Strategies for Treatment in Alexander Disease

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Summary: Alexander disease is a rare and generally fatal disorder of the CNS, originally classified among the leukodystrophies because of the prominent myelin deficits found in young patients. The most common form of this disease affects infants, who often have profound mental retardation and a variety of developmental delays, but later onset forms also occur, sometimes with little or no white matter pathology at all. The pathological hallmark of Alexander disease is the inclusion body, known as Rosenthal fiber, within the cell bodies and processes of astrocytes. Recent genetic studies identified heterozygous missense mutations in glial fibrillary acidic protein (GFAP), the major intermediate filament protein in astrocytes, as the cause of nearly all cases of Alexander disease. These studies have transformed our view of this disorder and opened new directions for investigation and clinical practice, particularly with respect to diagnosis. Mechanisms by which expression of mutant forms of glial fibrillary acidic protein (GFAP) lead to the pleiotropic manifestations of disease (afflicting cell types beyond the ones expressing the mutant gene) are slowly coming into focus. Ideas are beginning to emerge that suggest several compelling therapeutic targets for interventions that might slow or arrest the evolution of the disease. This review will outline the rationale for pursuing these strategies, and highlight some of the critical issues that must be addressed in the planning of future clinical trials. **Key Words:** GFAP, glial fibrillary acidic protein, αB-crystallin, glutamate transporters, Nrf2.

ALEXANDER DISEASE—OVERVIEW

Alexander disease (AxD) is now recognized as the first primary disorder of astrocytes, resulting as it does from mutations in the intermediate filament protein, glial fibrillary acidic protein (GFAP).[1] In the most widely recognized form, children present before the age of 2 years with megalencephaly and white matter loss, especially in the frontal lobes. Seizures and spasticity are prominent symptoms, as are hydrocephalus and psychomotor developmental delay. These children suffer progressive deterioration with death usually before the age of 10.

The key diagnostic feature of the neuropathology in AxD, highlighted in the first description of the disease,[2] is widespread deposition of Rosenthal fibers in subpial, periventricular, and white matter astrocytes throughout the CNS.[3] Morphologically, Rosenthal fibers consist of two components: bundles of intermediate filaments surrounding irregular deposits of dense material (FIG. 1). Biochemically, Rosenthal fibers are composed of a complex ubiquitinated mixture of GFAP in association with other constituents, especially the small stress proteins αB-crystallin and Hsp27.[3–5]

In 2001, we reported the results from sequencing the GFAP gene in a group of 13 patients who had died of biopsy-proven or autopsy-proven AxD.[6] Twelve of these patients carried nonconservative, heterozygous point mutations in the coding region of GFAP. After this initial report, a number of other studies have now confirmed and extended these findings (reviewed in Reference 1). A diagram showing the location of nearly all published (as well as some unpublished) mutations in relation to the protein domains of GFAP is shown in FIG. 2. Web sites that continue to provide current updates on GFAP mutations can be found at the University of Wisconsin-Madison (http://www.waisman.wisc.edu/alexander/) and the Human Intermediate Filament Mutation Database (http://www.interfil.org).

Mutations are present in nearly all (>90%) cases of AxD.[1] Nearly one-third involve either of two amino acids (R79 or R239), although it appears that mutations distributed throughout the protein produce essentially identical Rosenthal fibers and similar disease.[7] All of the mutations are heterozygous, acting in an autosomal dominant fashion. Nearly all mutations are missense muta-

