Non–cell autonomous toxicity in neurodegenerative disorders: ALS and beyond

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Selective degeneration and death of one or more classes of neurons is the defining feature of human neurodegenerative disease. Although traditionally viewed as diseases mainly affecting the most vulnerable neurons, in most instances of inherited disease the causative genes are widely—usually ubiquitously—expressed. Focusing on amyotrophic lateral sclerosis (ALS), especially disease caused by dominant mutations in Cu/Zn superoxide dismutase (SOD1), we review here the evidence that it is the convergence of damage developed within multiple cell types, including within neighboring nonneuronal supporting cells, which is crucial to neuronal dysfunction. Damage to a specific set of key partner cells as well as to vulnerable neurons may account for the selective susceptibility of neuronal subtypes in many human neurodegenerative diseases, including Huntington's disease (HD), Parkinson's disease (PD), prion disease, the spinal cerebellar ataxias (SCAs), and Alzheimer's disease (AD).

Introduction

The great cell biologists of the 19th century, including Rudolph Virchow, the German physician widely known as the father of pathology, and the French physiologist Claude Bernard established the pivotal idea that individual cells function autonomously, while being part of the whole organism. Since then, many pathological conditions including all major neurodegenerative diseases have traditionally been considered mechanistically cell autonomous, meaning that damage within a selective population of affected neurons alone suffices to produce disease.

For most neurodegenerative conditions, injury may originate either from unknown stressors in the case of sporadic disease, or from expression of a mutant gene in the familial forms. With the recognition that essentially all of the genes whose mutation causes the inherited forms of these diseases are widely or ubiquitously expressed, three questions are central for understanding disease mechanism (and for devising therapies). First, what are the mutant-driven toxic mechanisms that mediate disease? Second, is disease actually driven by cell autonomous mechanisms as has previously been widely assumed? Third, what explanation is there for the selectivity in neuronal killing from a widely expressed mutant?

A plethora of damage: eight proposed mechanisms for familial ALS

Amyotrophic lateral sclerosis (ALS) is characterized by selective, premature degeneration and death of motor neurons initiating in mid-adult life. The ensuing progressive paralysis is typically fatal within a handful of years due to respiratory failure. Although the majority of incidences have no apparent hereditary contribution, ∼10% of instances are dominantly inherited. A landmark discovery reported in 1993 initiated the molecular era of ALS research with identification of mutations in the gene encoding for superoxide dismutase 1 (SOD1) as causative in 20% of the inherited cases (Rosen et al., 1993). A major cytoplasmic antioxidant, the ubiquitously expressed SOD1's normal function is to catalytically convert highly reactive superoxide (oxygen with an extra electron) to either hydrogen peroxide or oxygen.

Mice expressing various ALS-related mutants of SOD1 have recapitulated the fatal paralysis seen in human patients, and use of them has been the most important contributor to defining familial ALS disease mechanisms. An important initial realization from these efforts is that disease is caused by one or more acquired toxicity(ies) of the mutant proteins, rather than reduced superoxide dismutase activity. A universal finding is that a proportion of the more than 150 SOD1 mutants (Turner and Talbot, 2008) fails to fold properly, thus implicating accumulation of misfolded SOD1 as a possible toxic contributor in ALS. The misfolded SOD1 forms ubiquitinated cytoplasmic inclusions that occur early in disease and escalate as disease progresses (Bruijn et al., 1997). A sobering reality, however, is that 16 years after the initiating discovery (Rosen et al., 1993) no consensus has yet emerged as to the primary toxicity of

Abbreviations used in this paper: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; HD, Huntington's disease; PD, Parkinson's disease; SCA, spinal cerebellar ataxia; SOD1, superoxide dismutase.

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mutant SOD1. Instead, a plethora of toxic mechanisms have been proposed to mediate pathogenesis, that is, the course of events that underlie the progressive fatal paralysis from degeneration and death of motor neurons (Fig. 1).

Excitotoxicity from mishandling of glutamate. One of the early proposed mechanisms—observed both in SOD1 mutant mouse models and in familial and sporadic ALS patient samples—is glutamate excitotoxicity, the excessive firing of motor neurons derived from failure to rapidly remove synaptic glutamate (Fig. 1 A). Overstimulation by glutamate, the neurotransmitter that triggers motor neurons to fire, can elicit a cascade of toxic events in the postsynaptic motor neuron including repetitive activation of glutamate receptors and the corresponding increase in calcium influx, thus overriding the storage abilities of mitochondria and endoplasmic reticulum (ER). Contributing to this phenomenon is a failure to rapidly clear extracellular glutamate through deficiency in the glutamate transporter EAAT2 in the astrocytic processes that surround synapses of motor neurons (Rothstein et al., 1995; Bruijn et al., 1997; Howland et al., 2002; Yang et al., 2009a,b). Recent evidence has shown that loss of connectivity between upper and lower motor neurons triggers reduced transcription of EAAT2 through reduced expression of a κB motif binding phosphoprotein (KBBP), the mouse homologue of hnRNP K, or human heterogeneous nuclear ribonucleoprotein K (Yang et al., 2009b). Caspase-3 activation has also been linked to production of a truncated form of EAAT2 in SOD1 mutant mouse spinal cords (Boston-Howes et al., 2006).

Mutant SOD1 causes ER stress. Mutant SOD1 has been argued to trigger ER stress via two different pathways (Fig. 1 B). Mutant SOD1 aggregates were found to accumulate in fractions that are enriched in ER membranes in the affected tissues, a phenomenon that intensifies as disease progresses. These ER-associated SOD1 aggregates bind to the ER-luminal polypeptide chain binding protein (BiP) (Kikuchi et al., 2006), a chaperone that regulates the activation of ER stress transducers such as IRE1, PERK, and ATF6. A later study showed that mutant SOD1 inhibits ER-associated degradation (ERAD), the cell's machinery for eliminating proteins that fail to fold properly inside the ER. The first critical step in the ERAD process is the retrograde transport of misfolded proteins out of the ER lumen into the cytosol, where they are ubiquitinated and subsequently degraded by the proteasome. Multiple ALS-associated mutants of SOD1—including dismutase-active (e.g., $SOD1^{G93A}$, $SOD1^{A4V}$) and -inactive ($SOD1^{G85R}$) mutants but not the wild-type SOD1—interact with derlin-1, a transmembrane ER protein that is instrumental for dislocation of misfolded proteins from the ER to the cytosol. Binding of mutant SOD1 to Derlin-1 inhibits ERAD and thereby generates ER stress (Nishitoh et al., 2008), but only after disease onset, consistent with this being a consequence secondary to some unidentified initiating trigger. Nevertheless, up-regulation of ER-related genes at presymptomatic stages is seen in a subset of vulnerable motor neurons in mice expressing either dismutase-active or -inactive mutants (Saxena et al., 2009). We would note that mutant SOD1 association with the ER may interfere with synthesis of any protein, like EAAT2, whose synthesis and maturation are dependent on passage through the ER.

