

Cell Death in the Oligodendrocyte Lineage: A Molecular Perspective of Life/Death Decisions in Development and Disease

PATRIZIA CASACCIA-BONNEFIL^{1,2*}

¹*Molecular Neurobiology Skirball Institute at NYU, New York, New York*

²*Department of Neuroscience, UMDNJ Robert Wood Johnson Medical School, Piscataway, New Jersey*

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ABSTRACT Cell death in the oligodendrocyte lineage occurs during development and in pathological conditions as the result of a balance between opposing molecular signals. This review focuses on the molecular mechanisms of activation of signal transduction pathways affecting life/death decisions in progenitor cells and in mature oligodendrocytes. Loss of trophic support, cytokine receptor activation, and oxidative stress may differentially contribute to the induction of cell death at specific stages of development and to the pathogenesis of demyelinating disorders. The execution of the death program leading to the morphological changes of apoptosis and/or necrosis is then determined by the generation of reactive oxygen species and the level of impairment of mitochondrial function. The final decision of a cell to die or survive is determined by a competition between survival and death signals. Depending on ligand availability, type, and levels of receptor expression and downstream cross-talks between distinct signaling pathways, the cell may activate a death execution program that will be affected by its stage of differentiation and its energetic metabolism. *GLIA* 29:124–135, 2000.

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INTRODUCTION

Two forms of death have been historically identified on the basis of morphological criteria: apoptosis and necrosis (Kerr et al., 1972). Apoptosis is characterized by nuclear pyknosis, chromatin condensation, intact plasma membrane, and cellular organelles (Wyllie et al., 1981). It requires active energy consumption and is characterized by internucleosomal DNA fragmentation, activation of specific cysteine proteases (caspases), and proteolytic cleavage of specific substrates (after aspartic acid residues). In contrast, necrosis is characterized by cytoplasmic swelling and disintegration of subcellular components and loss of plasma membrane integrity and is associated with energy loss (Fig. 1). These two morphological aspects of death may occur in the oligodendrocyte lineage during normal development and upon injury, depending on the type of stimulus and the downstream signaling intermediates involved in the execution process.

EFFECTORS OF DEATH: EXECUTION MACHINERY RESPONSIBLE FOR MORPHOLOGICAL CHANGES TYPICAL OF APOPTOSIS

Apoptosis is accomplished by an evolutionarily well conserved death machinery, composed of an elaborate proteolytic system of caspases all sharing similarities in amino acid sequence, protein structure, and substrate specificity (Alnemri et al., 1996). The cleavage consensus sequence (aspartic acid in the P1 position) within the molecule and the N-terminal domain of variable length are crucial structural features in the mechanism of activation of the proteolytic cascade. The cleavage consensus site within the proenzyme implies

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*Correspondence to: Patrizia Casaccia-Bonnel, Dept. of Neuroscience UMDNJ Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854-5635. E-mail: pcasacci@saturn.med.nyu.edu

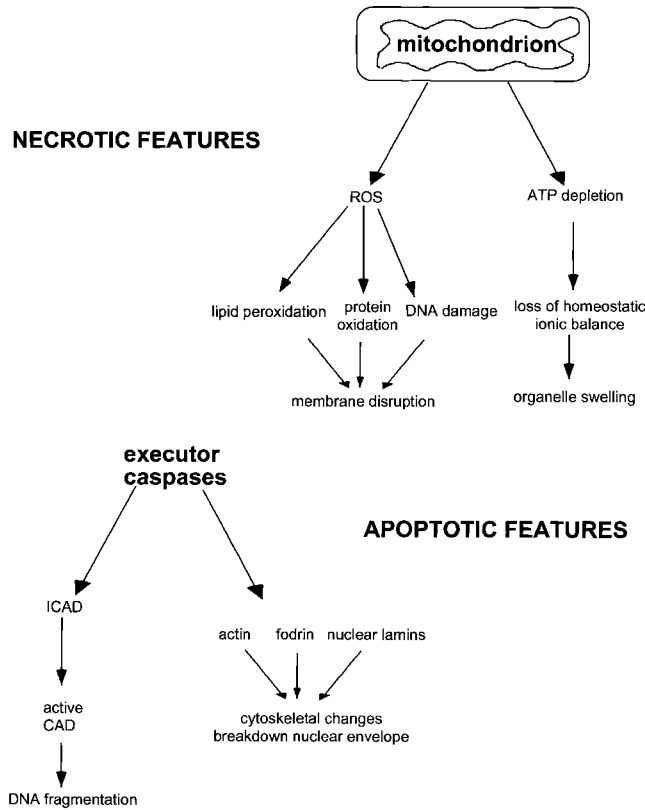


Fig. 1. Schematic representation of the two major modalities of cell death execution. Necrosis is characterized by loss of cellular integrity and severe mitochondrial impairment. In contrast, apoptosis is characterized by activation of specific proteases called caspases, which are responsible for cytoskeletal changes and DNA fragmentation. ROS = reactive oxygen species; CAD = caspase activated DNase.

that these enzymes can be activated either autocatalytically or by other proteases with similar specificity. Upon cleavage at the consensus site, two subunits are generated: a large 20 kDa (p20), and a small 10 kDa (p10) subunit; association of these subunits leads to formation of catalytically active heterotetramers. The presence of pro-enzymes with N-terminus of different length has led to the distinction between initiator and effector caspases, depending on their respective modality of activation. Initiator caspases are characterized by long N-terminal domains, containing specific protein-protein interaction motifs called DED (Death Effector Domain) in procaspase -8 and -10 and CARD (Caspase Recruitment Domain) in procaspases -1, -2, -9. The interaction of these domains with equivalent motifs present in the intracellular tail of death receptors or in adaptor molecules initiates a proteolytic cascade by activating the pro-enzyme due to intermolecular cleavage upon oligomerization. Two main modalities of activation of initiator caspases are presented: direct recruitment to activated death receptor (e.g., caspase-8) and formation of active complexes between cytosolic components and mitochondrially released cytochrome c (e.g., caspase-9).