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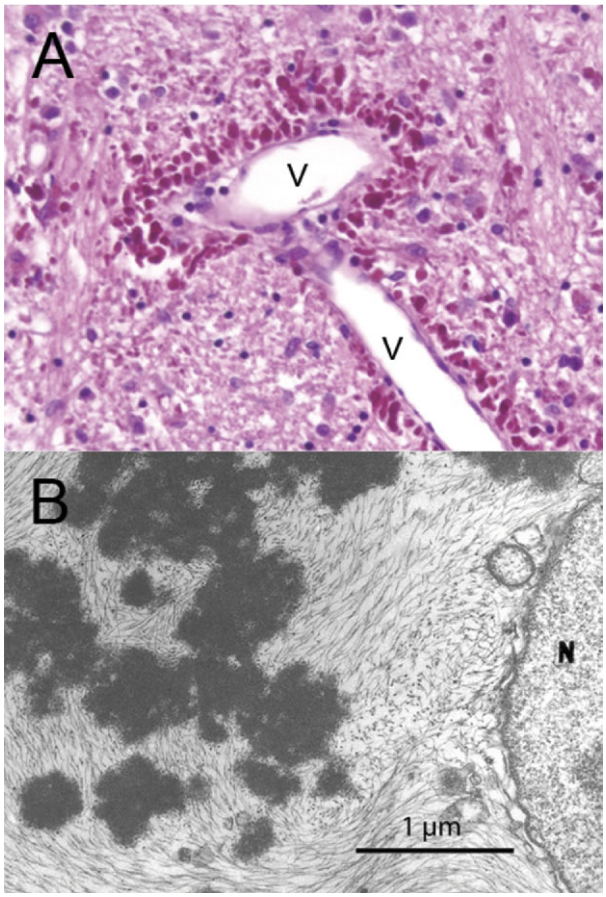

FIG. 1. Rosenthal fibers, shown as eosinophilic aggregates when stained with hematoxylin & eosin, in astrocyte end-feet surrounding a blood vessel in the brainstem of a child with an R239H mutation (A, reprinted from The Lancet Neurology [2003; 2:75], with permission from Elsevier). Ultrastructural appearance of Rosenthal fibers in an astrocyte cell body (B, reprinted from Eng et al. J Neurosci Res [1998;53:353-60], copyright by Wiley-Liss, Inc.).

tions, predicting a change in a single amino acid, although recently several other types of mutations have been described with short in-frame insertions or deletions. Proof that mutant protein is actually synthesized, and incorporated as part of the Rosenthal fibers, has been possible for one mutation, R416W.[8] Most mutations occur *de novo*, although some adult-onset forms of the disease (where patients live to reproductive age) are transmitted to subsequent generations. The penetrance also approaches 100%, the few exceptions being ones where it can be difficult to distinguish between variable penetrance and presymptomatic diagnosis.[1]

Clinical course and current treatments

Although AxD is genetically homogeneous, the clinical features of AxD are less consistent. Early descriptions emphasized macrocephaly, frontal leukodystrophy, and a variety of developmental delays; based on reliable genetic diagnoses and larger numbers of patients, it is now clear that these features are not always present.[1] In particular, later-onset patients often display a very different picture than early-onset patients, dominated by bulbar or pseudo-bulbar signs, atrophy of the cervical spinal cord, and sometimes little or even no leukodystrophy at all.[9,10] The disorder is typically progressive, with median survivals from the time of onset of 3.6 years for the infantile group, 8 years for the juvenile group, and highly variable durations for the adult group. MRI criteria for diagnosis have been published,[9,11] although atypical MRIs are also now recognized.[12] Brenner et al.[1] have argued that the age distribution of patients is almost certainly skewed by selection bias and that many adult-onset patients are missed due to ambiguities in presenting signs, atypical MRIs, and a failure to test for GFAP mutations.

Vanderver has recently proposed a slightly different classification system, focusing less on the age of onset than on constellations of symptoms and regions of MRI lesions that categorize patients as either type I (most of whom would have previously been considered "infantile") or type II (most of whom would have previously been considered juvenile or adult). (A. Vanderver, personal communication).