Mutant SOD1 inhibits the proteasome. A third proposal for SOD1 mutant toxicity is for misfolded mutant SOD1 inhibition of clearance of damaged proteins by the proteasome, the proteolysis machine for removing abnormally folded proteins from the cytoplasm (Fig. 1 C). In cell culture, mutant SOD1 turns over more rapidly than wild-type and turnover depends on proteasome activity (Hoffman et al., 1996). Intracellular accumulations in familial or sporadic ALS patients are not immunoreactive for proteasome components (Ii et al., 1997; Watanabe et al., 2001) but have been reported to contain Dorfin, a RING finger–type E3 ubiquitin ligase. At least in some instances, Dorfin physically binds and ubiquitinates various SOD1 mutants, thereby enhancing their degradation, but does not affect the stability of wild-type SOD1 (Niwa et al., 2002). A preponderance of biochemical evidence from spinal cords of SOD1 mice has reported decreased activities of the proteasome in lumbar spinal cords of SOD1 mutant mice (Kabashi et al., 2004; Cheroni et al., 2009) or after sustained expression of mutant SOD1 in a culture neuronal line (Urushitani et al., 2002). In this view, a vicious cycle can thus ensue in which protein aggregation not only increases the levels of misfolded mutant SOD1 (Hoffman et al., 1996), but also sequesters essential cellular components (including endogenous SOD1) within the aggregates, causing further damage to the affected cell (Bruijn et al., 1998).

Misfolded mutant SOD1 damages mitochondria. A fourth proposal is that misfolded mutant SOD1 damages mitochondria by its deposition onto the cytoplasmic face of the outer membrane (Fig. 1 D) of spinal cord mitochondria (Liu et al., 2004; Vande Velde et al., 2008). Apparent morphological damage to mitochondria is seen presymptomatically within motor neurons of some, but not all, lines of mice that develop SOD1 mutant-mediated ALS. A proportion of both dismutase-active and -inactive mutants, however, has been shown convincingly to be mitochondrially associated (Mattiazzi et al., 2002; Liu et al., 2004; Vijayvergiya et al., 2005; Vande Velde et al., 2008). Mitochondrial association of mutant SOD1 begins presymptomatically (Liu et al., 2004), firmly supporting an important mechanistic contribution to disease initiation.

There is no uniform view of how mitochondrial function is affected. ATP levels have been reported to be diminished in symptomatic (Mattiazzi et al., 2002) and presymptomatic (Browne et al., 2006) mutant spinal cords of one mouse model but to be unchanged in another (Damiano et al., 2006). Mitochondrial calcium buffering capacity was found affected in spinal cords of two different strains of mice and has direct connection to the excitotoxic hypothesis (Damiano et al., 2006). Mitochondrial damage seems not to be neuronally limited, but is also found in spinal cord astrocytes for at least one mutant (Cassina et al., 2008). Still untested is whether association of mutant SOD1 with the mitochondrial outer membrane could trigger changes in other functions vital for mitochondrial homeostasis such as protein import, mitochondrial fission/fusion, ionic balance, or regulation of apoptosis.

Extracellular toxicity from aberrant secretion of mutant SOD1. A fifth proposed mechanism involves interaction of misfolded mutant SOD1 with components of neurosecretory vesicles, chromogranin A (CgA) and chromogranin B

