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Essential Trans-synaptic Nonviability of Individual Neurons Characterized by Dysfunctional Neurofilament Aggregation in Amyotrophic Lateral Sclerosis

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Abstract: Amyotrophic lateral sclerosis would constitute a system complex of heightened neuronal susceptibility that is dependent on skeletal myofiber participation towards further progression of such neuronal injury. Indeed, a simple concept of neurofilament disorganization and abnormal protein trafficking might implicate systems of direct and indirect consequence in inducing both myofiber denervation atrophy on a selective axonal basis of involvement and also a possible pathway of susceptibility towards further intraneuronal disorganization. Indeed, in terms of neuropathologic lesions such as neurofilament skeins and axonal spheroids in a complex setting of aggregation and inclusion bodies in the perikaryon, one might perhaps strictly recognize skeletal myofiber atrophy as a central target of pathology of motoneuron injury in a context however of further induced injury to such motoneurons as resulting from active myofiber atrophy. In simple terms, individual skeletal myofibers that atrophy on an individual axonal basis of injury would constitute a main mechanism for further self-propagation of progressive injury to the supplying axon. Indeed, amyotrophic lateral sclerosis would appear a disease process with active overcompensatory neuronal attempts at recovery further contributing to the disease activity.

Key words: Neurofilament, amyotrophic lateral sclerosis, neuronal

INTRODUCTION

The actual cause and mechanisms of neuronal injury and death in amyotrophic lateral sclerosis remain unknown[1]. However, it would appear that an essentially disorganized assembly of neurofilaments would be a marker, or perhaps even an epigenetic effect, of a primary defect in viability of motor neurons in the central nervous system of patients with amyotrophic lateral sclerosis (ALS)[2]. It would further appear that a potentially wide variety of primary metabolic defects[3] could account for such defective viability of motor neuron in ALS, in association with a significant degree of pathological lesion heterogeneity in ALS patients coming to autopsy[4].

A fundamental problem regards primary pathologic or genetic defects[5] in viability of neurons that would constitute essential progression of the ALS state. What defective metabolic functions, highly selective for motor neurons, would help account for the pathologic pattern of involvement in ALS?

This regard, for example, glycation might act synergistically with oxidative stress towards the formation of neurofilament aggregates and thus would interfere with both axonal transport and intraneuronal protein traffic[6,17]. Also, so-called metal binding region mutants of Cu,Zn SOD (copper zinc superoxide dismutase) would involve especially compromised SOD activities and abnormal thermal stability as a susceptibility trait[8]. Indeed, aggregation of Cu, Zn superoxide dismutase (SOD1) is a pathologic hallmark of familial ALS linked to SOD1 gene mutations[9].

Certainly, it would seem that a phenomenon of rescue of the normal neuronal phenotype by increased neurofilaments NF-L (light subunit) in transgenic mice characterized by high levels of NF-H (heavy subunit) would reflect a preservation of subunit stoichiometry, and an abnormal neurofilament accumulation, as an effect and not as a primary mediator of the pathologic effects on the neuron in ALS. In fact, over expression of neurofilament NF-H would appear to protect neurons by sequestering peripherin in the neuronal perikaryon[10]. In a sense, perhaps, there is a concurrent series of events that would result in both disorganized assembly of neurofilaments and of a decreased viability of neurons, a dual situation whereby neurofilament accumulation would probably constitute a step subsequent to the disorganized assembly of the neurofilaments.

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In addition, there is evidence that neurotoxic splice variants of peripherin filament, a neuronal intermediate filament, might contribute to the neurodegeneration in ALS[11].

In simple terms, it would appear that disorganized assembly of neurofilaments might strongly promote, as a secondary event, the subsequent accumulation of neurofilaments. The precise role of neurofilaments in healthy neurons would implicate perhaps cytoskeletal mechanics linking cellular functions—hence the disassembly of neurofilaments would in itself presumably be reflected particularly in axonal dysfunction and in the formation of axonal spheroids. Particularly interesting in this regard is the observed disintegration of the Golgi apparatus in human ALS in conjunction with downregulation of mRNA expression for the NF light subunit.

Particularly in view of the non-toxicity of accumulated NFs it would appear that a primary form of disruption involving abnormal accumulation and abnormal metabolic synthesis of NF-L in particular would constitute an essential disorganization assembly of neurofilaments.