Activation of INITIATOR Caspases by Recruitment to Death Receptors via Adaptor Molecules

As activation of initiator caspases is consequent to ligand-dependent oligomerization of death receptors (p55TNFR, p75 NGFR, or Fas) and caspase recruitment to the membrane by engagement of specific adaptor molecules (Chinnaiyan et al., 1996; Hsu et al., 1995). A schematic and simplified version of this process is represented in Figure 2. Death receptors are type I transmembrane proteins that share a common cysteine motif in the extracellular domain and a region of weak homology in the intracellular portion, designated the death domain, which should be regarded as a protein-protein interaction region (Hofmann and Tschopp, 1995). Through this region, adaptor molecules (such as FADD for Fas or TRADD for the p55 TNFR) are recruited and they bind to the N-terminal domain of initiator pro-caspases, such as FLICE/caspase-8 (Muzio et al., 1996). Similarly, other initiator caspases, such as caspase-11 and ICE/caspase-1 can be activated by p55TNFR and p75 NGFR in oligodendrocytes, via recruitment of yet unidentified adaptor molecules (Gu et al., 1999; Hisahara et al., 1997).

Activation of INITIATOR Caspases Depending on Mitochondrial Release of Cytochrome C

Another pathway of activation of downstream effector caspases requires the formation of a complex between cytosolic factors and mitochondrially released cytochrome c (Li et al., 1997; Zou et al., 1997). The initiator caspase -9 is recruited by Apaf-1 only in the presence of cytochrome c and ATP in the cytosol; it is, therefore, crucial to understand the mechanisms of regulation of cytochrome c release. In normal conditions, this molecule resides in the mitochondrial intermembrane space where it participates in the respiratory chain (Matthews, 1985). Following changes in permeability of the outer mitochondrial membrane due to alterations of the Bcl-2/Bax/Bad system, cytochrome c can leak into the cytosol and bind to Apaf-1 and caspase-9 and initiate a proteolytic cascade in the presence of ATP.

Cytochrome C Release due to Altered Function of the Bcl-2/Bax/Bad System

Members of the anti-apoptotic BH family include Bcl-2 and Bcl-XL, all containing four BH-domains (Bcl-2 homology domains), designated BH1, BH2, BH3, BH4. Interestingly, all the four domains are required for the protective function, whereas BH3 alone is sufficient to induce death. In the oligodendrocyte lineage, the protective function of Bcl-2 has been described in vivo, in the optic nerve of bcl-2 transgenic mice (Burne et al., 1996) and in intact and enucleated CG-4 cells (Jacobson et al., 1994). Members of the Bcl-2

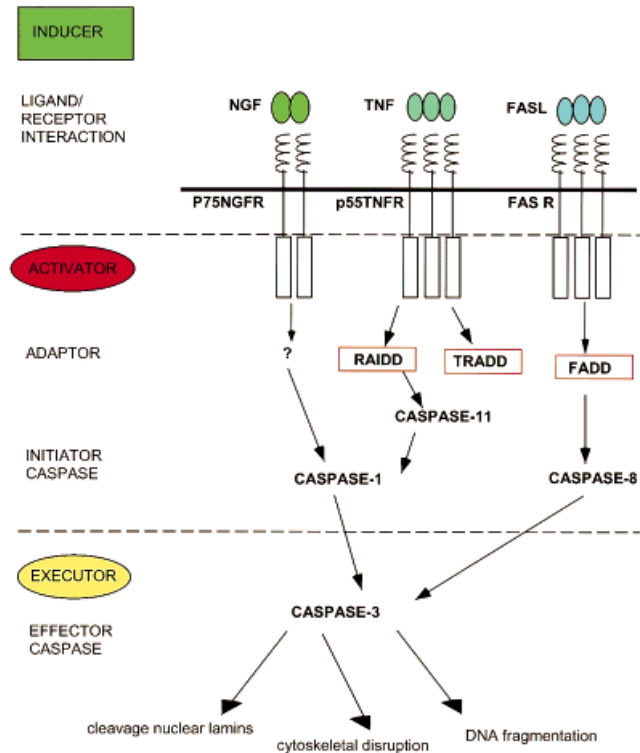


Fig. 2. Example of apoptotic cell death due to activation of death receptors. Ligand binding to the cognate death receptor leads to oligomerization of the intracellular domains and recruitment of adaptor molecules (TRADD or FADD) that recruit the proenzyme form of initiator caspases. The proteolytic cascade is then initiated by proteolytic intermolecular cleavage, leading to activation of caspase-3, a central executor caspase, responsible for the main morphological and biochemical changes of programmed cell death.

family can act at two levels: as cation transporters they contribute to preserve mitochondrial function by maintaining the appropriate ionic gradient, and as members of the BH family, they can heterodimerize and antagonize bax pro-apoptotic function (Kroemer et al., 1998; Reed, 1997). In addition, the bioavailability of the protective signals Bcl-2 and Bcl-XL is regulated by the formation of heterodimers, which is dependent on the phosphorylation state of Bad (Fig. 3). When dephosphorylated, Bad is cytosolic and, therefore, free to sequester Bcl-2 and Bcl-XL from the mitochondrial membrane. Phosphorylation of Baa is under strict control of survival pathways initiated by tyrosine kinase receptors. Signaling cascades initiated by ligand-dependent activation of tyrosine kinase receptors result in activation of the PI-3-Kinase/Akt pathway as well as activation of the Raf- kinases, both of which are able to interfere with activation of this pathway by controlling the phosphorylation state of Bad (Datta et al., 1996).

