'- phosphodiesterase in the CNS of adult rats. J Neurochem 52:296-304.

Butt AM, Colquhoun K, Tutton M, Berry M (1994) Three-dimensional morphology of astrocytes and oligodendrocytes in the intact mouse optic nerve. J Neurocytol 23:469-485.

Butt AM, Ibrahim M, Ruge FM, Berry M (1995) Biochemical subtypes of oligodendrocyte in the anterior medullary velum of the rat as revealed by the monoclonal antibody Rip. Glia 14:185-197.

Butt AM, Ransom BR (1993) Morphology of astrocytes and oligodendrocytes during development in the intact rat optic nerve. J Comp Neurol 338:141-158.

Cajal SR (1913) Sobre un nuevo proceder de impregnacion de la neuroglia y sus resultados en los centros nerviosos del hombre y animales. Trab Lab Invest Biol Univ Madrid 11:219-237.

Calof AL, Lander AD (1991) Relationship between neuronal migration and cell-substratum adhesion: laminin and merosin promote olfactory neuronal migration but are anti-adhesive. J Cell Biol 115:779-794.

Calver AR, Hall AC, Yu WP, Walsh FS, Heath JK, Betsholtz C, Richardson WD (1998) Oligodendrocyte population dynamics and the role of PDGF in vivo. Neuron 20:869-882.

Cannella B, Raine CS (1995) The adhesion molecule and cytokine profile of multiple sclerosis lesions. Ann Neurol 37:424-435.

Cardwell MC, Rome LH (1988) Evidence that an RGD-dependent receptor mediates the binding of oligodendrocytes to a novel ligand in a glial-derived matrix. J Cell Biol 107:1541-1549.

Canete SR, Gui YH, Linask KK, Muschel RJ (1995) MMP-9 (gelatinase B) mRNA is expressed during mouse neurogenesis and may be associated with vascularization. Dev Brain Res 88:37-52.

Carroll P, Sendtner M, Meyer M, Thoenen H (1993) Rat ciliary neurotrophic factor (CNTF): Gene structure and regulation of mRNA levels in glial cell cultures. Glia 9:176.

Chakraborty G, Drivas A, Ledeen R (1999) The phosphoinositide signaling cycle in myelin requires cooperative interaction with the axon. Neurochem Res 24:249-254.

Chambers AF, Matrisian LM (1997) Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 89:1260-1270.

Chan JR, Phillips LJ, Glaser M (1998) Glucocorticoids and progestins signal the initiation and enhance the rate of myelin formation. Proc Natl Acad Sci USA 95:10459-10464.

Chandler S, Miller KM, Clements JM, Lury J, Corkill D, Anthony DC, Adams SE, Gearing AJ (1997) Matrix metalloproteinases, tumor necrosis factor and multiple sclerosis: an overview. J Neuroimmunol 72:155-161.

Chapman HA (1997) Plasminogen activators, integrins, and the coordinated regulation of cell adhesion and migration. Curr Opin Cell Biol 9:714-724.

Chernausek SD (1993) Insulin-like growth factor-I (IGF-I) production by astroglial cells: Regulation and importance for epidermal growth factor-induced cell replication. J Neurosci Res 34:189-197.

Chida K, Hashiba H, Sasaki K, Kuroki T (1986) Activation of protein kinase C and specific phosphorylation of a Mr 90,000 membrane protein of promotable BALB/3T3 and C3H/10T1/2 cells by tumor promoters. Cancer Res 46:1055-1062.

Choi BH, Kim RC (1984) Expression of glial fibrillary acidic protein in immature oligodendroglia. Science 223:407-409.

Clements JM, Cossins JA, Wells GM, Corkill DJ, Helfrich K, Wood LM, Pigott R, Stabler G, Ward GA, Gearing AJ, Miller KM (1997) Matrix metalloproteinase expression during experimental autoimmune encephalomyelitis and effects of a combined matrix metalloproteinase and tumour necrosis factor-alpha inhibitor. J Neuroimmunol 74:85-94.

Condic ML, Letourneau PC (1997) Ligand-induced changes in integrin expression regulate neuronal adhesion and neurite outgrowth. Nature 389:852-856.

Cossins JA, Clements JM, Ford J, Miller KM, Pigott R, Vos W, Van d, De Groot CJ (1997) Enhanced expression of MMP-7 and MMP-9 in demyelinating multiple sclerosis lesions. Acta Neuropathol (Berl.) 94:590-598.

Curtis R, Cohen J, Fok-Seang J, Hanley MR, Gregson NA, Reynolds R, Wilkin GP (1988) Development of macroglial cells in rat cerebellum. I. Use of antibodies to follow early in vivo development and migration of oligodendrocytes. J Neurocytol 17:43-54.

Cuzner ML, Gveric D, Strand C, Loughlin AJ, Paemen L, Opdenakker G (1996) The expression of tissue type plasminogen activator, matrix metalloproteases and

endogenous inhibitors in the central nervous system in multiple sclerosis: comparison of stages in lesion evolution. J Neuropathol Exp Neurol 55:1194-1204.

Cuzner ML, Loughlin AJ, Mosley K, Woodroofe MN (1994) The role of microglia macrophages in the processes of inflammatory demyelination and remyelination. Neuropathol Appl Neurobiol 20:200-201.

Danilenko DM, Montestruque S, Philo JS, Li T, Hill D, Speakman J, Bahru M, Zhang M, Konishi M, Itoh N, Chirica M, Delaney J, Hernday N, Martin F, Hara S, Talvenheimo J, Narhi LO, Arakawa T (1999) Recombinant rat fibroblast growth factor-16: structure and biological activity. Arch Biochem Biophys 361:34-46.

De Angelis DA, Braun PE (1996) 2',3'-Cyclic nucleotide 3'-phosphodiesterase binds to actin-based cytoskeletal elements in an isoprenylation-independent manner. J Neurochem 67:943-951.

Deloulme JC, Janet T, Pettmann B, Laeng P, Knoetgen M-F, Sensenbrenner M, Baudier J (1992) Phosphorylation of the MARCKS protein (P87), a major protein kinase C substrate, is not an obligatory step in the mitogenic signaling pathway of basic fibroblast growth factor in rat oligodendrocytes. J Neurochem 58:567-578.

Deryugina EI, Bourdon MA, Luo GX, Reisfeld RA, Strongin A (1997) Matrix metalloproteinase-2 activation modulates glioma cell migration. J Cell Sci 110:2473-2482.

Deryugina EI, Bourdon MA, Reisfeld RA, Strongin A (1998) Remodeling of collagen matrix by human tumor cells requires activation and cell surface association of matrix metalloproteinase-2. Cancer Res 58:3743-3750.

Doherty P, Walsh FS (1989) Neurite guidance molecules. Curr Opin Cell Biol 1:1102-1106.

Doherty P, Williams E, Walsh FS (1995) A soluble chimeric form of the L1 glycoprotein stimulates neurite outgrowth. Neuron 14:57-66.

Dubois-Dalcq M (1987) Characterization of a slowly proliferative cell along the oligodendrocyte differentiation pathway. EMBO J 6:2587-2595.

Dubois-Dalcq M, Behar T, Hudson L, et al. (1986) Emergence of three myelin proteins in oligodendrocytes cultured without neurons. J Cell Biol 102:384-392.

Duncan ID, Hammang JP, Goda S, Quarles RH (1989) Myelination in the jimpy mouse in the absence of proteolipid protein. Glia 2:148-154.

Duncan ID, Paino C, Archer DR, Wood PM (1992) Functional capacities of transplanted cell-sorted adult oligodendrocytes. Dev Neurosci 14:114-122.

D'Souza SD, Bonetti B, Balasingam V, Cashman NR, Barker PA, Troutt AB, Raine CS, Antel JP (1996) Multiple sclerosis: Fas signaling in oligodendrocyte cell death. J Exp Med 184:2361-2370.

Dyer CA, Benjamins JA (1990) Glycolipids and transmembrane signaling: antibodies to galactocerebroside cause an influx of calcium in oligodendrocytes. J Cell Biol 111:625-633.

Dyer CA, Phillbotte T, Wolf MK, Billings-Gagliardi S (1997) Regulation of cytoskeleton by myelin components: studies on shiverer oligodendrocytes carrying an MBP transgene. Dev Neurosci 19:395-409.

Eccleston PA, Silberberg DH (1985) Fibroblast growth factor is a mitogen for oligodendrocytes in vitro. Dev Brain Res 21:315-318.

Eckenstein FP (1994) Fibroblast growth factors in the nervous system. J Neurobiol 25:1467-1480.

Emmert-Buck MR, Emonard HP, Corcoran ML, Krutzsch HC, Foidart JM, Stetler-Stevenson WG (1995) Cell surface binding of TIMP-2 and pro-MMP-2/TIMP-2 complex. FEBS Lett 364:28-32.

Enenstein J, Waleh NS, Kramer RH (1992) Basic FGF and TGF-beta differentially modulate integrin expression of human microvascular endothelial cells. Exp Cell Res 203:499-503.

Engel U, Wolswijk G (1996) Oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells derived from adult rat spinal cord: in vitro characteristics and response to PDGF, bFGF and NT-3. Glia 16:16-26.

Espinosa dlM A, Bernard R, Tiller B, Rouget P, de Vellis J (1993) Grafting of fast blue labeled glial cells into neonatal rat brain: differential survival and migration among cell types. Int J Dev Neurosci 11:625-639.