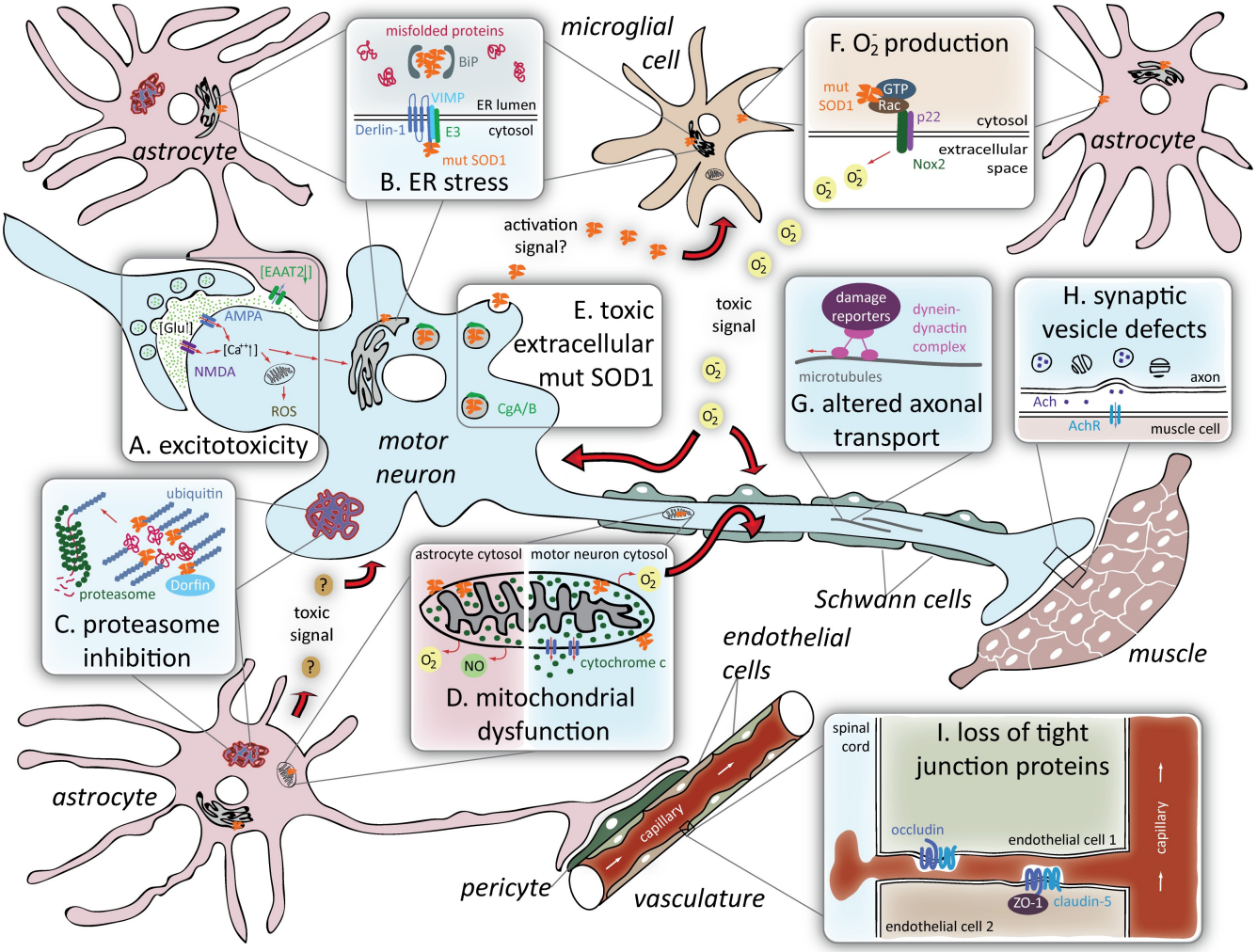

Figure 1. **Proposed mechanisms of toxicity in SOD1-mediated ALS.** (A) Excitotoxicity is the hyperactivation of motor neurons resulting from failure to rapidly remove neurotransmitter glutamate from synapses due to deficiency in the glutamate transporter EAAT2 in the neighboring astrocytes. (B) ER stress is induced by abnormal interactions of mutant SOD1 with ER proteins (see text for details). (C) Proteasome inhibition due to "overload" of the proteasome degradation pathway with ubiquitinated misfolded protein aggregates may damage astrocytes and motor neurons. (D) Mitochondrial dysfunction mediated by mutant SOD1 deposition on the mitochondrial membrane provokes release of cytochrome *c* in motor neurons, whereas in astrocytes it leads to nitroxidative stress. (E) Toxic extracellular mutant SOD1 is secreted from motor neurons and astrocytes (not depicted) after interaction with components of neurosecretory vesicles. (F) Superoxide production from microglia or astrocytes can damage neighboring motor neurons. (G) Altered axonal transport including an increase in retrogradely transported stress-related proteins was reported in mutant SOD1-expressing motor neurons. (H) Synaptic vesicle defects such as stalling and loss from distal synapse in vulnerable motor neurons is an early event in ALS. (I) Loss of tight junction proteins within capillary endothelial cells results in the disruption of the blood–spinal cord barrier and the occurrence of microhemorrhages within the spinal cord well before disease onset.

(CgB), which in turn can apparently direct the unexpected cosecretion of mutant SOD1 (Urushitani et al., 2006) by motor neurons or astrocytes (Fig. 1 D). Extracellular mutant SOD1 in turn acts to damage motor neurons through activation of microglia, the innate immunity cells within the spinal cord, so as to ultimately drive neuronal death (Zhao et al., 2009). Moreover, proteasome inhibition was reported to enhance aberrant secretion of mutant SOD1, suggesting a cross talk between these two pathways.

Mutant SOD1 generates extracellular superoxide. A sixth proposed mechanism of mutant SOD1 is counterintuitive: mutant stimulation of excessive extracellular production of superoxide (O^{-}_{2}) (Harraz et al., 2008). Both wild-type and mutant SOD1 can associate with Rac1, a small GTPase that controls the activation of NADPH oxidase (a multiprotein membrane-associated complex whose catalytic subunit is Nox2). A key normal role of Nox2 in phagocytic cells, including microglia, is to produce highly toxic extracellular superoxide, for example, in order to kill bacteria and other pathogens. Harraz et al. (2008) proposed that association of wild-type SOD1 with Rac1 participates in a tightly regulated mechanism that in reducing conditions activates Nox2. Mutant forms of SOD1 interact with Rac1 with apparent higher affinity, thereby locking Nox2 in its active, superoxide-producing form. Paradoxically, instead of its normal job of removing intracellular superoxide, mutant SOD1 may thus be responsible for driving extracellular production of superoxide (Fig. 1 F). It should be noted that for the proportion of Nox2 imbedded in internal

membranes—including newly made Nox2 transiting from the ER to the cell surface—Rac1 binding would stimulate high intracellular superoxide within the Nox2-containing vesicles. Like BiP and Derlin-1, still unexplained is why interaction between Rac1 and mutant SOD1 is not detectable at presymptomatic stages.

Mutant SOD1 causes axonal disorganization and disrupted transport. A seventh hypothesis for mutant SOD1-dependent toxicity is through interference with axonal cytoskeletal organization and/or inhibition of axonal transport. As the most asymmetric cells in nature, motor neurons have a crucial requirement for axonal transport to deliver the many components synthesized in the cell bodies to axons and synapses. SOD1 mutants have been demonstrated to slow both anterograde (Williamson and Cleveland, 1999) and retrograde (Murakami et al., 2001; Perlson et al., 2009) routes months before neurodegeneration. Indeed, axonal disorganization, especially neurofilament misaccumulation, is a hallmark of both sporadic and inherited forms of ALS. Mutations in neurofilaments are at best causes of a very small proportion of ALS, however (Marszalek et al., 1996). Reduction in retrograde transport by mutation in dynactin, an activator of the retrograde motor cytoplasmic dynein, provokes human motor neuron disease that is substantially less severe than ALS (Puls et al., 2003), whereas mutation in dynein provokes loss of sensory neurons that report position (proprioception), but not motor neurons (Chen et al., 2007; Ilieva et al., 2008). Similar decreases in overall retrograde flow in SOD1 mutant axons is, however, accompanied by an increase in retrogradely transported stress or cell death–related proteins (Fig. 1 G) (Perlson et al., 2009), presumably reflecting increased production of such factors in the distal axons and/or synapses. Likely coupled to errors in delivery early in disease are apparent synaptic vesicle stalling and depletion from distal synapses of vulnerable motor neurons (Pun et al., 2006) (Fig. 1 H). It is conceivable that affinity of misfolded mutant SOD1 for membranes may underlie these affects on synaptic vesicles and their normal synaptic trafficking.