Members of the pro-apoptotic family include bim, bik, blk, bax, bid. These proteins are characterized by the presence of a single Bcl-2 homology domain (BH-3) and the ability to form pores in the outer membrane of the mitochondria with selectivity for negatively charged molecules. More specifically, several studies have suggested a role for bid and bax as cytochrome c transporter in the absence of alterations in the mitochondrial

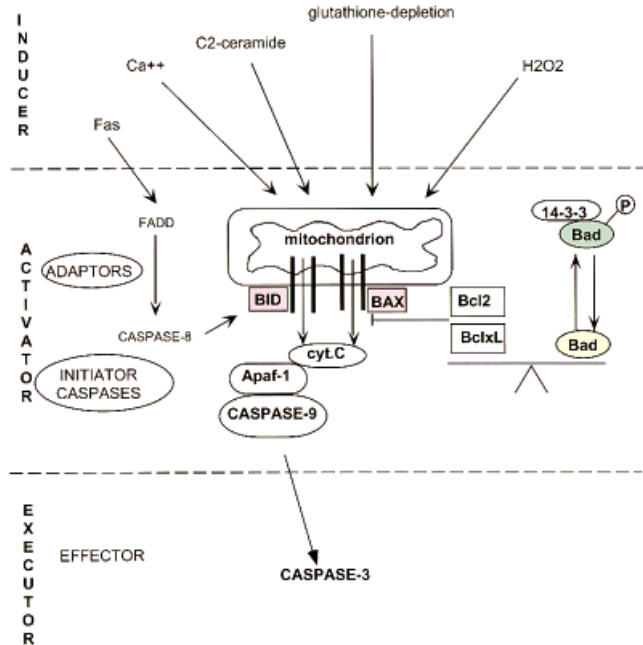


Fig. 3. The mitochondrion as cellular switch between apoptotic and necrotic modalities of death. The mitochondrion plays a major role in the activation of caspase-9 by releasing cytochrome c. Several modalities of cytochrome c release are illustrated. Death stimuli (INDUCER) may directly alter mitochondrial permeability (arrows) or affect the balance between pro-apoptotic proteins (Bid and Bax) and anti-apoptotic proteins (Bcl-2 and Bcl-xL). See text for details.

potential. Distinct death stimuli can, therefore, induce cytochrome c release by modulating the levels of BH3 proteins and/or their intracellular localization (Deckmerth et al., 1996). An example of this last modality of activation is the localization of bid. The full-length protein is localized in the cytosol, and only upon caspase-8 activation (by Fas or staurosporine) it can be translocated in the mitochondrial membrane where it allows cytochrome c release, thereby enhancing and amplifying the death signal (Fig. 3). In cultured oligodendrocytes, TNF has been shown to increase bax protein levels (Pulliam et al., 1998). In addition, dimeric IL-2 has been demonstrated to induce oligodendrocytic death mediated by p53, a well-known transcriptional activator of bax (Eizenberg et al., 1995).

EFFECTOR Caspases

Effector caspases are the enzymes directly responsible for the proteolytic cleavage of substrates involved in the induction of the morphological changes observed in apoptosis. They include caspase-3, -6, and -7, all of which share a relatively short N-terminal domain. Activation of these caspases depends on proteolytic cleavage of the pro-enzyme by activated initiator caspases. The number of cellular substrates for effector caspases is constantly increasing and can be grouped into two main categories: proteins responsible for apoptotic changes and proteins involved in antiapoptotic

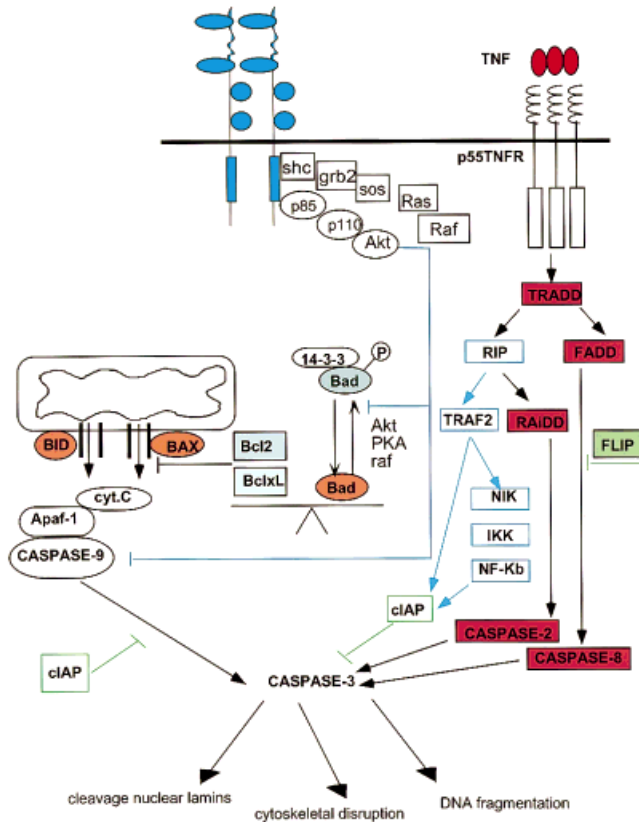


Fig. 4. Cross-talks between life and death signals. The main signaling pathways activated by tyrosine kinase receptors (blue) and cytokine receptors (white) are indicated. Protective/survival molecules are in green; death pathways are shown by black lines, and proapoptotic molecules are in orange or red.