Espinosa dlM A, Kumar S, Zhao P, Huang CJ, Nazarian R, Pan T, Scully S, Chang R, de Vellis J (1999) Transferrin is an essential factor for myelination. Neurochem.Res. 24:235-248.

Fagerstrom S, Pahlman S, Gestblom C, Nanberg E (1996) Protein kinase C- ε is implicated in neurite outgrowth in differentiating human neuroblastoma cells. Cell Growth Differ 7(6):775-785.

Fallon JR (1985) Preferential outgrowth of central nervous system neurites on astrocytes and Schwann cells as compared with nonglial cells in vitro. J Cell Biol 100:198-207.

Felsenfeld DP, Choquet D, Sheetz MP (1996) Ligand binding regulates the directed movement of beta1 integrins on fibroblasts. Nature 383:438-440.

Fernandez-Valle C, Gwynn L, Wood PM, Carbonetto S, Bunge MB (1994) Anti-β1 integrin antibody inhibits Schwann cell myelination. J Neurobiol 25:1207-1226.

Ferrara N, Ousley F, Gospodarowicz D (1988) Bovine brain astrocytes express basic fibroblast growth factor, a neurotropic and angiogenic mitogen. Brain Res 462:223-232.

ffrench-Constant C, Raff MC (1986) Proliferating bipotential glial progenitor cells in adult rat optic nerve. Nature 319:499-502.

Finch PW, Rubin JS, Miki T, Ron D, Aaronson SA (1989) Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. Science 245:752-755.

Finklestein SP, Apostolides PJ, Caday CG, Prosser J, Philips MF, Klagsbrun M (1988) Increased basic fibroblast growth factor (bFGF) immunoreactivity at the site of focal brain wounds. Brain Res 460:253-259.

Franklin RJ, Bayley SA, Milner R, ffrench-Constant C, Blakemore WF (1995) Differentiation of the O-2A progenitor cell line CG-4 into oligodendrocytes and astrocytes following transplantation into glia- deficient areas of CNS white matter. Glia 13:39-44.

Franklin RJ, Crang AJ, Blakemore WF (1993) The role of astrocytes in the remyelination of glia-free areas of demyelination. In Advances in Neurology (Seil FJ ed), pp 125-133. New York: Raven Press Ltd.

Franklin RJM, Blakemore WF (1997) Transplanting oligodendrocyte progenitors into the adult CNS. J Anatomy 190 Part 1:23-33.

Fressinaud C, Laeng P, Labourdette G, Durand J, Vallat JM (1993) The proliferation of mature oligodendrocytes in vitro is stimulated by basic fibroblast growth factor and inhibited by oligodendrocyte-type 2 astrocyte precursors. Dev Biol 158:317-329.

Fressinaud C, Vallat JM (1994) Basic fibroblast growth factor improves recovery after chemically induced breakdown of myélin-like membranes in pure oligodendrocyte cultures. J Neurosci Res 38:202-213.

Fressinaud C, Vallat JM, Labourdette G (1995) Basic fibroblast growth factor down-regulates myelin basic protein gene expression and alters myelin compaction of mature oligodendrocytes in vitro. J Neurosci Res, 40:285-293.

Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresh DA (1995) Definition of two angiogenic pathways by distinct alpha v integrins. Science 270:1500-1502.

Friedman B, Hockfield S, Black JA, Woodruff KA, Waxman SG (1989) In situ demonstration of mature oligodendrocytes and their processes: an immunocytochemical study with a new monoclonal antibody, RIP. Glia 2:380-390.

Friesel RE, Maciag T (1995) Molecular mechanisms of angiogenesis: fibroblast growth factor signal transduction. FASEB J 9:919-925.

Fruttiger M, Montag D, Schachner M, Martini R (1995) Crucial role for the myelin-associated glycoprotein in the maintenance of axon-myelin integrity. Eur J Neurosci 7:511-515.

Foran DR, Peterson AC (1992) Myelin acquisition in the central nervous system of the mouse revealed by an MBP-Lac Z transgene. J Neurosci 12:4890-4897.

Gadient RA, Cron KC, Otten U (1990) Interleukin-1β and tumor necrosis factor-α synergistically stimulate nerve growth factor (NGF) release from cultured rat astrocytes. Neurosci Lett 117:335-340.

Galzie Z, Kinsella AR, Smith JA (1997) Fibroblast growth factors and their receptors. Biochem Cell Biol 75:669-685.

Gard AL, Burrell MR, Pfeiffer SE, Rudge JS, Williams WC (1995) Astroglial control of oligodendrocyte survival mediated by PDGF and leukemia inhibitory factor-like protein. Development 121:2187-2197.

Gard AL, Pfeiffer SE (1990) Two proliferative stages of the oligodendrocyte lineage (A2B5+O4- and O4+GalC-) under different mitogenic control. Neuron 5:615-625.

Gensert JM, Goldman JE (1997) Endogenous progenitors remyelinate demyelinated axons in the adult CNS. Neuron 19:197-203.

Ghatak NR, Leshner RT, Price AN, Felton III WL (1989) Remyelination in the human central nervous system. J Neuropathol Exp Neurol 48:507-518.

Ghatak NR (1992) Occurrence of oligodendrocytes within astrocytes in demyelinating lesions. J Neuropathol Exp Neurol 51:40-46.

Giancotti FG (1997) Integrin signaling: specificity and control of cell survival and cell cycle progression. Curr Opin Cell Biol 9:691-700.

Giftochristos N, David S (1988) Laminin and heparan sulphate proteoglycan in the lesioned adult mammalian central nervous system and their possible relationship to axonal sprouting. J Neurocytol 17:385-397.

Gijbels K, Galardy RE, Steinman L (1994) Reversal of experimental autoimmune encephalomyelitis with a hydroxamate inhibitor of matrix metalloproteases. J Clin Invest 94:2177-2182.

Gijbels K, Proost P, Masure S, Carton H, Billiau A, Opdenakker G (1993) Gelatinase B is present in the cerebrospinal fluid during experimental autoimmune encephalomyelitis and cleaves myelin basic protein. J Neurosci Res 36:432-440.

Gilles FH, Shankle W, Dooling EC (1983) Myelinated tracts: Growth patterns. In The Developing Human Brain (Dooling EC, Gilles FH, Leviton A, eds), pp 117. Boston: John Wright & Co.

Giordano S, Sherman L, Lyman W, Morrison R (1992) Multiple molecular weight forms of basic fibroblast growth factor are developmentally regulated in the central nervous system. Dev Biol 152:293-303.

Giulian D, Vaca K, Corpuz M (1993) Brain glia release factors with opposing actions upon neuronal survival. J Neurosci 13:29-37.

Godfraind C, Friedrich VL, Holmes KV, Dubois-Dalcq M (1989) In vivo analysis of glial cell phenotypes during a viral demyelinating disease in mice. J Cell Biol 109:2405-2416.

Gogate N, Verma L, Zhou M, Milward E, Rusten R, O'Connor M, Kufta C, Kim J, Hudson L, Dubois-Dalcq M (1994) Plasticity in the adult human oligodendrocyte lineage. J Neurosci 14:4571-4587.

Goldman JE (1992) Regulation of oligodendrocyte differentiation. Trends Neurosci 15:359-362.

Gomez J, Martinez C, Garcia A, Rebollo A (1996) Association of phosphatidylinositol 3 kinase to protein kinase C zeta during interleukin-2 stimulation. Eur J Immunol 26:1781-1787.

Gómez-Pinilla F, Won-Kyun Lee J, Cotman CW (1992) Basic FGF in adult rat brain: cellular distribution and response to entorhinal lesion and fimbria-fornix transection. J Neurosci 12:345-355.

Gomis-Ruth FX, Maskos K, Betz M, Bergner A, Huber R, Suzuki K, Yoshida N, Nagase H, Brew K, Bourenkov GP, Bartunik H, Bode W (1997) Mechanism of inhibition of the human matrix metalloproteinase stromelysin-1 by TIMP-1. Nature 389:77-81.

Gonatas NK, Hirayama M, Stieber A, Silberberg DH (1982) The ultrastructure of isolated rat oligodendroglial cell cultures. J Neurocytol 11:997-1008.

Gonzalez AM, Berry M, Maher PA, Logan A, Baird A (1995) A comprehensive analysis of the distribution of FGF-2 and FGFR1 in the rat brain. Brain Res 701:201-226.

Gonzalez AM, Buscaglia M, Ong M, Baird A (1990) Distribution of basic fibroblast growth factor in the 18-day rat fetus: localization in the basement membranes of diverse tissues. J Cell Biol 110:753-765.

Gravel M, Peterson J, Yong VW, Kottis V, Trapp B, Braun PE (1996) Overexpression of 2',3'-cyclic nucleotide 3'-phosphodiesterase in transgenic mice alters oligodendrocyte development and produces aberrant myelination. Mol Cell Neurosci 7:453-466.

Grierson JP, Petroski RE, Ling DSF, Geller HM (1990) Astrocyte topography and tenascin/cytotactin expression: correlation with the ability to support neuritic outgrowth. Dev Brain Res 55:11-19.

Grinspan JB, Stern JL, Franceschini B, Pleasure D (1993) Trophic effects of basic fibroblast growth factor (bFGF) on differentiated oligodendroglia: a mechanism for regeneration of the oligodendroglial lineage. J Neurosci Res 36:672-680.

Grinspan JB, Stern JL, Pustilnik SM, Pleasure D (1990) Cerebral white matter contains PDGF-responsive precursors to O-2A cells. J Neurosci 10:1866-1873.