Microhemorrhages of spinal capillaries from mutant SOD1. A final eighth proposal is that mutant SOD1 damage within cells of the vasculature leads to leakage of toxic products, including iron complexes from hemoglobin, into the spinal cord. Indeed, microhemorrhages within the spinal cord that initiate well before disease onset have been seen in all models of SOD1 mutant-mediated disease in mice (Zhong et al., 2008). This disruption of the blood–spinal cord barrier is accompanied by loss of components of the tight junctions (including ZO-1, occludin, and claudin-5) between endothelial cells that maintain the blood–spinal cord barrier (Fig. 1 I). Indeed, decreased mRNA levels of ZO-1 and occludin have been found in lumbar spinal cords of ALS patients, compared with control samples, suggesting that this mechanism of toxicity is relevant for human disease (Henkel et al., 2009). Although efficient mutant SOD1 gene excision from the endothelial cells that line the capillaries does not affect disease course (Zhong et al., 2009), unresolved is how mutant SOD1 induces the loss of these tight junctions and whether the damage to the capillaries is mediated by mutant SOD1 synthesized within the pericytes or astrocytes that reinforce the initial endothelial cell barrier or SOD1 made outside of the vasculature.

Mutant SOD1 causes damage within multiple cell types

From the confusing diversity of proposed toxic pathways, which is correct? And which cells develop the crucial damage through their own synthesis of ubiquitously expressed mutant SOD1? Initial efforts assumed that disease was cell autonomous, that is, from mutant damage solely within motor neurons. Accordingly, attempts were made to generate disease from selective mutant SOD1 expression only in motor neurons. Although these efforts did not produce disease (Pramatarova et al., 2001; Lino et al., 2002), a later study succeeded with mutant synthesis largely restricted to neurons, using mutant SOD1 expression driven by neuron-specific Thy1.2 promoter (Jaarsma et al., 2008). In this latter paradigm, however, even animals with the highest level of mutant synthesis developed disease only at very late ages and disease progressed slowly without reaching the same degree of paralysis relative to lines expressing the same mutant ubiquitously.

It is now clear that toxicity of mutant SOD1 is not just within motor neurons. Analyses of chimeric mice that were mixtures of normal and SOD1 mutant-expressing cells revealed that high expression levels of mutant SOD1 in most (Clement et al., 2003) or all (Yamanaka et al., 2008a) motor neurons is not sufficient for early onset disease, clearly implicating mutant synthesis by nonmotor neurons in driving disease initiation. Lentiviral reduction of mutant SOD1 synthesis in motor neurons—produced through peripheral injection—led to long-term suppression in the central nervous system of mutant SOD1 synthesis selectively within motor neurons. This transcription-mediated mutant SOD1 suppression slowed disease onset very markedly when applied at a very young age, but was of no benefit at all in slowing the rate of disease progression after onset (Ralph et al., 2005).

Identities of cells beyond motor neurons whose mutant SOD1 synthesis contributes to disease has emerged by cell type–selective excision in mice expressing transgenes flanked by lox sites that permit deletion by action of Cre recombinase. As expected, expressing Cre recombinase under two different promoters largely selective to motor neurons in the central nervous system delayed disease onset from a dismutase-active ALS-linked SOD1 mutant (Fig. 2), but did not alter the rate of progression of disease after onset (Boillée et al., 2006; Yamanaka et al., 2008b). Similar deletion from motor neurons and interneurons (by a Lhx3-driven Cre transgene) primarily delayed disease onset and early disease progression (Wang et al., 2009). Taken together, mutant SOD1 expression in motor neurons determines the initial timing of disease onset and early progression, but very surprisingly does not contribute much to later disease progression.

Mutant SOD1 causes microglial damage, which drives rapid ALS progression. Mutant SOD1 expression in cells other than the motor neurons must therefore be the decisive source(s) that drive disease progression after onset. So which cell types develop damage that contributes to the

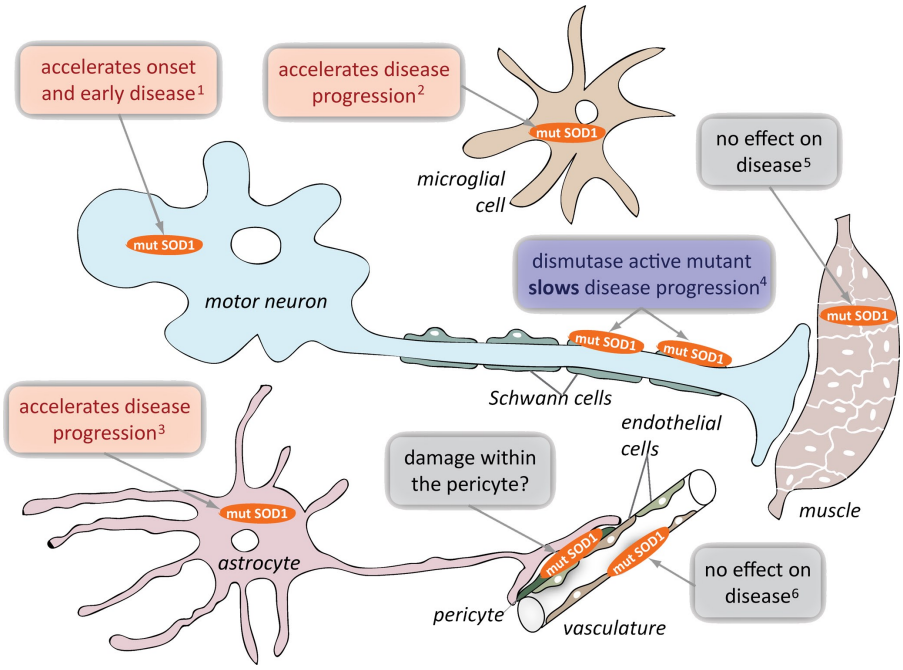

Figure 2. **Contribution of mutant SOD1 within different cell types in ALS.** Despite the apparent selectivity for motor neurons, multiple lines of evidence indicate that nonneuronal cell types contribute to pathogenesis and disease progression in SOD1-mediated neurodegeneration. Mutant SOD1 expression in motor neurons directs the onset and development of early disease, but does not influence its progression. In contrast, mutant SOD1 expression in microglia or astrocytes accelerates disease progression without affecting its onset. Expression of a dismutase-active mutant SOD1 specifically in Schwann cells was found to slow disease progression, but the role of a dismutase-inactive mutant in these cells has not been tested. Mutant SOD1 expression within muscle or endothelial cells does not affect ALS onset or progression, although some reports suggest that muscle might be a direct target of mutant SOD1 toxicity. Lastly, the vasculature is damaged very early in disease, leading to loss of tight junctions between endothelial cells and microhemorrhages, but whether any of this is from mutant SOD1 within pericytes, the terminal astrocyte, or coming from cells outside the vasculature is not established. [1](Ralph et al., 2005; Boillée et al., 2006; Jaarsma et al., 2008), [2](Beers et al., 2006; Boillée et al., 2006; Wang et al., 2009), [3](Yamanaka et al., 2008b), [4](Lobsiger et al., 2009), [5](Holzbaur et al., 2006; Miller et al., 2006; Dobrowolny et al., 2008; Towne et al., 2008), [6](Zhong et al., 2009).