signaling. Members of the first group of executor caspase substrates include: (1) the nuclear lamins, fodrin, actin, and the D4-GDI inhibitor of the Rho family of GTPases, which account for the disruption of the cytoskeleton and other morphological changes (Na et al., 1996), (2) ICAD, the inhibitor of Caspase Activated Dnase is responsible for DNA fragmentation (Enari et al., 1998), and (3) polyADP-ribose polymerase (PARP), and the catalytic subunit of DNA-PK, involved in repair of ds-DNA breaks (Lazebnik et al., 1994). Members of the second group of substrates include signal transduction molecules involved in maintenance of survival, such as Ras, Cbl, Raf-1, and Akt (Widmann et al., 1998). During the execution phase of apoptosis, those cellular substrates that usually are responsible for maintaining basic cellular function and structures are cleaved by the effector caspases, leading to the biochemical and morphological features of apoptosis (Fig. 1).

TRANSDUCERS OF LIFE: SURVIVAL SIGNALS INDUCED BY LIGAND-DEPENDENT ACTIVATION OF TYROSINE KINASE AND CYTOKINE RECEPTORS

Distinct mechanisms of “survival” may be active in the cell at different stages of development. Survival

signals may be activated simultaneously in a cell by tyrosine kinase receptors (e.g., Trks, PDGF receptors, IGF receptors, erbs, etc.), cytokine receptors (e.g., gp130 CNTF receptor, p55 and p75 TNFR, etc.), and distinct integrin subunits. The main signaling components responsible for the “protective” response of the cell to death stimuli are reviewed, with the exception of the Bcl-2/Bax/Bad system described in the previous section.

Signaling Cascades Downstream of Tyrosine Kinases

Binding of growth factors and cytokines to their receptors originates a cascade of events consequent to tyrosine phosphorylation catalyzed by ligand induced dimerization of specific membrane receptors (Heldin, 1995). In some cases, such as for the insulin or FGF receptors, ligand binding enhances the intrinsic tyrosine kinase catalytic activity of the receptor, leading to tyrosine phosphorylation of cytosolic substrates, which act as large “docking stations,” such as IRS-1 for the insulin receptor and FRS2 for the FGF receptor (Sun et al., 1991; Kouhara et al., 1997; Wang et al., 1996). In other cases, such as for the Trk neurotrophin receptors, phosphorylation of critical tyrosine residues on the receptor themselves serve as docking sites for adaptor proteins containing the src-homology (SH2) and/or the phosphotyrosine binding (PTB) domains (Segal and Greenberg, 1996). Upon binding of NGF to TrkA, several signaling pathways are activated. The enzyme phospholipase C_γ, PI3 Kinase and the adaptor protein shc interact with specific phosphorylated tyrosine residues on the receptor.

PhospholipaseC activation generates diacylglycerol, with consequent activation of protein kinase C and IP3, which leads to release of calcium from intracellular stores. PI-3-kinase activation results in the generation of the lipid second messenger PI 3,4, biphosphate and downstream activation of the serine/threonine kinase Akt (Segal and Greenberg, 1996; Franke et al., 1997). Activation of this kinase results in phosphorylation and inactivation of caspase-9 (Cardone et al., 1998) and bad (Datta et al., 1997), thus playing a crucial role in preventing cell death. Recruitment of the SH2 and PTB containing molecule shc allows interaction of the grb2-sos complex and activation of the ras-raf-MAPK cascade. Activated Raf can phosphorylate Bad, thus further enhancing the protective role on the cytochrome c induced activation of caspase-9. In addition, activation of the MAPK cascade seems to play a very important role in regulating survival of PC-12 cells deprived of growth factor and rat oligodendrocytes treated with NGF, by counteracting the proapoptotic action of stress activated kinases such as JNK (Xia et al., 1995; Yoon et al., 1998).

Survival Signals Activated by Cytokine Receptors

Cytokine receptors of the CNTF and IFN family can initiate survival cascades by recruiting JAK kinases