The current standard of treatment for AxD is symptomatic, focusing on major problems, such as seizure control, nutrition, and maintenance of pulmonary function. Only three reports describe attempts at alternative forms of therapy. One patient who was studied prior to the discovery of GFAP mutations as the cause of the disease was given bone marrow transplantation, which was based on the mistaken analogy of other leukodystrophies that are treated in such a manner.[13] The patient died 4.5 months after transplantation at the age of 1 year. A second patient (a 9-year-old girl with a D360V mutation) was treated with thyrotropin-releasing hormone (first intravenously, followed by an oral administration),[14] which was based on the apparent, but still poorly understood, neurotrophic functions of this hormone and its use in improving symptoms in mice and some human patients with other disorders of the hindbrain. One week of intravenous treatment was associated with a reduction in some of her most prominent symptoms, such as vomiting and truncal ataxia, and these improvements persisted through several months after a switch to oral therapy. Other manifestations of the disease were unchanged, such as EEG and MRI abnormalities. Most recently, a 39-year-old woman with an R70Q mutation was treated with a 20-month course of intravenous ceftriaxone, based on the proposed use of this drug for enhancing glutamate transport in astrocytes.[15] Compared with the 20-month period prior to initiation of treatment, deterioration in gait ataxia, dysarthria, and palatal myoclonus apparently slowed, and the amplitude of evoked nystagmus was reduced. Her ability to read improved, but the MRI was unchanged. Overall, the effectiveness