Groves AK, Barnett SC, Franklin RJ, Crang AJ, Mayer M, Blakemore WF, Noble M (1993) Repair of demyelinated lesions by transplantation of purified O-2A progenitor cells. Nature 362:453-455.

Guan JL (1997) Focal adhesion kinase in integrin signaling. Matrix Biol 16:195-200.

Haimovitz-Friedman A, Balaban N, McLoughlin M, Ehleiter D, Michaeli J, Vlodavsky I, Fuks Z (1994) Protein kinase C mediates basic fibroblast growth factor protection of endothelial cells against radiation-induced apoptosis. Cancer Res 54:2591-2597.

Haller H, Lindschau C, Maasch C, Olthoff H, Kurscheid D, Luft FC (1998) Integrin-induced protein kinase C alpha and epsilon translocation to focal adhesions mediates vascular smooth muscle cell spreading. Circ Res 82:157-165.

Halliday AL, Cepko CL (1992) Generation and migration of cells in the developing striatum. Neuron 9:15-26.

Hamilton SP, Rome LH (1994) Stimulation of in vitro myelin synthesis by microglia. Glia 11:326-335.

Han VKM, Smith A, Myint W, Nygard K, Bradshaw S (1992) Mitogenic activity of epidermal growth factor on newborn rat astroglia: interaction with insulin-like growth factors. Endocrinology 131:1134-1142.

Hardy R, Reynolds R (1993) Rat cerebral cortical neurons in primary culture release a mitogen specific for early (GD3+/04-) oligodendroglial progenitors. J Neurosci Res 34:589-600.

Hart IK, Richardson WD, Bolsover SR, Raff MC (1989) PDGF and intracellular signaling in the timing of oligodendrocyte differentiation. J Cell Biol 109:3411-3417.

Hart IK, Richardson WD, Heldin CH, Westermark B, Raff MC (1989) PDGF receptors on cells of the oligodendrocyte-type-2 astrocyte (O-2A) cell lineage. Development 105:595-603.

Hartwig JH, Thelen M, Rosen A, Janmey PA, Nairn AC, Aderem A (1992) MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium-calmodulin. Nature 356:618-622.

Hashimoto T, Ase K, Sawamura S, Kikkawa U, Saito N, Tanaka C, Nishizuka Y (1988) Postnatal development of a brain-specific subspecies of protein kinase C in rat. J Neurosci 8:1678-1683.

Hatten ME, Lynch M, Rydel RE, Sanchez J, Joseph-Silverstein J, Moscatelli D, Rifkin DB (1988) In vitro neurite extension by granule neurons is dependent upon astroglial-derived fibroblast growth factor. Dev Biol 125:280-289.

Heidecker G, Kolch W, Morrison DK, Rapp UR (1992) The role of Raf-1 phosphorylation in signal transduction. Adv Cancer Res 58:53-73.

Hewson AK, Smith T, Leonard JP, Cuzner ML (1995) Suppression of experimental allergic encephalomyelitis in the Lewis rat by the matrix metalloproteinase inhibitor Ro31-9790. Inflamm Res 44:345-349.

Hirokawa N (1998) Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science 279:519-526.

Hoffman KL, Duncan ID (1995) Canine oligodendrocytes undergo morphological changes in response to basic fibroblast growth factor (bFGF) in vitro. Glia 14:33-42.

Hu DE, Fan TP (1995) Protein kinase C inhibitor calphostin C prevents cytokine-induced angiogenesis in the rat. Inflamm 19:39-54.

Hua J, Muschel RJ (1996) Inhibition of matrix metalloproteinase 9 expression by a ribozyme blocks metastasis in a rat sarcoma model system. Cancer Res 56:5279-5284.

Hudson LD, Friedrich VLJ, Behar T, Dubois-Dalcq M, Lazzarini RA (1989) The initial events in myelin synthesis: orientation of proteolipid protein in the plasma membrane of cultured oligodendrocytes. J Cell Biol 109:717-727.

Huhtala P, Humphries MJ, McCarthy JB, Tremble PM, Werb Z, Damsky CH (1995) Cooperative signaling by alpha 5 beta 1 and alpha 4 beta 1 integrins regulates metalloproteinase gene expression in fibroblasts adhering to fibronectin. J Cell Biol 129:867-879.

Hynes RO (1992) Integrins: versatility, modulation, and signaling in cell adhesion. Cell 69:11-25.

Ikenaka K, Kagawa T, Mikoshiba K (1992) Selective expression of DM-20, an alternatively spliced myelin proteolipid protein gene product, in developing nervous system and in nonglial cells. J Neurochem 58:2248-2253.

Ilic D, Furuta Y, Kanazawa S, Takeda N, Sobue K, Nakatsuji N, Nomura S, Fujimoto J, Okada M, Yamamoto T (1995) Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature 377:539-544.

Jaramillo ML, Afar DE, Almazan G, Bell JC (1994) Identification of tyrosine 620 as the major phosphorylation site of myelin-associated glycoprotein and its implication in interacting with signaling molecules. J Biol Chem 269:27240-27245.

Javis MD, Turner AJ, Povirk LF, Traylor RS, Grant S (1994) Induction of apoptotic DNA fragmentation and cell death in HL-60 human promyelocytic leukemia cells by pharmacological inhibitors of protein kinase C. Cancer Res 54:1707-1714.

Johnson-Green PC, Dow KE, Riopelle RJ (1991) Characterization of glycosaminoglycans produced by primary astrocytes in vitro. Glia 4:314-321.

Johnson-Green PC, Dow KE, Riopelle RJ (1992) Neurite growth modulation associated with astrocyte proteoglycans: influence of activators of inflammation. Glia 5:33-42.

Jordan CA, Friedrich VLJ, de Ferra F, Weismiller DG, Holmes KV, Dubois-Dalcq M (1990) Differential exon expression in myelin basic protein transcripts during central nervous system (CNS) remyelination. Cell Mol Neurobiol 10:3-18.

Juliano RL, Haskill S (1993) Signal transduction from the extracellular matrix. J Cell Biol 120:577-585.

Kandel J, Bossy-Wetzel E, Radvanyi F, Klagsbrun M, Folkman J, Hanahan D (1991) Neovascularization is associated with a switch to the export of bFGF in the multistep development of fibrosarcoma. Cell 66:1095-1104.

Kapila YL, Kapila S, Johnson PW (1996) Fibronectin and fibronectin fragments modulate the expression of proteinases and proteinase inhibitors in human periodontal ligament cells. Matrix Biol 15:251-261.

Kaplan MR, Meyer-Franke A, Lambert S, Bennett V, Duncan ID, Levinson SR, Barres BA (1997) Induction of sodium channel clustering by oligodendrocytes. Nature 386:724-728.

Karin M, Liu Z, Zandi E (1997) AP-1 function and regulation. Curr Opin Cell Biol 9:240-246.

Kent KC, Mii S, Harrington EO, Chang JD, Mallette S, Ware JA (1995) Requirement for protein kinase C activation in basic fibroblast growth factor-induced human endothelial cell proliferation. Circ Res 77:231-237.

Khokha R, Martin DC, Fata JE (1995) Utilization of transgenic mice in the study of matrix degrading proteinases and their inhibitors. Cancer Metastasis Rev 14:97-111.

Kidd GJ, Hauer PE, Trapp BD (1990) Axons modulate myelin protein messenger RNA levels during central nervous system myelination in vivo. J Neurosci Res 26:409-418.

Kieseier BC, Clements JM, Pischel HB, Wells GM, Miller K, Gearing AJ, Hartung HP (1998) Matrix metalloproteinases MMP-9 and MMP-7 are expressed in experimental autoimmune neuritis and the Guillain-Barre syndrome. Ann Neurol 43:427-434.

Kimura M, Sato M, Akatsuka A, Nozawa-Kimura S, Takahashi R, Yokoyama M, Nomura T, Katsuki M (1989) Restoration of myelin formation by a single type of myelin basic protein in transgenic shiverer mice. Proc Natl Acad Sci USA 86:5661-5665.

Kirchhoff F, Hofer HW, Schachner M (1993) Myelin-associated glycoprotein is phosphorylated by protein kinase C. J Neurosci Res 36:368-381.

Klein S, Giancotti FG, Presta M, Albelda SM, Buck CA, Rifkin DB (1993) Basic fibroblast growth factor modulates integrin expression in microvascular endothelial cells. Mol Biol Cell 4:973-982.

Klint P, Claesson-Welsh L (1999) Signal transduction by fibroblast growth factor receptors. Front Biosci 4:D165-177

Kliot M, Smith GM, Siegal JD, Silver J (1990) Astrocyte-polymer implants promote regeneration of dorsal root fibers into the adult mammalian spinal cord. Exp Neurol 109:57-69.

Klugmann M, Schwab MH, Puhlhofer A, Schneider A, Zimmermann F, Griffiths IR, Nave KA (1997) Assembly of CNS myelin in the absence of proteolipid protein. Neuron 18:59-70.

Kobayashi E, Nakano H, Morimoto M, Tamaoki T (1989) Calphostin C (UCN-1028C), a novel microbial compound, is a highly potent and specific inhibitor of protein kinase C. Biochem Biophy Res Comm 159:548-553.

Kolanus W, Seed B (1997) Integrins and inside-out signal transduction: converging signals from PKC and PIP3. Curr Opin Cell Biol 9:725-731.

Kolch W, Heidecker G, Kochs G, Hummel R, Vahidi H, Mischak H, Finkenzeller G, Marme D, Rapp UR (1993) Protein kinase C alpha activates RAF-1 by direct phosphorylation. Nature 364:249-252.