What is the role of genetic defects particularly with reference to downregulation of mRNA expression of NF-L?

This might possibly evolve as a primary injury to subcellular organelles as for example a primary failure in protein synthesis affecting production of a specific subcomponent of NF-L.

In simple terms, a primary defect in synthesis or in metabolic processing towards the production of NF-L would be expected to result secondarily in disorganized assembly of neurofilaments in ALS. Is it possible that a defect of turnover or of reutilization of neurofilament subunit components underlies a fundamental defect in ALS?

Such failed reutilization of already present neurofilament subunits in maintaining and in replacing, periodically, worn-out neurofilaments could conceivably promote effective accumulation of large amounts of old worn-out neurofilament and also failure to resynthesize new neurofilament.

It is possible in addition that one of a possible series of primary defects, primarily inherited or acquired would result in a defective reprocessing and reutilization of neurofilament subunits, particularly affecting the assembly of the neurofilament light subunit.

One possible defective mechanism might relate specifically to abnormal intracellular trafficking of NF-L subunits, a process that could itself be especially disruptive, in terms of a secondarily compounded effect, affecting trafficking as an axonal phenomenon . Also, for example, impaired spinal cord glutamate transport capacity has been reported in a transgenic superoxide dismutase mutant rat model of ALS[12,13].

Perhaps, indeed, a relatively simple defect in intracellular trafficking could result in failed reutilization of NF-L subunits, with subsequent defective assembly of normal neurofilaments. Such failed reutilization of NF-L subunits would presumably induce pathologic effects in cases with a primary genetic defect impairing production of normal NF-L subunits.

Hence, a situation might actually arise whereby individuals with reduced genetic capability for the production of NF-L subunits, might induce progressively impaired intracellular trafficking and reutilization of NF-L, and result in abnormal neurofilament assembly and in its subsequent abnormal aggregation as skeins and axonal spheroids.

In a general sense, hence, the decreased viability of neurons would be perhaps more a direct consequence of the primary injury also causing abnormal intracellular trafficking—as such, two processes would result in the concurrent occurrence of both decreased neuronal viability and of abnormal accumulation of neurofilaments. In such a dual context, in sporadic ALS, apoptotic pathways of neuronal cell death would in particular implicate p53 and Bcl-2 family members; p53 would appear to become itself differentially involved in different mutant superoxide dismutase (SOD) mice[14] in possibly different apoptotic pathways of cell death.

Possible analogy between motor neuron disease and poliomyelitis: Motor neuron disease would essentially be recognized as a disease process affecting the lower motor neuron, and targeting the anterior horn cell body in the spinal cord. Such a form of disease involvement would help explain selective motor neuronal affliction, and also the atrophy and other denervation effects involving skeletal muscle. As such, the primary disease process might revolve around participation of the perikarya of anterior horn cells in the development of the disease process as a progressive phenomenon in ALS.

However, active morphologic features primarily involving the anterior horn cell might effectively evolve simply as a depletion of these cells through systems of participation of the perikaryon influencing dynamics of axonal neurofilament accumulation and disorganization.

It is interesting in this connection that apparent primary involvement of the neuronal perikaryon evolving dynamically as motor neuron disease would tend to implicate at times the motor cerebral cortex.

In overall general terms, the similarities pathobiologically between motor neuron disease and poliomyelitis might predominantly determine motor neuronal pathology, as the anterior horn cell loss, and as a possible concomitant involvement of the motor cortical neurons .

Such analogy would evolve as a possible viral mechanism in motor neuron disease showing relentless progression clinically. In fact, a modified viral agent closely related to the poliovirus itself might activate serial phenomena of latency and of reactivation strictly characterizing pathobiology in terms of selective neuronal injury.

Certainly, from a purely morphologic point of view, an essentially variable difference from poliomyelitis might arise as an inherently absent lymphocytic reaction affecting the anterior horn cells in amyotrophic lateral sclerosis.