that will phosphorylate specific transcription factors of the STAT family with consequent activation of transcriptional events associated with protection. Similarly, members of the death receptor superfamily, together with the ability to activate pro-apoptotic programs, can also initiate signaling events leading to activation of NF- κ B transcription of protective genes. This double feature of death receptors activating survival signals may explain the different response observed *in vitro* upon NGF treatment or IFN and TNF treatment of oligodendrocytes (Agresti et al., 1996; Ladiwala et al., 1998). Also in this case, ligand binding to the p55TNFR (Hsu et al., 1996), or p75NGFR (Khursigara et al., 1998) or p75TNFR (Rothe et al., 1994) induces oligomerization and recruitment of adaptor proteins called TRAFs. These molecules are involved in antiapoptotic signaling at several levels. On one hand, TRAFs have been shown to be upstream of NF- κ B activation, by forming a complex with NIK, the NF- κ B inducing kinase (Malinin et al., 1997). Activation of NF- κ B and translocation into the nucleus will then induce expression of anti-apoptotic genes, such as c-IAP2 (Chu et al., 1997). On the other hand, TRAF-1/TRAF-2 heterocomplexes have been shown directly to recruit antiapoptotic molecules to the membrane and, therefore, counteract activation of specific caspases (Rothe et al., 1995). This is of great relevance to oligodendrocytic death, since blockade of the TRAF-2 response in primary oligodendrocytes (using a dominant negative TRAF-2 retrovirus) and to a lesser degree interference with NF- κ B activation greatly enhanced the susceptibility of these cells to TNF-mediated cell death (Natoli et al., 1998).

Cellular Inhibitors of Death

The identification of caspase inhibitory molecules has led to the discovery of two classes of inhibitors: IAPs, with the ability to inhibit executor caspase 3, and initiator caspase-1 and FLIPs, with the ability to inhibit initiator caspases 8 and 10. The cellular IAPs are the mammalian homologues of the viral protein p35, originally identified in baculovirus. At least four different members of the IAP family have been described in mammalian cells: cIAP1, cIAP2, XIAP (which is the product of an X-linked gene) and NAIP (which is a neural specific isoform) (Deveraux and Reed, 1999). The FLIPs, in contrast, represent a more controversial family of "protective" molecules. Structurally are characterized by the presence of a DED, similar to that present on FADD and on initiator caspase-8. For this reason it is believed that their mechanism of action is to provide "anchoring" of a molecule that is structurally similar to caspase-8/FLICE, but without catalytic activity. This family of proteins include, however, a short and long form, both derived from alternative splicing of a single gene. Their role as promoters or inhibitors of apoptosis seems to be cell specific and dose dependent.

In conclusion, survival signals activated by growth factor and cytokine receptors exert a protective func-

tion either by promoting phosphorylation or transcriptional activation of protective genes. Direct phosphorylation of Bad by Raf or Akt prevents sequestration of Bcl-2 and, therefore, inhibits release of cytochrome c from the mitochondrial intermembrane space; direct phosphorylation of caspase-9 by Akt further controls this pathway. However, transcriptional activation of direct inhibitors of caspases provides an even tighter control over apoptotic programs.

CELL DEATH IN THE OLIGODENDROCYTE LINEAGE DURING DEVELOPMENT: APOPTOSIS DUE TO LACK OF SURVIVAL SIGNALS

The modality of activation of death programs, and therefore the relative contribution of apoptotic and necrotic changes in a given cell, may differ depending on the inducing stimuli. In normal development, for instance, death occurs mainly due to lack of trophic support or competition for limiting concentrations of growth factors.

Normal Development: Competition for Trophic Factor Support

Cell death in the oligodendrocyte lineage has been described in developing animals as a way to adjust the number of myelinating cells to that of the axons (Barres et al., 1992). In the rat developing optic nerve, approximately 50% of newly generated oligodendrocytes are eliminated (Barres et al. 1992; Raff et al., 1993; Raff, 1996). Similarly, in the developing neocortex, approximately 20% of premyelinating oligodendrocytes are lost between postnatal days 7–11 and 37% by day 28 (Trapp et al., 1997) (Table 1). These studies and several others (Levine, 1989; Dutly and Schwab, 1991; Hardy and Reynolds, 1993) led to the hypothesis that oligodendrocyte survival signals are generated upon axonal contact. Death in development, therefore, is mainly attributable to competition for limiting amounts of trophic factors provided by the axons (Barres et al., 1993). Support for this hypothesis is provided by Calver et al. (1998) using transgenic mice overexpressing PDGF-AA from a neuronal specific enolase promoter. In this study, the insertion of multiple copies of the transgene results in 30–100-fold more PDGF-AA mRNA in the spinal cord which correlates with a linear increase in number of progenitors. As gliogenesis continues, however, excess progenitors are eliminated, due to competition for PDGF or lack of additional growth factors that may be supplied at limiting concentration in ectopic locations (e.g., newly generated oligodendrocytes in gray matter). *In vitro* studies have begun to address the molecular identity of these additional survival signals, which include IGF-1 (Barres et al., 1993; McMorris and Dubois-Dalcq, 1988), neurotrophin-3 (Althaus 1992; Bertollini et al., 1997; Cohen et al., 1996; Kumar et al.,

TABLE 1. *Oligodendrocyte cell death in development*

Developmental stage	Reference
Optic nerve	Barres et al., 1992 Burne et al., 1996 Raff et al., 1993
Developing cortex and hippocampus	Trapp et al., 1997 Bertollini et al., 1997
Spinal cord	Calver et al., 1998
Loss of trophic support	Barres et al., 1993 Cohen et al., 1996 Dell'Albani et al., 1998 Kumar et al., 1998
Myelin mutants (e.g., jimpy mice, md rats)	Grinspan et al., 1998 Knapp et al., 1990, 1999 Lipsitz et al., 1998 Vermeesh et al., 1990 Williams and Gard, 1997
Transgenics	Jensen et al., 1998