Komoly S, Hudson LD, deF Webster H, Bondy CA (1992) Insulin-like growth factor I gene expression is induced in astrocytes during experimental demyelination. Proc Natl Acad Sci USA 89:1894.

Kossakowska AE, Edwards DR, Lee SS, Urbanski LS, Stabbler AL, Zhang CL, Phillips BW, Zhang Y, Urbanski SJ (1998) Altered balance between matrix metalloproteinases and their inhibitors in experimental biliary fibrosis. Am J Pathol 153:1895-1902.

Kraft AS, Anderson WB (1983) Phorbol esters increase the amount of Ca2+, phospholipid-dependent protein kinase associated with plasma membrane. Nature 301:621-623.

Krystosek A, Seeds NW (1981) Plasminogen activator release at the neuronal growth cone. Science 213:1532-1534.

Larjava H, Lyons JG, Salo T, Makela M, Koivisto L, Birkedal-Hansen H, Akiyama SK, Yamada KM, Heino J (1993) Anti-integrin antibodies induce type IV collagenase expression in keratinocytes. J Cell Physiol 157:190-200.

Leco KJ, Khokha R, Pavloff N, Hawkes SP, Edwards DR (1994) Tissue inhibitor of metalloproteinases-3 (TIMP-3) is an extracellular matrix-associated protein with a distinctive pattern of expression in mouse cells and tissues. J Biol Chem 269:9352-9360.

Leli U, Cataldo A, Shea TB, Nixon RA, Hauser G (1992) Distinct mechanisms of differentiation of SH-SY5Y neuroblastoma cells by protein kinase C activators and inhibitors. J Neurochem 58:1191- 1198.

Lentz SI, Miner JH, Sanes JR, Snider W (1997) Distribution of the ten known laminin chains in the pathways and targets of developing sensory axons. J Comp Neurol 378:547-561.

Letourneau PC, Condic ML, Snow DM (1992) Extracellular matrix and neurite outgrowth. Curr Opin Genet Dev 2:625-634.

Levi G, Aloisi F, Wilkin GP (1987) Differentiation of cerebellar bipotential glial precursors into oligodendrocytes in primary culture: developmental profile of surface antigens and mitotic activity. J Neurosci Res 18:407-417.

Levine JM, Stallcup WB (1987) Plasticity of developing cerebellar cells in vitro studied with antibodies against the NG2 antigen. J Neurosci 7:2721-2731.

Lewis JM, Cheresh DA, Schwartz MA (1996) Protein kinase C regulates alpha v beta 5-dependent cytoskeletal associations and focal adhesion kinase phosphorylation. J Cell Biol 134:1323-1332.

Librach CL, Werb Z, Fitzgerald ML, Chiu K, Corwin NM, Esteves RA, Grobelny D, Galardy R, Damsky CH, Fisher SJ (1991). 92-kD type IV collagenase mediates invasion of human cytotrophoblasts. J Cell Biol 113:437-449.

Liedtke W, Cannella B, Mazzaccaro RJ, Clements JM, Miller KM, Wucherpfennig KW, Gearing AJ, Raine CS (1998) Effective treatment of models of multiple sclerosis by matrix metalloproteinase inhibitors. Ann Neurol 44:35-46.

Liesi P (1985) Laminin-immunoreactive glia distinguish regenerative adult CNS systems from non-regenerative ones. EMBO J 4:2505-2511.

Liesi P, Dahl D, Vaheri A (1983) Laminin is produced by early rat astrocytes in primary culture. J Cell Biol 96:920-924.

Liesi P, Kaakkola S, Dahl D, Vaheri A (1984) Laminin is induced in astrocytes of adult brain by injury. EMBO J 3:683-686.

Liesi P, Kirkwood T, Vaheri A (1986) Fibronectin is expressed by astrocytes cultured from embryonic and early postnatal rat brain. Exp Cell Res 163:175-185.

Liesi P, Risteli L (1989) Glial cells of mammalian brain produce a variant form of laminin. Glial cells of mammalian brain produce a variant form of laminin. Exp Neurol 105:86-92.

Liesi P, Silver J (1988) Is astrocyte laminin involved in axon guidance in the mammalian CNS? Dev Biol 130:774-785.

Lindsay RM (1979) Adult rat brain astrocytes support survival of both NGF-dependent and NGF-insensitive neurones. Nature 282:80-82.

Lisak RP, Pleasure DE, Silberberg DH, Manning MC, Saida T (1981) Long term culture of bovine oligodendroglia isolated with a Percoll gradient. Brain Res 223:107-122.

Lotem J, Cragoe EJ Jr., Sachs L (1991) Rescue from programmed cell death in leukemic and normal myeloid cells. Blood 78:953-960.

Loughlin AJ, Honeggar P, Woodroofe MN, Comte V, Matthieu JM, Cuzner ML (1994) Myelin basic protein content of aggregating rat brain cell cultures treated with cytokines and/or demyelinating antibody: Effects of macrophage enrichment. J Neurosci Res 37:647-653.

Louis JC, Magal E, Takayama S, Varon S (1993) CNTF protection of oligodendrocytes against natural and tumor necrosis factor-induced death. Science 259:689-692.

Lubetzki-Korn I, Ovadia H, Vlodavsky I, Fuks Z, Abramsky O (1983) Enhanced growth and morphological differentiation of isolated adult rat oligodendrocytes in vitro: use of a naturally produced extracellular matrix. Brain Res 267:151-155.

Lucchinetti CF, Bruck W, Rodriguez M, Lassmann H (1996) Distinct patterns of multiple sclerosis pathology indicates heterogeneity in pathogenesis. Brain Pathol 6:259-274.

Lucchinetti CF, Noseworthy JH, Rodriguez M (1997) Promotion of endogenous remyelination in multiple sclerosis. Mult Scler 3:71-75.

Luckenbill-Edds L (1997) Laminin and the mechanism of neuronal outgrowth. Brain Res 23:1-27.

Ludwin SK (1988) Remyelination in the central nervous system and the peripheral nervous system. Adv Neurol 47:215-254.

Ludwin SK (1989) Evolving concepts and issues in remyelination. Dev Neurosci 11:140-148.

Ludwin SK (1997) The pathobiology of the oligodendrocyte. J Neuropathol Exp Neurol 56:111-124.

Ludwin SK (1981) Pathology of demyelination and remyelination. In Demyelinating disease: basic and clinical electrophysiology (Waxman SG, Ritchie JM eds), pp 123-168. New York: Raven.

Ludwin SK, Bakker DA (1988) Can oligodendrocytes attached to myelin proliferate? J Neurosci 8:1239-1244.

Ludwin SK, Sternberger NH (1984) An immunohistochemical study of myelin proteins during remyelination in the central nervous system. Acta Neuropathol (Berl.) 63:240-248.

Ludwin SK, Szuchet S (1993) Myelination by mature ovine oligodendrocytes in vivo and in vitro: evidence that different steps in the myelination process are independently controlled. Glia 8:219-231.

Lunn KF, Baas PW, Duncan ID (1997) Microtubule organization and stability in the oligodendrocyte. J Neurosci 17:4921-4932.

MacDougall JR, Kerbel RS (1995) Constitutive production of 92-kDa gelatinase B can be suppressed by alterations in cell shape. Exp Cell Res 218:508-515.

Machida CM, Rodland KD, Matrisian L, Magun BE, Ciment G (1989) NGF induction of the gene encoding the protease transin accompanies neuronal differentiation in PC12 cells. Neuron 2:1587-1596.

Maeda A, Sobel RA (1996) Matrix metalloproteinases in the normal human central nervous system, microglial nodules, and multiple sclerosis lesions. J Neuropathol Exp Neurol 55:300-309.

Malek-Hedayat S, Rome LH (1994) Expression of beta 1-related integrin by oligodendroglia in primary culture: Evidence for a functional role in myelination. J Cell Biol 124:1039-1046.

Marais R, Light Y, Mason C, Paterson H, Olson MF, Marshall CJ (1998) Requirement of Ras-GTP-Raf complexes for activation of Raf-1 by protein kinase C. Science 280:109-112.

Marshall CJ (1995) Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. Cell 80:179-185.

Mason IJ (1994) The ins and outs of fibroblast growth factors. Cell 78:547-552.

Mason IJ (1994) Cell signalling. Do adhesion molecules signal via FGF receptors? Curr Biol 4:1158-1161.

Massa PT, Mugnaini E (1982) Cell junctions and intramembrane particles of astrocytes and oligodendrocytes: a freeze-fracture study. Neurosci 7:523-538.

Matrisian LM (1994) Matrix Metalloproteinase Gene Expression. Ann NY Acad Sci 732:42-50

Matsuda Y, Koito H, Yamamoto H (1997) Induction of myelin-associated glycoprotein expression through neuron-oligodendrocyte contact. Brain Res Dev Brain Res 100:110-116.

Matsuzaki K, Yoshitake Y, Matuo Y, Sasaki H, Nishikawa K (1989) Monoclonal antibodies against heparin-binding growth factor II/bFGF that block its biological activity: Invalidity of the antibodies for tumor angiogenesis. Proc Natl Acad Sci USA 86:9911-9915.

Matthew WD, Greenspan RJ, Lander AD, Reichardt LF (1985) Immunopurification and characterization of a neuronal heparan sulfate proteoglycan. J Neurosci 5:1842-1850.

Matthiessen HP, Schmalenbach C, Muller HW (1989) Astroglia-released neurite growth-inducing activity for embryonic hippocampal neurons is associated with laminin bound in a sulfated complex and free fibronectin. Glia 2:177-188.