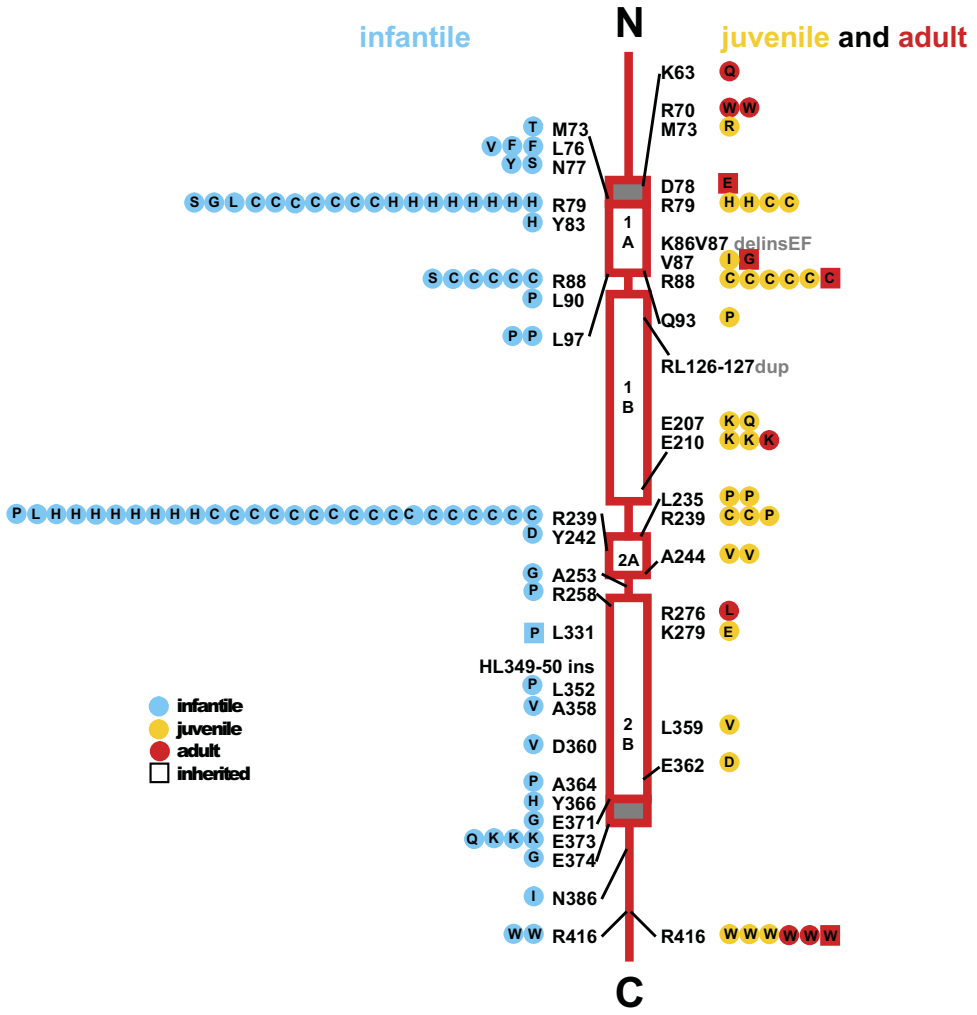

FIG. 2. Locations of published Alexander disease-associated mutations in glial fibrillary acidic protein (GFAP) in relation to protein domain structure of intermediate filaments. The boxes indicate the four α-helical subdomains within the central rod domain, separated by nonhelical linkers. The gray box just before segment 1A is a nonconserved prehelical sequence important for initiation of rod formation at the start of 1A; the gray box at the end of 2B represents the highly conserved 365TYRKLLEGEE374 sequence that includes the end of the coiled coil 2B segment. Each symbol represents an individual patient, or a family in the case of inherited mutations, or identical twins. Multiple independent occurrences of a mutation are indicated by the number of symbols. Symbols are color coded for clinical category based on age of onset (infantile, juvenile, or adult). The wild type amino acid is indicated next to the structure, and amino acid replacements within symbols on either side. (Adapted and reprinted from Reference 1, with permission from Springer.)

of these latter two treatments is difficult to judge from studies of single patients, especially in the absence of standardized clinical measures and clearly recognized benchmarks for success.

GFAP mutations and gain-of-function hypothesis

Many of the GFAP mutations associated with AxD occur within amino acid sequences that are highly conserved among intermediate filament proteins.[16] However, in contrast to most mutations of other intermediate filaments, which lead to loss of function, GFAP mutations appear to produce a toxic gain of function (for review, see Reference 1). The implication of these concepts for devising treatment strategies is that rather than replacing GFAP, we should strive to eliminate the mutant protein, prevent its initial expression, or interfere with the consequences of its expression.

As previously noted, the hallmark feature of the pathology, with or without significant leukodystrophy, is the presence of abundant Rosenthal fibers throughout the CNS. What role the Rosenthal fibers play in disease pathogenesis is much less certain.[7,17] Rosenthal fibers clearly occur in contexts other than AxD, and transgenic mouse studies demonstrate that sufficient over-expression of wild-type human GFAP will produce Rosenthal fibers.[18] Both knock-in and transgenic studies prove that expression of mutant protein leads to Rosenthal fibers, but again in the context of overexpression.[19,20] Surprisingly, none of the existing mouse models, while forming

Rosenthal fibers, have any apparent deficits in white matter, although the ones expressing mutant GFAPs do exhibit abnormal sensitivity to kainic acid-induced seizures, which might model the seizures in the human phenotype.[19,20] Cell culture studies implicate overexpression and accumulation of GFAP above a currently unknown toxic threshold in the activation of multiple stress pathways and inhibition of proteasome function, with the likelihood of positive feedback loops to cause still further accumulation.[21] One hypothesis regarding Rosenthal fibers is that the aggregates sequester certain proteins away from critical functions elsewhere in the cell (ie, see below for αB-crystallin).[7] Another is that the aggregates remove mutant GFAP from the cytoskeleton; thus they have a protective function. The composition of Rosenthal fibers is a topic of active investigation, but at the very least they are now known to contain both wild type and mutant GFAP, stress proteins such as αB-crystallin, Hsp27, intermediate filament-binding partners such as plectin,[22] and other proteins such as p62.[23]

Mechanisms of leukodystrophy

Why there is a leukodystrophy in Alexander disease is not currently understood. Depending on the age of onset one can imagine either hypomyelination or demyelination taking place. One possible mechanism for oligodendrocyte or myelin toxicity is exposure to excess levels of glutamate.[24–27] Astrocyte expression of their major glutamate transporter (Glt-1) is impaired in Alexander disease,[28] which could pose particular problems for white matter where local release of glutamate occurs from axons, astrocytes, or oligodendrocytes themselves.[24,29,30] Another possible mechanism for oligodendrocyte toxicity is tumor necrosis factor-α,[31] the expression of which is elevated in tissue samples from Alexander disease patients and mouse models of the disorder.[32] It is also conceivable that astrocyte dysfunction in Alexander disease extends to gap junctional communication, and recent studies indicate that astrocyte-targeted double deletion of connexins 43 and 30 results in vacuolation in oligodendrocyte cell bodies and intra-myelin edema.[33]