spread of motor neuron injury? Three independent studies reached a common conclusion: damage within mutant-expressing microglial cells—the macrophages of the central nervous system—is key to rapid disease progression (Fig. 2). Using a CD11b-Cre transgene that yields excision of "floxed" genes only in the myeloid lineage, selective mutant SOD1 gene excision provided the most substantial slowing of disease progression yet seen in a rodent model of inherited ALS. Survival after disease onset was almost tripled (Boillée et al., 2006). Slowed progression after reduced mutant SOD1 synthesis in microglia was subsequently confirmed by an analogous approach in a different mutant transgenic line (Wang et al., 2009). Similarly, bone marrow transplantation to generate complete replacement of mutant SOD1-expressing microglial cells with nontransgenic ones did not affect disease onset, but slowed progression (Beers et al., 2006). Driving disease progression does not seem to require the proliferation of mutant microglia in response to an initial injury, as killing of about half of proliferating microglia did not affect the rate of progression (Gowing et al., 2008).

A controversial point has been whether microglial cells from the periphery enter the brain and spinal cord during disease, and if so, whether they participate in driving or ameliorating pathogenesis. Evidence in favor of both roles has emerged using irradiation and grafting (Kang and Rivest, 2007), but this outcome is likely the result of irradiation-induced disruption of the blood–brain barrier (Mildner et al., 2007). A complex experiment which used parabiosis (thereby avoiding irradiation and bone marrow transplantation) found almost no contribution of peripherally circulating macrophages to microglial proliferation and activation during SOD1 mutant-mediated disease (Ajami et al., 2007).

Astrocytes expressing mutant SOD1 drive disease progression. Astrocytes are one of the most abundant cell types in the adult nervous system. Closely apposed to motor neurons, they are responsible for supplying nutrients, buffering ions, recycling neurotransmitter precursors, and limiting motor neuron firing through rapid recovery of synaptic glutamate with their glutamate transporters. Although restricting mutant SOD1 expression in astrocytes is not sufficient for disease (Gong et al., 2000), selective reduction of mutant SOD1 in astrocytes (Yamanaka et al., 2008b) slowed disease progression and doubled the length of disease duration after onset (Fig. 2). This was accompanied by delayed microglial activation, demonstrating a functional cross talk between mutant astrocytes and microglia.

Mutant astrocytes also induce changes in their partner motor neurons. In cell culture experiments, normal (but not mutant SOD1-expressing) astrocytes induce the up-regulation of the glutamate receptor subunit GluR2 in neighboring (cocultured) motor neurons through an unidentified soluble factor(s). The GluR2 subunit produces receptors that are impermeable to Ca^{2+}, thereby protecting the motor neurons from excitotoxic damage. Mutant SOD1 expression in astrocytes abrogated their GluR2-regulating capacity, rendering motor neurons vulnerable to excitotoxicity (Van Damme et al., 2007). Activation of the redox-sensitive nuclear factor erythroid-2–related transcription factor 2 (Nrf2) in astrocytes is thought to coordinate the up-regulation of antioxidant defenses, thereby conferring protection to neighboring neurons. Indeed, astrocyte-selective up-regulation

of Nrf2 produced significant delay in disease onset in mouse models overexpressing dismutase-active and -inactive SOD1 mutants (Vargas et al., 2008). Perhaps most importantly, cervical transplantation of lineage-restricted astrocyte precursors delayed progression of mutant SOD1-mediated disease after onset, not only reinforcing the influence of astrocytes on disease progression, but demonstrating the feasibility of cell replacement therapies focused on astrocytes (Lepore et al., 2008).

A cascade of oxidative damage from motor axons to myelinating Schwann cells. Schwann cells are in intimate contact with the full length of the axons of lower motor neurons. The multiple wraps of myelin provide the electrical insulation essential for rapid signal conduction. (A different cell, the oligodendrocyte, has the job of myelination of upper motor axons.) After axonal damage, Schwann cells also participate—in concert with peripheral macrophages—in clearing debris and in guiding the recovering axon. Selective excision of a dismutase-active mutant SOD1 from a substantial proportion of Schwann cells (70%) (through P0-Cre–mediated excision) yielded a highly unexpected outcome: not only did removal of the mutant gene fail to slow any aspect of disease, it generated a substantial acceleration of the late phase of disease (Lobsiger et al., 2009). Indeed, a retrospective look at disease progression in dismutase-active and -inactive SOD1 mutants confirmed that inactive mutants uniformly generate more rapidly progressing disease, consistent with an ameliorating influence of increased dismutase activity in Schwann cells. These findings implicate an oxidative cascade during disease progression that is triggered within axon-ensheathing Schwann cells and that can be ameliorated by elevated dismutase activity (Fig. 1 D). This provocative possibility now awaits a direct test, which could be posed by elevating dismutase activity selectively within Schwann cells as a means to slow disease progression.

A controversial role of mutant SOD1 damage within muscle. A mechanistic role for mutant SOD1 in muscle remains controversial. Diminished mutant synthesis by 50–60% in muscle (Miller et al., 2006; Towne et al., 2008) did not affect any aspect of mutant SOD1-mediated disease, findings inconsistent with muscle cells as a direct target of mutant SOD1 toxicity (Fig. 2). Indeed, stimulation of myogenesis to produce chronic muscle hypertrophy (by inhibition of the inhibitory hormone myostatin) provided no benefit in slowing disease (Holzbaur et al., 2006; Miller et al., 2006). In contrast, mutant synthesis selectively within skeletal muscle did provoke damage to muscle (Dobrowolny et al., 2008) that was reminiscent of initial damage seen in SOD1 mutant-mediated ALS. A resolution of the conflicting views should now be undertaken by testing whether increased mutant synthesis in muscle can affect onset or progression in a SOD1 mutant mouse that generates very late disease—as in mice with mutant synthesis restricted to neurons (Jaarsma et al., 2008).