Matyszak MK, Perry VH (1996) Delayed-type hypersensitivity lesions in the central nervous system are prevented by inhibitors of matrix metalloproteinases. J Neuroimmunol 69:141-149.

Mayer M, Bhakoo K, Noble M (1994) Ciliary neurotrophic factor and leukemia inhibitory factor promote the generation, maturation and survival of oligodendrocytes in vitro. Development 120:143-153.

Mayer M, Bögler O, Noble M (1993) The inhibition of oligodendrocytic differentiation of O-2A progenitors caused by basic fibroblast growth factor is overridden by astrocytes. Glia 8:12-19.

McCarthy KD, de Vellis J (1980) Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. J Cell Biol 85:890-902.

McGuire PG, Seeds NW (1990) Degradation of underlying extracellular matrix by sensory neurons during neurite outgrowth. Neuron 4:633-642.

McKeon RJ, Schreiber RC, Rudge JS, Silver J (1991) Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of inhibitory molecules on reactive astrocytes. J Neurosci 11:3398-3411.

McKinnon RD, Matsui T, Aranda M, Dubois-Dalcq M (1991) A role for fibroblast growth factor in oligodendrocyte development. Ann NY Acad Sci 638:378-386.

McKinnon RD, Matsui T, Dubois-Dalq M, Aaronson SA (1990) FGF modulates the PDGF-driven pathway of oligodendrocyte development. Neuron 5:603-614.

McKinnon RD, Piras G, Ida JJA, Dubois-Dalcq M (1993) A role for TGF-β in oligodendrocyte differentiation. J Cell Biol 121:1397-1407.

McLaurin J, Antel JP, Yong VW (1995) Immune and non-immune actions of interferon-β-1b on primary human neural cells. Mult Scler 1:10-19.

McLaurin J, Yong VW (1995) Oligodendrocytes and myelin. In Neurologic Clinics of North America: Multiple Sclerosis (Antel JP, ed), pp 23-49. Philadelphia: W.B. Saunders Co.

McLoon SC, McLoon LK, Palm SL, Furcht LT (1988) Transient expression of laminin in the optic nerve of the developing rat. J Neurosci 8:1981-1990.

Mcmorris FA, Smith TM, DeSalvo S, Furlanetto RW (1986) Insulin-like growth factor I/somatomedin C: a potent inducer of oligodendrocyte development. Proc Natl Acad Sci USA 83:822-826.

McPhilemy K, Mitchell LS, Griffiths IR, Morrison S, Deary AW, Sommer I, Kennedy PG (1990) Effect of optic nerve transection upon myelin protein gene expression by oligodendrocytes: evidence for axonai influences on gene expression. J Neurocytol 19:494-503.

Merrill JE, Ignarro LJ, Sherman MP, Melinek J, Lane TE (1993) Microglial cell cytotoxicity of oligodendrocytes is mediated through nitric oxide. J Immunol 151:2132-2141.

Merrill JE, Zimmerman RP (1991) Natural and induced cytotoxicity of oligodendrocytes by microglia is inhibitable by TGFβ. Glia 4:327-331.

Meyer T, Regenass U, Fabbro D, Alter E, Rosel J, Muller M, Caravatti G, Matter (1989) A derivative of staurosporine (CGP 41 251) shows selectivity for protein kinase C inhibition and in vitro anti-proliferative as well as in vivo anti-tumor activity. Int J Cancer 43:851-856.

Miller RH, Abney ER, David S, ffrench-Constant C, Lindsay R, Patel R, Stone J, Raff MC (1986) Is reactive gliosis a property of a distinct subpopulation of astrocytes? J Neurosci 6:22-29.

Millichip MI, Dallas DJ, Wu E, Dale S, McKie N (1998) The metallo-disintegrin ADAM10 (MADM) from bovine kidney has type IV collagenase activity in vitro. Biochem Biophys Res Commun 245:594-598.

Milner R, Edwards G, Streuli C, ffrench-Constant C (1996) A role in migration for the alpha-v-beta-1 integrin expressed on oligodendrocyte precursors. J Neurosci 16:7240-7252.

Milner R, ffrench-Constant C (1994) A developmental analysis of oligodendroglial integrins in primary cells: changes in αv-associated β subunits during differentiation. Development 120:3497-3506.

Milner R, Frost E, Nishimura S, Delcommenne M, Streuli C, Pytela R, ffrench-Constant C (1997) Expression of alphav beta3 and alphav beta8 integrins during oligodendrocyte precursor differentiation in the presence and absence of axons. Glia 21:350-360.

Miyake A, Hattori Y, Ohta M, Itoh N (1996) Rat oligodendrocytes and astrocytes preferentially express fibroblast growth factor receptor-2 and -3 mRNAs. J Neurosci Res 45:534-541.

Miyamoto M, Naruo K, Seko C, Matsumoto S, Kondo T, Kurokawa T (1993) Molecular cloning of a novel cytokine cDNA encoding the ninth member of the fibroblast growth factor family, which has a unique secretion property. Mol Cell Biol 13:4251-4259.

Mogi A, Hatai M, Soga H, Takenoshita S, Nagamachi Y, Fujimoto J, Yamamoto T, Yokota J, Yaoi Y (1995) Possible role of protein kinase C in the regulation of intracellular stability of focal adhesion kinase in mouse 3T3 cells. FEBS Lett 373:135-140.

Monard D (1988) Cell-derived proteases and protease inhibitors as regulators of neurite outgrowth. Trends Neurosci 11:541-544.

Monge M, Kadiiski D, Jacque CM, Zalc B (1986) Oligodendroglial expression and deposition of four major myelin constituents in the myelin sheath during development. An in vivo study. Dev Neurosci 8:222-235.

Monsky WL, Kelly T, Lin CY, Yeh Y, Stetler-Stevenson WG, Mueller SC, Chen WT (1993) Binding and localization of M(r) 72,000 matrix metalloproteinase at cell surface invadopodia. Cancer Res 53:3159-3164.

Monsky WL, Lin CY, Aoyama A, Kelly T, Akiyama SK, Mueller SC, Chen WT (1994) A potential marker protease of invasiveness, seprase, is localized on invadopodia of human malignant melanoma cells. Cancer Res 54:5702-5710.

Morell P, Quarles RH, Norton WT (1994) Myelin formation, structure and biochemistry In Basic Neurochemistry: Molecular, Cellular, and Medical Aspects. (Siegel GJ, ed), pp 117-144. New York: Raven Press Ltd.

Muir D (1994) Metalloproteinase-dependent neurite outgrowth within a synthetic extracellular matrix is induced by nerve growth factor. Exp Cell Res 210:243-252.

Muller HW, Junghans U, Kappler J (1995) Astroglial neurotrophic and neurite-promoting factors. Pharmacol Ther 65:1-18.

Murphy G, Knauper V (1997) Relating matrix metalloproteinase structure to function: why the "hemopexin" domain? Matrix Biol 15:511-518.

Ngase H (1997) Activation mechanism of matrix metalloproteinases. Biol Chem 378:1475-1480

Neugebauer KM, Tomaselli KJ, Lilien J, Reichardt LF (1988) N-cadherin, NCAM and integrins promote retinal neurite outgrowth on astrocytes in vitro. J Cell Biol 107:1177-1187.

Newton AC (1995) Protein kinase C: Structure, function, and regulation. J Biol Chem 270:28495-28498.

Niebroj-Dobosz I, Fidzianska A, Rafalowska J, Sawicka E (1980) Correlative biochemical and morphological studies of myelination in human ontogenesis. I. Myelination of the spinal cord. Acta Neuropathol (Berl.) 49:145-152.

Nishizuka Y (1992) Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258:607-614.

Nishizuka Y (1995) Protein kinase C and lipid signaling for sustained cellular responses. FASEB J 9:484-496.

Noble M, Fok-Seang J, Cohen J (1984) Glia are a unique substrate for the in vitro growth of central nervous system neurons. J Neurosci 4:1892-1903.

Norton WT, Poduslo SE (1973) Myelination in rat brain: changes in myelin composition during brain maturation. J Neurochem 21:759-773.

Oh LYS, Goodyer CG, Olivier A, Yong VW (1997) The promoting effects of bFGF and astrocyte extracellular matrix on process outgrowth by adult human oligodendrocytes are mediated by protein kinase C. Brain Res 757:236-244.

Oh LYS, Yong VW (1996) Astrocytes promote process outgrowth by adult human oligodendrocytes in vitro through interaction between bFGF and astrocyte extracellular matrix Glia 17:237-253.

O'Neill RC, Minuk J, Cox ME, Braun PE, Gravel M (1997) CNP2 mRNA directs synthesis of both CNP1 and CNP2 polypeptides. J Neurosci Res 50:248-257.

Osterhout DJ, Ebner S, Xu J, Ornitz DM, Zazanis GA, McKinnon RD (1997) Transplanted oligodendrocyte progenitor cells expressing a dominant- negative FGF receptor transgene fail to migrate in vivo. J Neurosci 17:9122-9132.

Oury F, Faucher C, Rives I, Bensaid M, Bouche G, Darbon JM (1992) Regulation of cyclic adenosine 3', 5'-monophosphate-dependent protein kinase activity and regulatory subunit RII beta content by basic fibroblast growth factor (bFGF)

during granulosa cell differentiation: possible implication of protein kinase C in bFGF action. Biol Reprod 47:202-212.