TABLE 2. *Oligodendrocyte cell death in pathological conditions*

Pathological condition	Reference
Excitotoxicity	Matute et al., 1997 McDonald et al., 1998 Yoshioka et al., 1996, 1998
Oxidative stress	
By glutathione depletion	Back et al., 1998
By hydrogen peroxide	Richter-Landsberg and Vollgraf, 1998 Uberti et al., 1999
By nitric oxide	Boullerne et al., 1999 Merrill et al., 1993 Mitrovic et al., 1996
Activation of cytokine receptors	
TNF	Akassoglou et al., 1997, 1998 D'Souza et al., 1995 Hisahara et al., 1997 McLaurin et al., 1995 Merril, 1991 Probert et al., 1995 Selmaj et al., 1988, 1991
Fas	D'Souza et al., 1996 Dowling et al., 1996
IFN-gamma	Andrews et al., 1998 Baerwald and Popko, 1998 Corbin et al., 1996 Orwitz et al., 1997 Vartanian et al., 1995
NGF (in the absence of TrkA)	Casaccia-Bonnet et al., 1996b Gu et al., 1999 Dowling et al., 1997
Other stimuli	
Irradiation	Li et al., 1996
C2 ceramide	Casaccia-Bonnet et al., 1996a Brogi et al., 1997
Exogenous MBP expression	Tzeng et al., 1995
PLP misfolding	Gow et al., 1998 Jung et al., 1996
Wortmannin	Vemuri et al., 1996

1998), neuregulins (Canoll et al., 1996), and CNTF (Dell'Albani et al., 1998; Louis et al., 1993; Mayer et al., 1994).

Prior to contact with the axon, however, oligodendrocyte progenitor cell number may also be affected by the presence of survival signal present on the migratory route to a specific destination. Contact with the extracellular matrix may play an important role in regulating survival of these progenitors. Loss of trophic support due to loss of contact with a specific substrate ("anoikosis") may deprive the cell of intracellular signals that are crucial for maintenance of survival. In support of

this view, studies from Buttery et al. (1999) in the oligodendrocyte lineage, have clearly indicated that cell-permeable peptides mimicking the N-terminal region of the cytoplasmic domain of beta 1 integrin induce apoptosis, presumably by competing with survival signals generated by activation of the focal adhesion kinases.

Normal Development: Inappropriate Coordination Between Mitogenic and Antimitogenic Signals

In some cases, developmental cell death is independent of trophic support and activation of an apoptotic program occurs when a cell receives opposing signals regulating proliferation or growth arrest. Two paradigmatic situations can be described: progenitor cells expressing cell cycle molecules inducing proliferation that are eliminated upon exposure to differentiative cues (Casaccia-Bonnet et al., 1997, 1999; Jensen et al., 1998), and postmitotic cells eliminated upon exposure to strong mitogens (Muir and Compston, 1996). In the MBP-c-myc transgenic mice, for instance, apoptosis likely occurs as a consequence of heterochronic and heterotopic expression of the *myc* oncogene in cells of the oligodendrocyte lineage at a late stage of maturation (Jensen et al., 1998). Similarly, incoordination between exogenous signals and intrinsic alterations of the cell cycle machinery is observed in the p27Kip1 null mice (Casaccia-Bonnet et al., 1997). In these mice, loss of the cell cycle inhibitor p27Kip1 results in lower threshold to mitogenic stimulation and enhanced proliferative potential in vitro (Casaccia-Bonnet et al., 1997; Durand et al., 1998). In vivo, this translates into an initial expansion of the oligodendrocyte progenitor pool due to increased proliferation. However, these excess cells are still exposed to differentiative cues from the environment, leading to activation of compensatory feedback loops or elimination of cells that are unable to growth arrest (Casaccia-Bonnet et al., 1997, 1999). The converse situation also results in cell death. Oligodendrocyte progenitor cells induced to growth arrest by overexpressing p27Kip1 (using adenoviral vectors) and grown in the presence of strong mitogenic signals are programmed to die (unpublished observations). In all these cases, the inappropriate coordination between the intrinsic components of the cell cycle machinery and the signal transduction pathways activated by exogenous stimuli may create a genotoxic stimulus leading to activation of a p53-dependent death program. In support of this view, mice lacking p53 are resistant to *myc*-induced death and overexpression of p53 in the oligodendrocyte lineage is sufficient to induce an apoptotic program (Eizenberg et al., 1996). In general, however, it is very important to distinguish between manipulation of the intrinsic cell cycle machinery (either using transgenic approaches or viral-mediated gene transfer) and conflicting growth factor

stimulation. In the latter case, the determination of an apoptotic phenotype is strongly dependent on the balance between apoptotic signals induced by the genotoxic damage (as discussed above) and the activation of signal transduction pathways leading to survival. The cross-talk between multiple tyrosine kinase receptors in cultured cells may explain differences in susceptibility to apoptosis, as reported in the literature (Bansal and Pfeiffer, 1997; Fressineaud et al., 1995; Grinspan et al., 1996).

In summary, in the developing brain, life/death decisions of progenitor cells and newly generated oligodendrocytes are mainly regulated by activation of survival pathways, initiated by tyrosine kinase receptors and/or contact with the extracellular matrix and recruitment of soluble tyrosine kinases (of the src family). Growth factor availability, expression of functional tyrosine kinase and cytokine receptors, switch in the composition of integrin subunits may all affect the final survival/death decision of a cell by initiating signaling cascades that require integration with the cell cycle machinery responsible for proliferative control.