STRATEGIES FOR THERAPY

Despite the incomplete state of our knowledge regarding how GFAP mutations cause AxD, a number of useful themes have emerged that suggest potentially effective strategies for therapy. These are presented below in three general groups: 1) reducing the initial insult, 2) enhancing protective stress responses, and 3) minimizing detrimental downstream effects. We are still at an early stage of thinking these problems through, and we offer the following ideas as a guide for future research.

Reducing the initial insult—GFAP

Expression of mutant GFAP is the root cause of AxD, and accumulation (of both wild type and mutant protein) above a toxic threshold is believed to be an essential element in pathogenesis. Hence, reducing the level or expression of GFAP is one obvious strategy for treatment. An ideal drug might selectively prevent expression of just the mutant allele, but this goal is technically challenging and also involves approaches (such as RNA interference) that have not yet reached clinical practice for any disease. Alternatively, and especially in light of the fact that complete absence of GFAP produces such a mild phenotype in the mouse,[34–37] one approach being pursued is to screen libraries of drugs or compounds for suppressors of GFAP expression from both alleles. New tools for evaluating regulation of the GFAP promoter in transgenic mice using luciferase reporters have been developed that make such screening feasible.[38,39] Cho et al.[39a] have recently completed a screen using primary cultures of astrocytes derived from normal newborn mice, and they found several well-known drugs that reduce GFAP levels in vitro by 50% or more. Whether these findings can be easily translated into in vivo treatments, and eventually to humans, remains to be seen.

Instead of reducing initial GFAP synthesis through regulation of the GFAP promoter, an alternative strategy to reduce protein levels is to increase degradation. Although the normal pathways of GFAP degradation are not fully understood, impairment of proteasome function has been proposed as both a cause and a consequence of excess GFAP.[21] Pharmacological enhancers of proteasome function are therefore a theoretical possibility for treating AxD, but few studies exist on this topic.[40]

In contrast, autophagy appears to be enhanced in AxD, based on data from cell cultures and mouse models, as well as tissues from patients.[41] One strategy for treatment may be to boost the autophagic response still further, and a number of drugs have already been studied for such properties in the treatment of other neurological disorders. For instance, rapamycin and its analogues can increase autophagy through inhibition of the mammalian target of rapamycin (mTOR), and have recently shown beneficial effects when tested in mouse models of Huntington's disease,[42] tuberous sclerosis,[43,44] and spinocerebellar ataxia type 3.[45] Chronic treatments with rapamycin are tolerated, and even extend lifespan in mice.[46] Rapamycin also decreased GFAP levels in astrocytoma cell lines expressing AxD mutant GFAP.[41]

A second autophagy-inducing drug is lithium, which reduced cell death and enhanced the clearance of aggregate-prone mutant huntingtin and α-synuclein in cell culture models of Huntington's and Parkinson's disease.[47,48] Lithium also increased autophagy, delayed motoneuron death, increased lifespan, and reduced SOD1 aggregates in a mouse model of amyotrophic lateral scle-

rosis,[49] reduced toxicity in a *Drosophila* model of Huntington's disease,[50] and reduced degeneration and aggregated tau in a mouse tauopathy model.[51] Lithium increases autophagy in an mTOR-independent manner through the inhibition of inositol monophosphatase 1, which leads to a depletion of free inositol and myo-inositol-1,4,5-triphosphate.[48] Interestingly, lithium also decreases autophagy by activating mTOR through a pathway involving inhibition of glycogen synthase kinase-3β.[52] Because lithium has opposing effects on autophagy through the inositol monophosphatase 1 and mTOR pathways, it has been proposed that dual treatment with lithium and rapamycin should cause an additive increase in autophagy through their actions on both the inositol monophosphatase 1 and mTOR pathways. When this strategy was tested in cell culture and *Drosophila*, lithium and rapamycin together did have an additive effect for increasing autophagy and decreasing mutant protein.[48,52] Hence combination therapies may ultimately prove necessary for effective treatment of AxD.