Ovadia H, Lubetzki-Korn I, Brenner T, Abramsky O, Fridman R, Vlodavsky I (1984) Adult rat oligodendrocytes grown in vitro upon an extracellular matrix have the ability to proliferate. Brain Res 322:93-100.

Quarles RH (1997) Glycoproteins of myelin sheaths. J Mol Neurosci 8:1-12.

Pesheva P, Gloor S, Schachner M, Probstmeier R (1997) Tenascin-R is an intrinsic autocrine factor for oligodendrocyte differentiation and promotes cell adhesion by a sulfatide- mediated mechanism. J Neurosci 17:4642-4651.

Peters A, Vaughn JE (1970) Morphology and development of the myelin sheath. In: Myelination (Davison AN, Peters A eds), pp 3. Springfield, IL: Charles C Thomas.

Pfeiffer SE, Warrington AE, Bansal R (1993) The oligodendrocyte and its many cellular processes. Trends Cell Biol 3:191-197.

Prekeris R, Mayhew MW, Cooper JB, Terrian DM (1996) Identification and localization of an actin-binding motif that is unique to the epsilon isoform of protein kinase C and participates in the regulation of synaptic function. J Cell Biol 132:77-90.

Presta M, Maier JAM, Rignotti G (1989) The mitogenic signaling pathway but not the plasminogen activator-inducing pathway of basic fibroblast growth factor is mediated through protein kinase C in fetal bovine aortic endothelial cells. J Cell Biol 109:1877-1884.

Presta M, Tiberio L, Rusnati M, Dell'Era P, Ragnotti G (1991) Basic fibroblast growth factor requires a long-lasting activation of protein kinase C to induce cell proliferation in transformed fetal bovine aortic endothelial cells. Cell Regul 2:719-726.

Price J, Hynes RO (1985) Astrocytes in culture synthesize and secrete a variant form of fibronectin. J Neurosci 5:2205-2211.

Prineas JW, Barnard RO, Kwon EE, Sharer LR, Cho ES (1993) Multiple sclerosis: Remyelination of nascent lesions. Ann Neurol 33:137-151.

Prineas JW, Kwon EE, Goldenberg PZ, Cho E-S, Sharer LR (1990) Interaction of astrocytes and newly formed oligodendrocytes in resolving multiple sclerosis lesions. Lab Invest 63:624-636.

Prineas JW, Kwon EE, Goldenberg PZ, Ilyas AA, Quarles RH, Benjamins JA, Sprinkle TJ (1989) Multiple sclerosis: Oligodendrocyte proliferation and differentiation in fresh lesions. Lab Invest 61:489-503.

Pringle NP, Guthrie S, Lumsden A, Richardson WD (1998) Dorsal spinal cord neuroepithelium generates astrocytes but not oligodendrocytes. Neuron 20:883-893.

Radhakrishna M, Almazan G (1994) Protein kinases mediate basic fibroblast growth factor's stimulation of proliferation and c-fos induction in oligodendrocyte progenitors. Brain Res 24:118-128.

Raff MC (1989) Glial cell diversification in the rat optic nerve. Science 243:1450-1455.

Raff MC, Abney ER, Cohen J, Lindsay R, Noble M (1983) Two types of astrocytes in cultures of developing rat white matter: differences in morphology, surface gangliosides, and growth characteristics. J Neurosci 3:1289-1300.

Raff MC, Miller RH, Noble M (1983) A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium. Nature 303:390-396.

Raine CS (1984) Morphology of myelin and myelination. In Myelin (Morrell P ed), pp 1 New York: Plenum Press.

Raine CS, Scheinberg L, Waltz JM (1981) Oligodendrocyte survival and proliferation in an active established lesion. Lab Invest 45:534-546.

Raine CS, Wu E (1993) Multiple sclerosis: Remyelination in acute lesions. J Neuropathol Exp Neurol 52:199-204.

Redwine JM, Blinder KL, Armstrong RC (1997) In situ expression of fibroblast growth factor receptors by oligodendrocyte progenitors and oligodendrocytes in adult mouse central nervous system. J Neurosci Res 50:229-237.

Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255:1707-1710.

Richardson WD, Pringle N, Mosley MJ, Westermark B, Dubois-Dalcq M (1988) A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. Cell 53:309-319.

Rifkin DB, Moscatelli D (1989) Recent developments in the cell biology of basic fibroblast growth factor. J Cell Biol 109:1-6.

Rio Hortega PD (1921) La glia de escasas radiacions (oligodendroglia). Bol R Soc Esp Hist Nat 21:63-92.

Rio Hortega PD (1928) Tercera aportacion al conocimiento morfologico e interpretacion funcional de la oligodendroglia. Mem R Soc Esp Hist Nat 14:5-122.

Roach A, Boylan K, Horvath S, Prusiner SB, Hood LE (1983) Characterization of cloned cDNA representing rat myelin basic protein: absence of expression in brain of shiverer mutant mice. Cell 34:799-806.

Robertson W (1899) On a new method of obtaining a black reaction in certain tissue-elements of the central nervous system (platinum method). Scott Med Surg J 4:23-30.

Rodriguez M (1991) Immunoglobulins stimulate central nervous system remyelination: electron microscopic and morphometric analysis of proliferating cells. Lab Invest 64:358-370.

Rodriguez M, Scheithauer B (1994) Ultrastructure of multiple sclerosis. Ultrastruct Pathol 18:3-13.

Rodriguez M, Scheithauer BW, Forbes G, Kelly PJ (1993) Oligodendrocyte injury is an early event in lesions of multiple sclerosis. Mayo Clin Proc 68:627-636.

Roivainen R, Hundle B, Messing RO (1995) Ethanol enhances growth factor activation of mitogen-activated protein kinases by a protein kinase C-dependent mechanism. Proc Natl Acad Sci USA 92(6):1891-1895.

Rosen CL, Bunge RP, Ard MD, Wood PM (1989) Type 1 astrocytes inhibit myelination by adult rat oligodendrocytes in vitro. J Neurosci 9:3371-3379.

Rosenberg GA, Kornfeld M, Estrada E, Kelley RO, Liotta LA, Stetler-Stevenson WG (1992) TIMP-2 reduces proteolytic opening of blood-brain barrier by type IV collagenase. Brain Res 576:203-207.

Rudge JS, Alderson RF, Pasnikowski E, McClain J, Ip NY, Lindsay RM (1992) Expression of ciliary neurotrophic factor and the neurotrophins - nerve growth factor, brain-derived neurotrophic factor and neurotrophin 3 - in cultured rat hippocampal astrocytes. Eur J Neurosci 4:459-471.

Rumbsy MG (1984) Structural organization and stability of central nervous system myelin. In A Multidisciplinary Approach to Myelin Diseases (Crescenzi GS ed), pp 111 New York: Plenum Press.

Ruoslahti E, Yamaguchi Y (1991) Proteoglycans as modulators of growth factor activities. Cell 64:867-869.

Rutka JT, Giblin J, Dougherty DV, McCulloch JR, DeArmond SJ, Rosenblum ML (1986) An ultrastructural and immunocytochemical analysis of leptomeningeal and meningioma cultures. J Neuropath Exp Neurol 45:285-303.

Sanes JR (1989) Extracellular matrix molecules that influence neural development. Ann Rev Neurosci 12:491-516.

Saneto RP, de Vellis J (1985) Characterization of cultured rat oligodendrocytes proliferating in a serum-free, chemically defined medium. Proc Natl Acad Sci USA 82:3509-3513.

Satoh J, Kim SU (1994) Proliferation and differentiation of fetal human oligodendrocytes in culture. J Neurosci Res 39:260-272.

Schmidt-Schultz T, Althaus HH (1994) Monogalactosyl diglyceride, a marker for myelination, activates oligodendroglial protein kinase C. J Neurochem 62:1578-1585.

Schonwasser DC, Marais RM, Marshall CJ, Parker PJ (1998) Activation of the mitogen-activated protein kinase/extracellular signal- regulated kinase pathway by conventional, novel, and atypical protein kinase C isotypes. Mol Cell Biol 18:790-798.

Schwartz MA, Schaller MD, Ginsberg MH (1995) Integrins: emerging paradigms of signal transduction. Annu Rev Cell Dev Biol 11:549-599.

Schwartz JH (1991) The cytology of neurons. In Principles of neural science (Kandel ER, Schwartz JH, Jessell TM eds), pp 37-48. Norwalk: Appleton & Lange.

Scolding N, Franklin R, Stevens S, Heldin CH, Compston A, Newcombe J (1998) Oligodendrocyte progenitors are present in the normal adult human CNS and in the lesions of multiple sclerosis. Brain 121:2221-2228.

Scolding NJ, Rayner PJ, Sussman J, Shaw C, Compston DA (1995) A proliferative adult human oligodendrocyte progenitor. Neuroreport 6:441-445.

Sehgal G, Hua J, Bernhard EJ, Sehgal I, Thompson TC, Muschel RJ (1998) Requirement for matrix metalloproteinase-9 (gelatinase B) expression in metastasis by murine prostate carcinoma. Am J Pathol 152:591-596.

Selak I, Foidart JM, Moonen G (1985) Laminin promotes cerebellar granule cells migration in vitro and is synthesized by cultured astrocytes. Dev Neurosci 7:278-285.

Selmaj K, Raine CS, Cross AH (1991) Anti-tumor necrosis factor therapy abrogates autoimmune demyelination. Ann Neurol 30:694-700.

Seniuk NA, Henderson JT, Tatton WG, Roder JC (1994) Increased CNTF gene expression in process-bearing astrocytes following injury is augmented by R(-)-Deprenyl. J Neurosci Res 37:278-286.