CELL DEATH IN DYSMYELINATING DISEASES: MISFOLDING OF MYELIN CONSTITUENTS AND ER STRESS

Developmental cell death can be exacerbated in specific pathological conditions, such as the Pelizaeus-Merzbacher disease, an X-linked genetic mutation in the PLP locus (Nave, 1994; Nave and Boespflug-Tanguy, 1996). Animal models of this disease include the *jimpy* (Knapp et al., 1986) and the *msd* mice, characterized, respectively, by a point mutation and a missense mutation in the PLP gene, and the *myelin deficient* rat (Jackson and Duncan, 1988). A prominent feature of these naturally occurring PLP mutants is increased oligodendrocytic death coincident with the onset of myelination (Knapp et al., 1986; Lipsitz et al., 1998; Skoff, 1995), and the morphological characteristic of apoptotic death (Castellano et al., 1996; Gow et al., 1998; Grinspan et al., 1998; Lipsitz et al., 1998; Skoff, 1995), although DNA fragmentation has not been consistently detected (Knapp et al., 1999; Owen and Skoff, 1995; Williams and Gard, 1995). In vitro studies on cells isolated from these mutants have confirmed that oligodendrocytic death correlates with the expression of PLP (Bongarzone et al., 1997; Knapp et al., 1996; Williams and Gard, 1997) and its accumulation in the endoplasmic reticulum (Gow et al., 1998), due to misfolding (Jung et al., 1996).

Although the signaling components linking altered PLP synthesis to the cell death execution machinery are yet to be identified, recent developments in the field of signaling pathways induced by endoplasmic reticulum stress may provide intriguing cues (Kaufman, 1999). Altered protein folding of PLP and trafficking

can be interpreted by the cell as stress signal originating from the endoplasmic reticulum and, therefore, activate two protein kinases called PERK and PKR, as shown in other cellular systems. These kinases, will then be responsible for the phosphorylation of the eIF-2 translation factor, with the direct consequential reduction of the translation rate as a way to protect the cell from additional accumulation of unfolded protein. In case of severe endoplasmic reticulum stress, however, the cell may not be able to overcome the level of damage thus resulting in apoptosis, probably mediated by phosphorylation of eIF-2 itself and the potential involvement of the transcriptional activator CHOP (reviewed in Kaufman, 1999). This model awaits further experimental evidence.

CELL DEATH INITIATORS IN THE ADULT BRAIN: EXCITOTOXICITY AND OXIDATIVE STRESS

The modality of activation of a death program in the diseased adult brain may significantly differ from that occurring in the developing brain. In support of this hypothesis, studies on long-term survival of oligodendrocytes following Wallerian degeneration in the optic nerve have indicated that a large proportion of adult, but not neonatal oligodendrocytes remained intact even after loss of axonal stimuli (Ludwin, 1990). Interestingly, also the susceptibility to glutamate-dependent toxicity is highly dependent on the developmental stage since kainate application on cultured immature progenitors leads to inhibition of proliferation and lineage progression (Gallo et al., 1996; Ghiani et al., 1999; Yuan et al., 1998), whereas AMPA treatment of mature oligodendrocytes (McDonald et al., 1998) or differentiated CG-4 cells (Yoshioka et al., 1996) leads to cell death (Yoshioka et al., 1998). These data suggest that, although loss of trophic support may contribute to decreased inhibitory control over the "intrinsic death machinery," this is not a primary modality of death in mature oligodendrocytes.

In the adult animal, injection of glutamate agonists in the optic nerve (Matute et al., 1997) or in the subcortical white matter (McDonald et al., 1998) leads to excitotoxic death of mature oligodendrocytes. The primary event leading to this type of death is calcium entry into the cell, via calcium-permeable glutamate receptors and voltage activated calcium channels. Increased intracellular calcium leads to activation of a broad spectrum of effectors, including calpain, a calcium-dependent protease responsible for degradation of cytoskeletal components (Siman and Noszek, 1988), a calcium-activated endonuclease and calcineurin, a phosphatase responsible for dephosphorylation of Bad (Wang et al., 1999). Excessive calcium load inside the cell may also result in overstimulation of mitochondrial buffering capacity, uncoupling electron transport from

energy production and consequent release of reactive oxygen species (Gunter and Pfeiffer, 1990).

In addition to excitotoxic damage, in ischemic and inflammatory conditions demyelination has been also associated with generation of reactive oxygen and nitrogen species (Merril et al., 1991; Brosnan et al., 1994; Merrill and Benveniste, 1996; Smith et al., 1999) and alteration of the redox potential of the cell (Back et al., 1998). Nitric oxide produced by microglial cells is highly toxic to cultured oligodendrocytes and to cell lines of oligodendrocyte origin (Mitrovic et al., 1996; Boullerne et al., 1999). Oxidative stress due to glutathione depletion (Back et al., 1998) or hydrogen peroxide (Richter-Landsberg and Vollgraf, 1998; Uberti et al., 1999) may induce progressive impairment of mitochondrial function with consecutive induction of apoptotic and necrotic death.

In mild cases, oxidative stress or excess intracellular calcium may alter the permeability of the inner mitochondrial membrane with consequent dissipation of the proton gradient. Loss of the inner transmembrane potential will favor leakage of cytochrome c and activation of caspase cascades followed by disruption of membrane integrity (Fig. 3). In more severe cases, however, generated superoxide radicals and nitric oxide can react with other reactive oxygen species and generate peroxynitrites, a powerful oxidants leading to lipid peroxidation, protein oxidation and DNA damage. The disruption of plasma membrane integrity and loss of energetic metabolism, due to mitochondrial damage leads to the morphological changes characteristic of necrosis (Fig. 1).