A protective role of T lymphocytes in mutant SOD1-mediated ALS. T lymphocytes appear to play a protective role in SOD1 mutant-mediated ALS. Preventing T lymphocyte recruitment to the spinal cord of the mutant SOD1 mouse (Beers et al., 2008; Chiu et al., 2008) accelerated disease progression. Lack of T lymphocytes was accompanied by a remarkably blunted microglial proliferation, decrease in mRNA levels for neurotrophic factors (BDNF, GDNF, IGF-1), for EAAT2, for immunomodulatory IL-4, and TGF-β, but increased level of proinflammatory mRNA for TNF-α and especially for Nox2 (100-fold!). These results demonstrate that the presence of T lymphocytes, through interactions with microglia and astrocytes and/or directly with motor neurons, modifies the local environment to promote neuroprotection (Beers et al., 2008).

Selectivity in ALS: damage-producing pathways converge in different cells

The collective evidence for SOD1 mutant-mediated ALS is that the disease process is decidedly non-cell autonomous (Fig. 2). Synthesis of mutant SOD1 within motor neurons is a primary determinant of driving disease onset. Mutant synthesis by other cells (the most directly implicated include interneurons and cells that comprise the blood–brain barrier) also contributes in a substantial way to disease initiation. Neighboring glial cells, especially the astrocytes and the microglia, develop mutant damage within them, causing them to accelerate disease progression—very markedly so—whereas mutant synthesis in motor neurons has little influence on progression. Schwann cells are recipients of damage, presumably from the damaged motor axons to which they are partnered, and such damage is at least in part from oxidative species that can be diminished by SOD1 activity within the Schwann cells.

Combining the contributions from different cell types with the diversity in proposed toxic mechanisms (Fig. 1), we propose an explanation for preferential toxicity to motor neurons from ubiquitously expressed mutant SOD1. All of the proposed mechanisms are probably contributors to pathogenesis, but they generate their damage within different cell types. Initiating damage takes place within the motor neurons most susceptible to ALS: here it is hard to imagine that misfolded mutant SOD1 aberrantly aggregated onto mitochondria does not damage their function and/or distribution within the motor neuron and its highly extended axon. Damage to one or more cell types of the blood–brain barrier leads to very early microhemorrhaging within the spinal cord, with release of neurotoxic hemoglobin products, that tips the balance of already damaged motor neurons, thereby driving disease initiation.

ER stress is generated in motor neurons and probably other cell types by misfolded mutant SOD1 bound to either Derlin-1 or BiP. This may affect many proteins that mature in the ER, including those contributing to synaptic vesicles. Not yet established is whether ER stress is also generated by mutant SOD1 within astrocytes, but fully consistent with this is the loss of the mature EAAT2 glutamate transporter, whose reduction is sure to enhance calcium-dependent excitotoxicity within the juxtaposed motor neuron. Damage to both intracellular calcium stores, the ER and the mitochondria, would then accelerate damage within the motor neurons, fueling a feed-forward mechanism through which mutant astrocytes promote disease progression. Misfolded mutant SOD1 within microglia—which become activated and migrate to initial sites of cell injury—triggers unregulated and high production of extracellular superoxide, thereby enflaming the initial injury.

How does this help with understanding the basis of selectivity in neuronal loss? Recognizing the contributions of multiple mechanisms and direct involvement of multiple cell types in disease initiation and progression, the selective vulnerability of motor neurons to toxicity from a ubiquitously expressed mutant can be explained by the accidental convergence of the motor neuron's own inherent functional properties and the combination of mutant damage developed within it and its multiple cell partners.

ALS as the tip of the iceberg: non-cell autonomy in neurodegenerative disease

Although our understanding of non-cell autonomy is most advanced for cases of SOD1-mediated familial ALS, it is likely that this is going to be a unifying theme in many other neurodegenerative diseases as almost all of the causative proteins are widely or ubiquitously expressed. For ALS, within the last 18 months, dominant mutations in two other widely expressed genes, TDP-43 (Sreedharan et al., 2008; Van Deerlin et al., 2008) and FUS/TLS (Kwiatkowski et al., 2009; Vance et al., 2009), have been reported. Both of these new ALS-causing genes encode proteins intimately associated with RNA processing and are at least partially depleted from nuclei in mutant-expressing cells. It remains to be determined if the underlying disease mechanism is from loss of nuclear function, gain of one or more toxic properties, or both. Moreover, TDP-43 misaccumulation in motor neurons and astrocytes (Neumann et al., 2006; Cairns et al., 2007) is seen in most instances of sporadic or familial ALS, so noncell-autonomous disease seems likely to be a universal feature of ALS. The first report of a prion promoter-driven mutant TDP-43 mouse line has just been published (Wegorzewska et al., 2009) and will assuredly be followed by further work assessing if the neurological phenotype described is mutant specific.

Parkinson's disease and multiple system atrophy. A hallmark of the second most common age-related neurodegenerative disease—Parkinson's disease (PD)—is the loss of dopaminergic neurons in a brain region called the substantia nigra (for review see Dauer and Przedborski, 2003). Intraneuronal accumulations of α-synuclein, a highly abundant presynaptic protein, are a defining pathology of sporadic and many inherited instances of PD. Although most incidences of disease are sporadic, multiple genetic causes are known (for review see Hardy et al., 2009), including dominant mutations in α-synuclein or increased synthesis of normal α-synuclein. Although increased intraneuronal synthesis of α-synuclein can damage those neurons (Masliah et al., 2000), elevated expression of α-synuclein selectively within the axon-ensheathing oligodendrocytes can also induce neurodegeneration of the associated neurons (Yazawa et al., 2005), firmly suggesting a non–cell autonomous component to pathogenesis. Indeed, in multiple system atrophy (MSA), whose clinical presentation includes Parkinsonism, ataxia, and autonomic failure, α-synuclein–containing inclusions are actually more prominent in oligodendrocytes (for review see Dauer and Przedborski, 2003).