Shapiro SD (1998) Matrix metalloproteinase degradation of extracellular matrix: biological consequences. Curr Opin Cell Biol 10:602-608.

Shea TB, Cressman CM, Spencer MJ, Beermann ML, Nixon RA (1995) Enhancement of neurite outgrowth following calpain inhibition is mediated by protein kinase C. J Neurochem 65:517-527.

Sheffield JB, Krasnopolsky V, Dehlinger E (1994) Inhibition of retinal growth cone activity by specific metalloproteinase inhibitors in vitro. Dev Dyn 200:79-88.

Sheppard AM, Hamilton SK, Pearlman AL (1991) Changes in the distribution of extracellular matrix components accompany early morphogenetic events of mammalian cortical development. J Neurosci 11:3928-3942.

Shine HD, Readhead C, Popko B, Hood L, Sidman RL (1992) Morphometric analysis of normal, mutant, and transgenic CNS: correlation of myelin basic protein expression to myelinogenesis. J Neurochem 58:342-349.

Shoyab M, Warren TC, Todaro GJ (1976) Tissue and species distribution and development variation of specific receptors for biologically active phorbol and ingenol esters. Carcinogenesis 2:273-276

Silver J, Ogawa MY (1983) Postnatally induced formation of the corpus callosum in acallosal mice in glial-coated cellulose bridges. Science 220:1067-1069.

Skoff RP, Knapp PE (1991) Division of astroblasts and oligodendroblasts in postnatal rodent brain: evidence for separate astrocyte and oligodendrocyte lineages. Glia 4:165-174.

Smith GM, Rutishauser U, Silver J, Miller RH (1990) Maturation of astrocytes in vitro alters the extent and molecular basis of neurite outgrowth. Dev Biol 138:377-390.

Snow DM, Lemmon V, Carrino DA, Caplan AI, Silver J (1990) Sulfated proteoglycans in astroglial barriers inhibit neurite outgrowth in vitro. Exp Neurol 109:111-130.

Soloway PD, Alexander CM, Werb Z, Jaenisch R (1996) Targeted mutagenesis of Timp-1 reveals that lung tumor invasion is influenced by Timp-1 genotype of the tumor but not by that of the host. Oncogene 13:2307-2314.

Spivak-Kroizman T, Lemmon MA, Dikic I, Ladbury JE, Pinchasi D, Huang J, Jaye M, Crumley G, Schlessinger J, Lax I (1994) Heparin-induced oligomerization of FGF molecules is responsible for FGF receptor dimerization, activation, and cell proliferation. Cell 79:1015-1024.

Sommer I, Schachner M (1981) Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: An immunocytochemical study of the central nervous system. Dev Biol 83:311-327.

Springman EB, Angleton EL, Birkedal-Hansen H, Van Wart HE (1990) Multiple modes of activation of latent human fibroblast collagenase: evidence for the role of a Cys73 active-site zinc complex in latency and a "cysteine switch" mechanism for activation. Proc Natl Acad Sci USA 87:364-368.

Steinfeld R, Van DBH, David G (1996) Stimulation of fibroblast growth factor receptor-1 occupancy and signaling by cell surface-associated syndecans and glypican. J Cell Biol 133:405-416.

Sternberger NH, Itoyama Y, Kies MW, Webster H de F (1978) Myelin basic protein demonstrated immunocytochemically in oligodendroglia prior to myelin sheath formation. Proc Natl Acad Sci USA 75:2521-2524.

Stevens B, Tanner S, Fields RD (1998) Control of myelination by specific patterns of neural impulses. J Neurosci 18:9303-9311.

Stewart M, Thiel M, Hogg N (1995) Leukocyte integrins. Curr Opin Cell Biol 7:690-696.

Stuve O, Dooley NP, Uhm JH, Antel JP, Francis GS, Williams G, Yong VW (1996). Interferon beta 1b decreases the migration of T lymphocytes in vitro: effects on matrix metalloproteinase 9. Ann Neurol 40:853-863.

Suzuki K, Andrews JM, Waltz JM, Terry RD (1969) Ultrastructural studies of multiple sclerosis. Lab Invest 20:444-454.

Suzuki M, Raisman G (1992) The glial framework of central white matter tracts: segmented rows of contiguous interfascicular oligodendrocytes and solitary astrocytes give rise to a continuous meshwork of transverse and longitudinal processes in the adult rat fimbria. Glia 6:222-235.

Szuchet S (1987) The plasticity of mature oligodendrocytes: a role for substratum in phenotype expression. In A Multidisciplinary Approach to Myelin Diseases. (Crescenzi GS, ed), pp 143-159. New York: Plenum Press.

Szuchet S, Stefansson K, Wollmann RL, Dawson G, Arnason BG (1980) Maintenance of isolated oligodendrocytes in long-term culture. Brain Res 200:151-164.

Tang DG, Diglio CA, Bazaz R, Honn KV (1995) Transcriptional activation of endothelial cell integrin alpha v by protein kinase C activator 12(S)-HETE. J Cell Sci 108:2629-2644.

Thomas D, Caruelle JP, Lachapelle F, Barritault D, Boully B (1991) Acidic fibroblast growth factor in normal, injured and jimpy mutant developing mouse brain. Ann NY Acad Sci 648:161-166.

Tomasek JJ, Halliday NL, Updike DL, Ahern-Moore JS, Vu TK, Liu RW, Howard EW (1997) Gelatinase A activation is regulated by the organization of the polymerized actin cytoskeleton. J Biol Chem 272:7482-7487.

Tomaselli KJ, Neugebauer KM, Bixby JL, Lilein J, Reichardt LF (1988) N-cadherin and integrins: Two receptor systems that mediate neuronal process outgrowth on astrocyte surfaces. Neuron 1:33-43.

Toker A (1998) Signaling through protein kinase C. Front Biosci 3:D1134-1147.

Trapp BD, Andrews SB, Cootauco C, Quarles R (1989) The myelin-associated glycoprotein is enriched in multivesicular bodies and periaxonal membranes of actively myelinating oligodendrocytes. J Cell Biol 109:2417-2426.

Trapp BD, Nishiyama A, Cheng D, Macklin W (1997) Differentiation and death of premyelinating oligodendrocytes in developing rodent brain. J Cell Biol 137:459-468.

Tremble P, Chiquet-Ehrismann R, Werb Z (1994) The extracellular matrix ligands fibronectin and tenascin collaborate in regulating collagenase gene expression in fibroblasts. Mol Biol Cell 5:439-453.

Tropak MB, Johnson PW, Dunn RJ, Roder JC (1988) Differential splicing of MAG transcripts during CNS and PNS development. Brain Res 464:143-155.

Turner RS, Chou CH, Kibler RF, Kuo JF (1982) Basic protein in brain myelin is phosphorylated by endogenous phospholipid-sensitive Ca2+-dependent protein kinase. J Neurochem 39:1397-1404.

Uhm JH, Dooley NP, Oh LY, Yong VW (1998) Oligodendrocytes utilize a matrix metalloproteinase, MMP-9, to extend processes along an astrocyte extracellular matrix. Glia 22:53-63.

Uhm JH, Dooley NP, Villemure JG, Yong VW (1996) Glioma invasion in vitro: regulation by matrix metalloprotease 2 and protein kinase C. Clin Exp Metastasis 14:421-433.

Uhm JH, Dooley NP, Villemure JG, Yong VW (1997) Mechanisms of glioma invasion: role of matrix metalloproteinases. Can J Neurol Sci 24:3-15.

Unemori EN, Werb Z (1986) Reorganization of polymerized actin: a possible trigger for induction of procollagenase in fibroblasts cultured in and on collagen gels. J Cell Biol 103:1021-1031.

Umemori H, Sato S, Yagi T, Aizawa S, Yamamoto T (1994) Initial events of myelination involve Fyn tyrosine kinase signalling. Nature 367:572-576.

Utzschneider DA, Archer DR, Kocsis JD, Waxman SG, Duncan ID (1994) Transplantation of glial cells enhances action potential conduction of amyelinated spinal cord axons in the myelin-deficient rat. Proc Natl Acad Sci USA 91:53-57.

Van Wart HE, Birkedal-Hansen H (1990) The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc Natl Acad Sci USA 87:5578-5582.

Varnum-Finney B, Venstrom K, Muller U, Kypta R, Backus C, Chiquet M, Reichardt LF (1995) The integrin receptor alpha 8 beta 1 mediates interactions of embryonic chick motor and sensory neurons with tenascin-C. Neuron 14:1213-1222.

Vartanian T, Szuchet S, Dawson G (1992) Oligodendrocyte-substratum adhesion activates the synthesis of specific lipid species involved in cell signaling. J Neurosci Res 32:69-78.

Vartanian T, Szuchet S, Dawson G, Campagnoni AT (1986) Oligodendrocyte adhesion activates protein kinase C-meidated phosphorylation of myelin basic protein. Science 234 (1986) 1395-1398.

Venstrom KA, Reichardt LF (1993) Extracellular matrix. 2: Role of extracellular matrix molecules and their receptors in the nervous system. FASEB J 7:996-1003.

Vicks RS, DeVries GH (1992) Mitotic potential of adult rat oligodendrocytes in culture. J Neurosci Res 33:68-74.

Vlodavsky I, Folkman J, Sullivan R, Fridman R, Ishai-Michaeli R, Sasse J, Klagsbrun M (1987) Endothelial cell-derived basic fibroblast growth factor: synthesis and deposition into subendothelial extracellular matrix. Proc Natl Acad Sci USA 84:2292-2296.