Enhancing protective stress responses—αB-crystallin

Induction of various stress proteins is a prominent feature of protein aggregation disorders, where they are believed to represent an attempt by cells to deal with the challenge of mis-folded or mutant proteins. Hsp70 in particular has been the focus of considerable attention for the treatment of neurodegenerative diseases. Interestingly, Hsp70 is not induced in AxD, and instead the stress response is dominated by small stress proteins such as αB-crystallin and Hsp27.[8,53] We focus here on αB-crystallin, although many of the same concepts might apply to Hsp27 as well.

αB-crystallin is normally expressed at low levels in the brain, but it is increased in a variety of neurodegenerative disorders[54] and has been of particular interest for astrocytes and AxD since it was identified as a major component of Rosenthal fibers.[4] It is also upregulated in the mouse models that develop Rosenthal fibers.[18,55] In several types of cell culture models of injury or stress, including heat shock (glioma cells),[56] hypertonicity (kidney, primary astrocytes, glioma cells, heart),[57–60] and ischemia/hypoxia (heart),[60,61] the expression of αB-crystallin is markedly upregulated and increases cellular resistance. In some cases αB-crystallin is coordinately upregulated along with Hsp27, as with cadmium exposure in astrocytes,[58] but in other situations αB-crystallin is upregulated by itself, as with exposures to hypertonic stress and tumor necrosis factor-α.[58,62]

That αB-crystallin might offer therapeutic benefit in AxD is suggested by two sets of observations. First, αB-crystallin promotes disassembly of preformed GFAP filaments in cell-free systems,[63] and co-transfection of

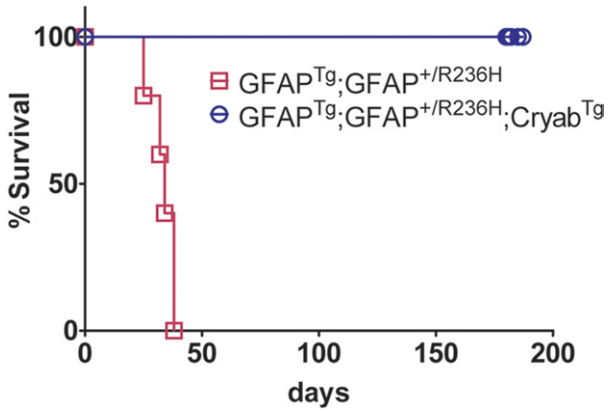

FIG. 3. Increased Cryab expression rescues lethal seizures, and reduces glial fibrillary acidic protein (GFAP) levels and Rosenthal fibers in GFAP^{Tg};GFAP$^{+/R236H}$ mice. 100% of GFAP^{Tg};GFAP$^{+/R236H}$ mice die at an early age (for littermates shown [n = 5], median age of death = 34) compared with 100% survival in GFAP^{Tg};GFAP$^{+/R236H}$ mice expressing Cryab^{Tg} (n = 8; p = 0.0002, log rank Mantel-Cox test). (Reprinted with permission from Figure 3A of Reference 45, Oxford University Press.)

αB-crystallin, along with wild type GFAP into cultured cells promotes dissolution of the filamentous aggregates that typically form in these situations.[64,65] Using transgenic methods, increasing expression of αB-crystallin in the heart conferred protection against ischemic injury in vivo.[66]

Most recently, Hagemann et al.[55] reported two key findings relating to the role of αB-crystallin in AxD. First, mice forming Rosenthal fibers from overexpression of wild-type human GFAP had increased mortality when crossed into an αB-crystallin-null background. This result supports the idea the AxD could result, in part, from depletion of αB-crystallin levels in astrocytes due to sequestration of this stress protein in Rosenthal fibers. Second, and most dramatically, forcing constitutive overexpression of αB-crystallin in astrocytes beyond its natural levels afforded complete rescue from the otherwise 100% mortality observed in a cross between two AxD mouse models (FIG. 3). The mechanisms by which αB-crystallin achieves this rescue are not clear, although recent data suggest that its binding to oligomeric forms of GFAP shifts the equilibrium to smaller forms and mitigates the effects of mutant GFAP on the proteasome.[67] Whether pharmacologic enhancers of αB-crystallin expression can be found to mimic these transgenic studies is an area of active investigation.