Additional evidence for a non–cell autonomous mechanism has come from chemically induced PD. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) can induce a Parkinsonian syndrome in humans and rodents almost indistinguishable from PD (Dauer and Przedborski, 2003). The conversion of MPTP to the damaging MPP^+ depends on monoamine oxidase B, an enzyme expressed predominantly by astrocytes (Nakamura et al., 1990; Ekblom et al., 1993) and serotoninergic neurons, but not by the affected dopaminergic neurons (Kitahama et al., 1991; Luque et al., 1995; Jahng et al., 1997). Myeloperoxidase, an oxidant-producing enzyme, is expressed by the neighboring astrocytes in brains of both PD patients and MPTP-induced animal models, and its genetic knockdown increases resistance to MPTP toxicity (Choi et al., 2005). Microglial activation precedes neurodegeneration (at least in animal studies) and genetic reduction of the inducible nitric oxide synthase expressed by microglial cells or inhibition of microglial activation (by the antibiotic minocycline) ameliorates dopaminergic neurodegeneration (Liberatore et al., 1999; Wu et al., 2002). T lymphocytes were shown to have invaded the brain in both postmortem human PD specimens and in the MPTP-treated mice during the course of neurodegeneration (Brochard et al., 2009). Moreover, MPTP-induced dopaminergic cell death was markedly attenuated in the absence of mature T lymphocytes (Brochard et al., 2009). Thus, in this chemically induced PD model, converging damage within astrocytes, microglia, and invading T cells, as well as the target neurons, creates an oxidative milieu detrimental to the neurons and which is probably further exacerbated by neuronally restricted oxidative pathways (Teismann et al., 2003; Hunot et al., 2004).

Huntington's disease. Huntington's disease (HD) is a dominant, fatal, progressive disease characterized by prominent, age-dependent degeneration and death of striatal medium spiny neurons. The lesion that underlies nearly all instances of HD is CAG expansion within the widely expressed huntingtin gene. Many more cells beyond the striatum are affected, especially cortical pyramidal neurons, which send their axons to synapse on striatal neurons. Indeed, one third of total brain mass is lost by end-stage disease (Zoghbi and Orr, 2000).

Like the preceding diseases covered in this review, HD disease mechanism is non-cell autonomous and based upon pathological cell–cell interactions. Progressive motor deficits and striato-cortical neuropathology in mice have been observed when mutant huntingtin expression was activated (with nervous system–specific nestin-Cre) (Gu et al., 2007) in multiple neuronal and glial cell types, including striatal medium spiny neurons, cortical interneurons, and cortical pyramidal neurons. Conversely, when Cre synthesis was restricted to cortical pyramidal neurons (Gu et al., 2005) or striatal medium spiny neurons (Gu et al., 2007), no motor deficits or cortical neuropathology were observed despite mutant huntingtin aggregation.

Substantial evidence also has revealed that mutant huntingtin damage within microglia and astrocytes are likely pathogenic contributors, including progressive reactive microgliosis (Sapp et al., 2001). The inflammatory modulator minocycline delays disease in mice generated by widespread expression of mutant

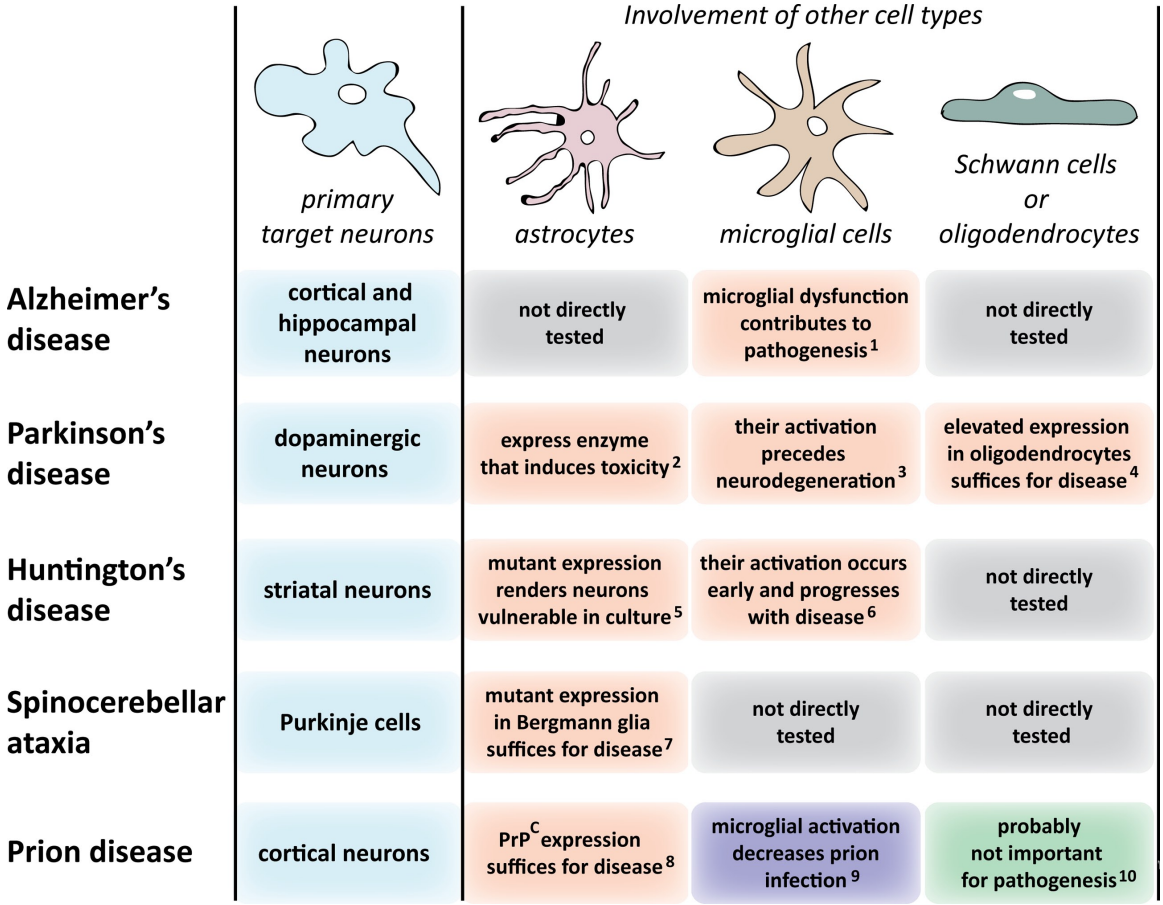

Figure 3. **Non–cell autonomous pathogenesis in neurodegenerative diseases.** This figure summarizes current evidence suggesting the contribution of apparently unaffected cell types in pathogenic mechanisms of neurodegenerative diseases, other than ALS. [1](Choi et al., 2008; Streit et al., 2009), [2](Nakamura et al., 1990; Ekblom et al., 1993), [3](Liberatore et al., 1999), [4](Yazawa et al., 2005), [5](Shin et al., 2005), [6](Sapp et al., 2001), [7](Custer et al., 2006), [8](Raeber et al., 1997; Jeffrey et al., 2004), [9](Falsig et al., 2008), [10](Prinz et al., 2004).

huntingtin exon 1 (R6/2), accompanied by decreased accumulation of microglial-derived iNOS activity (Chen et al., 2000). Mutant huntingtin accumulates in astroglial nuclei of diseased brains, accompanied by decreased levels of the EAAT2 glutamate transporter and transporter activity in HD mouse models (Shin et al., 2005), and mutant astrocytes increase neuronal vulnerability to excitotoxicity in cell culture (Shin et al., 2005).