Vu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, Shapiro SD, Senior RM, Werb Z (1998) MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell 93:411-422.

Vuori K, Ruoslahti E (1993) Activation of protein kinase C precedes alpha 5 beta 1 integrin- mediated cell spreading on fibronectin. J Biol Chem 268:21459-21462.

Wang S, Sdrulla AD, diSibio G, Bush G, Nofziger D, Hicks C, Weinmaster G, Barres BA (1998) Notch receptor activation inhibits oligodendrocyte differentiation. Neuron 21:63-75.

Warrington AE, Pfeiffer SE (1992) Proliferation and differentiation of O4+ oligodendrocytes in postnatal rat cerebellum: analysis in unfixed tissue slices using anti-glycolipid antibodies. J Neurosci Res 33:338-353.

Weaver CD, Yoshida CK, de CI, Reichardt LF (1995) Expression and in vitro function of beta 1-integrin laminin receptors in the developing avian ciliary ganglion. J Neurosci 15:5275-5285.

Weidenheim KM, Kress Y, Epshteyn I, Rashbaum WK, Lyman WD (1992) Early myelination in the human fetal lumbosacral spinal cord: characterization by light and electron microscopy. J Neuropathol Exp Neurol 51:142-149.

Werb Z, Tremble PM, Behrendtsen O, Crowley E, Damsky CH (1989) Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. J Cell Biol 109:877-889.

Weston CA, Weeks BS (1996) bFGF stimulates u937 cell adhesion to fibronectin and secretion of of gelatinase B. Biochem Biophy Res Comm 228:318-323.

Williams K, Bar-Or A, Ulvestad E, Olivier A, Antel JP, Yong VW (1992) Biology of adult human microglia in culture: comparisons with peripheral blood monocytes and astrocytes. J Neuropathol Exp Neurol 51:538-549.

Williams EJ, Furness J, Walsh FS, Doherty P (1994) Activation of the FGF receptor underlies neurite outgrowth stimulated by L1, N-CAM, and N-cadherin. Neuron 13:583-594.

Williams BP, Price J (1992) What have tissue culture studies told us about the development of oligodendrocytes? Bioessays 14:693-698.

Wilson R, Brophy PJ (1989) Role for the oligodendrocyte cytoskeleton in myelination. J Neurosci Res 22:439-448.

Wintergerst ES, Fuss B, Bartsch U (1993) Localization of janusin mRNA in the central nervous system of the developing and adult mouse. Eur J Neurosci 5:299-310.

Woessner JF Jr. (1994) The Family of Matrix Metalloproteinases. Ann NY Acad Sci 11-21.

Wolswijk G (1995) Strongly GD3+ cells in the developing and adult rat cerebellum belong to the microglial lineage rather than to the oligodendrocyte lineage. Glia 13:13-26.

Wolswijk G (1998) Chronic stage multiple sclerosis lesions contain a relatively quiescent population of oligodendrocyte precursor cells. J Neurosci 18:601-609.

Wolswijk G, Noble M (1989) Identification of an adult-specific glial progenitor cell. Development 105:387-400.

Wolswijk G, Noble M (1992) Cooperation between PDGF and FGF converts slowly dividing O-2A adult progenitor cells to rapidly dividing cells with characteristics of O- 2A perinatal progenitor cells. J Cell Biol 118:889-900.

Wood PM, Bunge RP (1991) The origin of remyelinating cells in the adult central nervous system: the role of the mature oligodendrocyte. Glia 4:225-232.

Wood P, Bunge RP (1984) The biology of the oligodendrocyte. In Oligodendroglia, Advances in Neurochemistry (Norton WT ed), pp 1-10. New York: Plenum Publishing Co.

Wood PM, Bunge RP (1986) Evidence that axons are mitogenic for oligodendrocytes isolated from adult animals. Nature 320:756-758.

Woods A, Couchman JR (1992) Protein kinase C involvement in focal adhesion formation. J Cell Sci 101:277-290.

Wood DD, Moscarello MA (1989) The isolation, characterization, and lipid-aggregating properties of a citrulline containing myelin basic protein. J Biol Chem 264:5121-5127.

Wren D, Wolswijk G, Noble M (1992) In vitro analysis of the origin and maintenance of O-2A adult progenitor cells. J Cell Biol 116:167-176.

Wu E, Raine C, Raine CS (1992) Multiple sclerosis: Interaction between oligodendrocytes and hypertrophic astrocytes and their occurrence in other, non-demyelinating conditions. Lab Invest 67:88-99.

Wujek JR, Akeson RA (1987) Extracellular matrix derived from astrocytes stimulates neuritic outgrowth from PC12 cells in vitro. Brain Res 431:87-97.

Xu J, Clark RA (1997) A three-dimensional collagen lattice induces protein kinase C-zeta activity: role in alpha2 integrin and collagenase mRNA expression. J Cell Biol 136:473-483.

Yamada KM, Miyamoto S (1995) Integrin transmembrane signaling and cytoskeletal control. Curr Opin Cell Biol 7:681-689.

Yamashita K, Suzuki M, Iwata H, Koike T, Hamaguchi M, Shinagawa A, Noguchi T, Hayakawa T (1996) Tyrosine phosphorylation is crucial for growth signaling by tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2). FEBS Lett 396:103-107.

Yasuda T, Grinspan J, Stern J, Franceschini B, Bannerman O, Pleasure D (1995) Apoptosis occurs in the oligodendroglial lineage, and is prevented by basic fibroblast growth factor. J Neurosci Res 40:306-317.

Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM (1991) Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. Cell 64:841-848.

Yin X, Peterson J, Gravel M, Braun PE, Trapp BD (1997) CNP overexpression induces aberrant oligodendrocyte membranes and inhibits MBP accumulation and myelin compaction. J Neurosci Res 50:238-247.

Yim SH, Toda K, Goda S, Quarles RH (1995) Comparison of the phosphorylation of myelin-associated glycoprotein in cultured oligodendrocytes and Schwann cells. J Mol Neurosci 6:63-74.

Yong VW, Antel JP (1992) Culture of glial cells from human brain biopsies. In Protocols for Neural Cell Culture. (Fedoroff S, Richardson A, eds), pp 81-96. New Jersey: Humana Press.

Yong VW, Antel JP (1997) Culture of glial cells from human brain biopsies. In Protocols for Neural Cell Culture. (Fedoroff S, Richardson A, eds), pp 81-96. New Jersey: Humana Press.

Yong VW, Cheung JCB, Uhm JH, Kim SU (1991) Age-dependent decrease of process formation by cultured oligodendrocytes is augmented by protein kinase C stimulation. J Neurosci Res 29:87-99.

Yong VW, Dooley NP, Noble PG (1994) Protein kinase C in cultured adult human oligodendrocytes: Characteristics, lack of down-regulation and isoform a as a mediator of fiber outgrowth. J Neurosci Res 39:83-96.

Yong VW, Kim SU, Kim MW, Shin DH (1988) Growth factors for human glial cells in culture. Glia 1:113-123.

Yong VW, Krekoski CA, Forsyth PA, Bell R, Edwards DR (1998) Matrix metalloproteinases and diseases of the CNS. Trends Neurosci 21:75-80.

Yong VW, Sekiguchi S, Kim MW, Kim SU (1988) Phorbol ester enhances morphological differentiation of oligodendrocytes in culture. J Neurosci Res 19:187-194.

Yong VW, Tejada-Berges T, Goodyer CG, Antel JP, Yong FP (1992) Differential proliferative response of human and mouse astrocytes to gamma-interferon. Glia 6:269-280.

Yong VW, Yong FP, Olivier A, Robitaille Y, Antel JP (1990) Morphologic heterogeneity of human adult astrocytes in culture: Correlation with HLA-DR expression. J Neurosci Res 27:678-688.

Yoshida K, Gage FH (1991) Fibroblast growth factors stimulate nerve growth factor synthesis and secretion by astrocytes. Brain Res 538:118-126.

Yoshimura T, Kobayashi T, Goto I (1992) Protein kinase C in rat brain myelin. Neurochem Res 17:1021-1027.

Yu Q, Stamenkovic I (1999) Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. Genes Dev 13:35-48

Zalc B, Monge M, Jacque C (1987) Oligodendroglial emergence and deposition of four major myelin constituents in the myelin sheath during development: An in vivo study. In A Multidisciplinary Approach to Myelin Diseases. (Crescenzi GS, ed), pp 77-85. New York: Plenum Press.

Zeller NK, Behar TN, Dubois-Dalcq ME, Lazzarini RA (1985) The timely expression of myelin basic protein gene in cultured rat brain oligodendrocytes is independent of continuous neuronal influences. J Neurosci 5:2955-2962.

Zhang C, Qiu HE, Krafft GA, Klein WL (1996) Protein kinase C and F-actin are essential for stimulation of neuronal FAK tyrosine phosphorylation by G-proteins and amyloid beta protein. FEBS Lett 386:185-188.

Zhang Z, Tarone G, Turner DC (1993) Expression of integrin alpha 1 beta 1 is regulated by nerve growth factor and dexamethasone in PC12 cells. Functional consequences for adhesion and neurite outgrowth. J Biol Chem 268:5557-5565.

Zuo J, Ferguson TA, Hernandez YJ, Stetler-Stevenson WG, Muir D (1998) Neuronal matrix metalloproteinase-2 degrades and inactivates a neurite-inhibiting chondroitin sulfate proteoglycan. J Neurosci 18:5203-5211.