Enhancing protective stress responses—Nrf2

A second stress pathway worth highlighting is that represented by the transcription factor Nrf2 (otherwise known as Nfe2l2). Normally Nrf2 is inactive and confined to the cytoplasm through binding to a Kelch-like protein, Keap-1. In response to a wide variety of stresses and especially oxidative stress, Nrf2 dissociates from

Keap-1 and translocates to the nucleus, where it binds to a short anti-oxidant response element found in the promoters of a number of stress response genes, such as NQO1, glutathione-S-transferase, heme oxygenase-1, and ferritin heavy and light chains, thus activating their expression.[68]

In the CNS, activation of Nrf2 in response to toxic stress occurs primarily in astrocytes.[69,70] Evidence that enhancing astrocytic expression of Nrf2 pathways in vivo might be neuroprotective has come from transplantation studies of adenoviral infected astrocytes into brains of animals given neurotoxic doses of malonate and 3-nitropropionic acid.[71,72] Most recently, transgenic studies have demonstrated that constitutive enhancement of Nrf2 expression in astrocytes using the human GFAP promoter provides significant neuroprotection in two mouse models of neurodegenerative disease, the SOD1 G93A mutant model of amyotrophic lateral sclerosis,[73] and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxin model of Parkinson's disease[74] (see also reference 65, in this volume).

We have found marked induction of the anti-oxidant response element pathways in human brain samples from Alexander diseased patients, as well as in our mouse models of AxD.[19,32,55] It is interesting that one mechanism by which Nrf2 might be elevated is through impairment of the ubiquitin-proteasome system,[75] a common feature of protein aggregation disorders that is likely occurring in AxD as well.[21] Given the common activation of Nrf2 in a number of CNS disorders, the potential neuroprotective effects of enhancing expression, and the existence of several natural as well as synthetic inducers of Nrf2 activity that could have therapeutic applications,[76] there is considerable appeal to further exploring its role in AxD.

Minimizing detrimental downstream effects

An alternative approach for treatment is to focus on the downstream detrimental effects of mutant GFAP expression, assuming one can identify those critical astrocyte function(s) that are compromised. As with many neurological disorders where astrocyte dysfunction is implicated, attention has focused on glutamate transport, since impaired transport could cause prolonged elevations of glutamate in the extracellular space with excitotoxic injury to neighboring cells (as previously noted). Glutamate transport in astrocytes is mediated predominately by EAAT2 (also known as Glt-1), and to a lesser extent by EAAT1 (also known as GLAST), with some variation depending on anatomical location and stage of development. Complete deficiency of EAAT2 in the mouse results in 94% reduction in glutamate transport in cerebral cortex, and ~50% of the mice die from seizures by 6 weeks after birth.[77] Interestingly, modest reductions in EAAT2 mRNA and protein are seen in the hippocampus of knock-in mouse models that carry a point mutation mimicking the R239H mutation in human patients (R236H in the mouse).[55] These mice display enhanced severity and duration of seizures after exposure to kainic acid. Further reductions in EAAT2 expression are seen in mice that express both the point mutant and excess wild type human GFAP (from a transgene) that consistently die at 4 to 5 weeks after birth.

Reduced EAAT2 is also observed in humans with AxD, where loss of hippocampal neurons correlate with reactive astrocytes and diminished transporter expression.[28] Astrocytic cell lines expressing R239C mutant GFAP show a significant decrease in EAAT2 expression with a reduced capacity to transport glutamate, and fail to rescue hippocampal neurons from glutamate toxicity in co-culture assays.[28] One possible explanation for decreased EAAT2 expression is altered activation of NF-κB, perhaps by reduction in signaling via phosphorylated Akt.[41,78] EAAT2 is also regulated by ubiquitination and lysosomal degradation.[79,80] Given the roles of proteasome function and autophagy in AxD,[21,41] these pathways may also affect EAAT2 protein levels, in addition to reduced transcription.