Spinocerebellar ataxias. Spinocerebellar ataxias (SCAs), characterized by cerebellar degeneration, lead to progressive motor incoordination (for review see Taroni and DiDonato, 2004). The most affected cells are the large, cerebellar Purkinje neurons. Intimate nonneuronal neighbors to these neurons are the Bergmann glia, the cerebellum's specialized astrocytes that use long finger-like processes to enwrap the huge dendritic trees of Purkinje cells. Mutations in at least 25 genes cause ataxias, 6 of which (SCA1, 2, 3, 6, 7, and 17) represent dominant, polyglutamine repeat expansions (polyQ). Each of these mutant gene products is widely expressed. The Purkinje neurons are killed even when they do not make the mutant ataxin (Garden et al., 2002) or, even more provocatively, when mutant ataxin is expressed only within the Bergmann glia (Custer et al., 2006), demonstrating a non–cell autonomous disease mechanism.

Prion diseases. Transmissible spongiform encephalopathies, including the bovine spongiform encephalopathy (BSE; i.e., mad cow disease), originate not just sporadically or through inherited mutations but notably also by an infectious agent, the prion (for review see Aguzzi and Calella, 2009). Prions consist of an aggregated form of a ubiquitously expressed cellular prion protein (PrP^C) (Prusiner, 1982; Basler et al., 1986). PrP^C expression is necessary both for toxicity and prion replication because mice devoid of PrP^C ($Prnp^{o/o}$) are resistant to infection and disease (Büeler et al., 1993). A cell's susceptibility to prion toxicity depends on the localization of PrP^C on the plasma membrane (Chesebro et al., 2005). Although clinical symptoms of prion diseases stem from neuronal injury, neighboring glial cells seem to play a decisive role in prion pathogenesis. Non-cell autonomy in disease mechanism has been shown in at least two contexts. First, for infection, as in the recent outbreak of bovine spongiform encephalopathy in Britain or the current epidemic of prion-mediated wasting disease in American deer (Tamgüney et al., 2009), an initial noncell-autonomous contribution is after ingestion of prions. The route of prion movement from the digestive system to the brain, a process called neuroinvasion, has been shown to generally use prion amplification in the

spleen and lymph nodes in order to deliver an effective dose to the nervous system (for review see Nuvolone et al., 2009). Second, astrocytes show morphological abnormalities early in disease (Eklund et al., 1967), and in some cases they are the first places of prion aggregation (Diedrich et al., 1991). Neuronal synthesis of PrP^C can be critical, as demonstrated by reversal of prion disease through excision of neuronal PrP^C in mice with established prion infection (Mallucci et al., 2003). Nevertheless, mice expressing PrP^C at higher than normal levels but exclusively in astrocytes develop prion disease despite lack of neuronal PrP^C (Raeber et al., 1997). In this paradigm, accumulated prion aggregates lead to reactive changes in astrocytes without damaging them, but trigger toxic events in nearby PrP^C-negative neurons (Jeffrey et al., 2004). Microglia cells may play the opposite role in prion diseases, possibly containing prion infections, because microglial ablation markedly increased prion titers in a slice culture system (Falsig et al., 2008).

Alzheimer's disease and tauopathies. For Alzheimer's disease (AD), the high frequency of instances of this well-known progressive dementia makes it the disease with the highest burden on society. Genetics has uncovered gene products central to pathogenesis, including α-secretase, β-secretase, γ-secretase, and amyloid precursor protein (APP). All are widely or ubiquitously expressed. An aberrant processing product of APP (Aβ) is thought by most investigators to be the pathogenic species. There are clear hints that pathogenesis may be noncell autonomous. Mutations in presenilin 1 (PS1), the catalytic part of the γ-secretase complex, cause familial AD. Co-culture of wild-type neural progenitor cells with microglia expressing PS1 mutations or with conditioned media from those microglia—but not normal microglia—impairs proliferation of the neural cells (Choi et al., 2008). Although neuroinflammation is increasingly recognized as a pathological component of AD, provocative recent evidence has supported microglial dysfunction—rather than activation—as a probable, primary event in sporadic AD (Streit et al., 2009). In this comprehensive histopathological study, analysis of 19 human AD specimens from patients with a broad range of AD pathology showed that degenerating neuronal structures positive for tau are invariably colocalized with severely dystrophic, fragmented microglial cells. A microglial contribution to pathogenesis is also supported by recent genome-wide association studies in which two of three genes linked to AD (clusterin [also known as ApoJ] and complement component [3b/4b] receptor 1) are involved in neuroinflammatory responses of microglia and astrocytes (Harold et al., 2009; Lambert et al., 2009).

Finally, the group of disorders that share misaccumulation of tau—the axonal microtubule-associated protein—are collectively referred to as tauopathies. Included here are a proportion of frontal temporal dementia caused by mutation in tau, progressive supranuclear palsy, and corticobasal degeneration. Intracellular tau accumulations are not restricted to the vulnerable neurons, but are found also in astrocytes and oligodendrocytes (Forman et al., 2005), suggestive of damage to multiple cell types as contributors to pathogenesis.

Selective neuronal vulnerability in neurodegenerative disease: the neighborhood matters

Disease mechanism in each of the major neurodegenerative diseases we have discussed is almost assuredly non-cell autonomous. We propose that neuronal selectivity in each disorder can be most persuasively explained not by unique vulnerability to damage developed solely within the neurons whose loss is characteristic of each disorder, but by the coincidental convergence of multiple disease-causing mutant gene products provoking damage within the vulnerable neuron and multiple neighboring cell types (Fig. 3). This is a very positive insight for the prospects of effective therapies, as they could be envisioned by targeting any of the mechanisms and cell types involved.

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