CELL DEATH INITIATORS IN THE ADULT BRAIN: OXIDATIVE STRESS AND ACTIVATION OF DEATH RECEPTORS IN THE PATHOGENESIS OF MULTIPLE SCLEROSIS

Several neuropathological studies on patients affected by multiple sclerosis or other inflammatory conditions have reported infiltration of immune cells and myelin damage around blood vessels with altered blood-brain-barrier (Prineas, 1985; Raine, 1997) and death of oligodendrocytes (Lucchinetti et al., 1996) in the area of demyelinating lesions. Two major mechanisms have been accounted responsible for the neuropathological lesions: a direct attack to myelin components by the immune system (Brosnan and Raine, 1996) and death of oligodendrocytes due to activation of cytokine receptors (Akassoglou et al., 1977,1978; D'Souza et al., 1995, 1996; Dowling et al., 1996; Probert et al., 1995), or generation of oxygen and nitrogen reactive species (Merril and Benveniste, 1996). There is a general consensus on the presence of CD4⁺/Thy1⁺ cells in the perivascular infiltrates in the site of lesion. Activated T cells can produce TNF-alpha, IFN-gamma (interferon-gamma), and IL-2 (interleukin-2); these cytokine will interact with specific death receptors that

are upregulated on the surface of oligodendrocytes and initiate a death program as previously described. TNF and TNF receptor (Hofmann et al., 1989; Selmaj et al., 1991) FasL and Fas receptor (Dowling et al., 1996; D'Souza et al., 1996); NGF (Bracci Laudiero et al., 1992), and its p75NGFR (Dowling et al., 1997) have been found in patients with multiple sclerosis. Several lines of research have clearly indicated a role of TNF-alpha and IFN-gamma in the induction of primary demyelination. Overexpression of IFN-gamma in developing (Corbin et al., 1996) and adult (Horwitz et al., 1997) mice results in myelin abnormalities and clinical symptoms. Similarly, transgenic mice overexpressing transmembrane TNF on the surface of astrocytes show severe demyelination, which is dependent on the expression of p55TNFR (Akassoglou et al., 1997,1998; Probert et al., 1995). These *in vivo* data supported *in vitro* observations that TNF-alpha (D'Souza et al., 1995; Hisahara et al., 1997; Selmaj et al., 1988) and IFN-gamma (Vartanian et al., 1995) can be toxic to cultured oligodendrocytes. In addition, clinical trials with IFN-gamma have clearly indicated an exacerbation of the attack in patients with multiple sclerosis (Panitch et al., 1987). IFN-gamma receptors are expressed on the membrane of cultured oligodendrocytes (Torres et al., 1996) and upon ligand activation of JAK tyrosine kinases with consequent phosphorylation of STATs transcription factors (Schindler and Darnell, 1995; Schindler, 1998), they upregulate TNF receptors, ICE and NOS (Andrews et al., 1998; Dopp et al., 1997; Merrill and Benveniste, 1996), therefore enhancing the susceptibility of oligodendrocytes to cell death.

The presence of oligodendrocytic death in multiple sclerosis, therefore, should be envisioned as a continuum between apoptotic and necrotic death, resulting from specific insults differentially affecting mitochondrial function. In the presence of TNF or FasL, activation of death receptors leads to an apoptotic program that can be initiated with or without the participation of the mitochondrion (Figs. 2,3). As mentioned above, cytochrome c release can initially occur without alteration of the mitochondrial potential. In this case, the balance between proapoptotic and antiapoptotic members of the Bcl-2 family will determine the activation of initiator caspase-9 and consequent activation of the proteolytic cascade leading to apoptotic features. In a transient activation of death receptor, the oligodendrocyte may be able to survive the "attack" by activating survival pathways depending on the presence of tyrosine kinases and cytokine receptors. However, in case of prolonged activation of death pathways (due, e.g., to continuous production of cytokines) efflux of cytochrome c can result in impaired proton flow, generation of reactive oxygen species, and cessation of ATP synthesis, eventually leading to necrotic changes. Similarly, in a severe attack of demyelination, the presence of oxygen reactive species and other stimuli leading to persistent changes in mitochondrial permeability will cause depletion of nonoxidized glutathione, production of

superoxide anion, depletion of NADPH2 and ATP, with major changes in redox potentials, loss of energetic metabolism, organelle swelling, generation of additional reactive oxygen species, and necrotic death.

In summary, the detection of apoptotic and/or necrotic changes in a dying cell is the result of the intensity and duration of the death signal and its repercussion on mitochondrial function (Eguchi et al., 1997).

CONCLUSION

Life/death decisions in the oligodendrocyte lineage are the result of a balance between signals promoting death and mechanisms of survival. Competitive signaling between transduction pathways is the result of differential activation of distinct classes of receptors. Cross-talks between apoptotic and antiapoptotic pathways will, therefore, be affected by the availability of ligands and the receptor composition on the cell surface. Integration of the distinct signals will be affected by the differentiative state of the cell, which may affect mitochondrial function and relative levels of signaling components. In pathological conditions, depending on the intensity and duration of the noxious stimulus, apoptotic features or necrotic changes are determined by the level of mitochondrial impairment.

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