Fortunately, EAAT2 previously emerged as a key therapeutic target for amyotrophic lateral sclerosis, and Rothstein et al.[81] conducted a screen of drugs and compounds (approved by the Food and Drug Administration) using explant cultures of rat spinal cord to search for enhancers of EAAT2 expression. Several compounds belonging to the class of β-lactam antibiotics were effective in this screen. Further validation was performed using mice carrying an EGFP reporter under the control of the EAAT2 promoter. One of these antibiotics, ceftriaxone, increased expression of the EGFP reporter in the hippocampus of mice, and partially restored the decrease in EAAT2 levels that is otherwise seen in the spinal cords of the G93A SOD mouse mutant. Ceftriaxone is now in phase I clinical trials for amyotrophic lateral sclerosis to determine safety in the chronic dosing schedules that would be required in such a disease (as would also be true for AxD). It is interesting, but puzzling, that several studies have now appeared showing the efficacy of ceftriaxone for the treatment of other disorders, sometimes without any change in expression of EAAT2.[82] Hence, the beneficial effects of this antibiotic may involve pathways that have not yet been identified, although one possibility is activation of the Nrf2 pathway previously described.[83]

HOW TO MEASURE SUCCESS?

What criteria should we use to choose among these therapeutic strategies? Cell cultures have provided most of the preliminary data in support of these approaches to

therapy, but these have primarily involved the use of transfected cell lines, which are convenient but highly artificial in several respects. Even primary cultures of astrocytes derived from one of the existing mouse models of AxD, while producing aggregates that display the same ultrastructural characteristics of bona fide Rosenthal fibers,[84] still consist of cells that are isolated from their normal anatomical context and interactions with other cell types. Mouse models have been created via both transgenic and knock-in approaches that reproduce key aspects of the Alexander phenotype, particularly the formation of Rosenthal fibers identical to those found in human disease[18] and increased seizure susceptibility.[19,20] However, none of the existing mouse models developed a leukodystrophy. Nevertheless, despite the imperfections of the mouse models, results of testing *in vivo* will have more validity than findings developed solely from cultured astrocytes.

Should any proposed therapy reach the stage of clinical trials, a number of other issues arise. The rarity of the disorder makes the idea of double-blind, placebo-controlled trials unrealistic. Instead, patients will likely have to serve as their own controls by comparing status pre- and post-treatment. However, although existing MRI criteria are highly reliable as diagnostic tools, they are not suitable for the purposes of quantifying disease severity or monitoring disease progression. Hence, any future clinical trials would be greatly facilitated by the identification of biomarkers that could serve as surrogate indicators of the response to therapy. One potential biomarker is GFAP itself. GFAP is normally present only at low levels in the CSF, but increases in the context of a number of diseases or injuries.[85] Kyllerman et al.[86] studied three AxD patients, and found elevations of GFAP in all, although only one measurement was made for each patient. Studies are now underway to replicate these studies in a larger cohort of patients and to determine whether GFAP is elevated in more convenient body fluids, such as blood.

CONCLUSIONS

The past 10 years have witnessed enormous progress in the understanding of AxD, with clinical practice transformed by the relative ease of genetic diagnosis and new ideas coming forth in regard to the effects that expression of mutant GFAP have on astrocyte biology. Whether these ideas can be translated into an effective therapy is the next, and perhaps most difficult, challenge.

Acknowledgements: We thank Michael Brenner, Shing-Yan Chiu, and James Goldman for comments on the article. This work was supported by the National Institutes of Health (Grants No. HD046599, No. NS060120, and No. NS042803 to AM, and Grant No. HD03352 to the Waisman Center). Additional support was provided by the Palamaro and Juanma Funds.

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