However, the phenomenon of latency and reactivation of putative virus might even incorporate the possible development of rapid neuronal degeneration and death without a recognizable inflammatory cell component, particularly if one considers a phenomenon of reactivation after a long period of symbiosis of the virus within the neuronal prikaryon. Such a mechanism, for example, might involve a cell cycle signaling at the neuronal G1-S checkpoint subsequent to cyclin-dependent kinase Cdk5 deregulation as reported in cases of ALS associated with mutant SOD1[15]. On the other hand, neuroinflammation in particular has been suggested as a characteristic pathologic form of involvement in ALS[17]. Reactivation of latent virus as demonstrated in cases of herpes zoster cases, would occur as a real phenomenon, one that often cannot be directly attributed to an acute precipitating event in the period immediately preceding the onset of clinical signs and symptoms.

Additionally, viral latency as a basic pathobiologic phenomenon would perhaps allow preservation of an ability to resume viral replication and in promoting at times even a fulminant viral infection in neurons.

Certain morphologic features of neuronal degeneration in some cases of motor neuron disease might include chromatolysis, in an absence of viral inclusion bodies. Certainly, the commonly held view of a possible association of motor neuron disease with oxygen-related damage to neurons as a secondary pathogenesis in the course of a viral infection might implicate reactive changes characteristic of an active viral infection.

Epidemiologic studies have been suggestive of a high incidence of exposure to poliovirus in the past history of several patients who subsequently developed motor neuron disease, although repeated attempts at transmitting the disease in the laboratory have not been substantiated.

The commonly observed phenomenon of neuronophagia in motor neuron disease is particularly interesting as a highly focused response on the part of the host in terms specifically directed against the neuronal perikarya, of anterior horn cells. As such, this would be suggestive of a foreign agent that strongly stimulates the microglial and immune systems against neurons transformed antigenically, as by an ongoing viral infection.

Indeed, for example, there has been reported a significant positive correlation between the cerebrospinal fluid Transforming Growth Factor-beta 1 and the duration of ALS[18].

Even the highly variable extent of involvement of the central nervous system in motor neuron disease is such that some process of spread of a specific agent within the spinal cord and brain would evolve as a replicative process with possible subsequent extension to brain stem or precentral motor cortex.

Additionally, infantile forms of disease very similar pathologically to motor neuron disease, as with Werdnig-Hoffmann disease and Welander variants, might actually constitute subcomponent systems in an overall pathogenic scheme strongly analogous to infection with poliovirus or to a closely related enterovirus[19].

Certainly, viral latency and reactivation might be strongly linked to atypical clinical and pathologic features of involvement once the latent infection assumes a putative transformation to an actively progressive course towards neuronal cell death and loss.

Loss of neurofilament integrity associated with trans-synaptic degeneration in motor neuron disease with a prominent form of involvement particularly at the neuromuscular junction: Any hypothesis regarding pathogenesis in a neurodegenerative disorder as typified by motor neuron disease would have to incorporate a main active agent that progressively overwhelms normal neuronal protective mechanisms, a phenomenon that

would evolve as an age-related cumulative effect. It is, for example, conceivable that axonal neurofilament would represent the primary target leading directly both to the skeletal muscle denervation atrophy and also to the death of the ventral horn cells in the spinal cord. Also, as a secondary consequence, degeneration of neurons in the brain stem and motor cortex[20] might constitute a phenomenon of transmitted effect.

Hence, motor neuron disease would constitute a particularly striking example of a pathology specific for the corticospinal system that shows dynamics and progression of possible trans-synaptic degenerative type.

In essence, therefore, motor neuron disease might evolve as a fundamental involvement of distal neurofilaments in axons that is progressive and relentless. Even the prominent clinical manifestations involving foot muscles and cramps would be suggestive of a defect in distal axonal neurofilament integrity, somehow extending proximally.

It is such a process of dying back of neurofilaments implicating motor neuron disease, as a distal degeneration of long axonal tracts would in addition help account for essential dynamics of relentless progressiveness of the disease process. What process would render the distal motor nerve axons particularly susceptible? These distal far-extended axons would presumably be particularly vulnerable to neurofilament degeneration perhaps mainly as a function of long axonal distances from the perikaryon of the parent neuron, a phenomenon developing concomitantly with generally good preservation of myelin around such axons. In this regard, glial cell-line-derived neurotrophic factor protein has been reported to prevent motor neuron loss in a transgenic mouse model of amyotrophic lateral sclerosis[21]. In addition, aberrant neurotrophin signaling via the low-affinity neurotrophin receptor p75 has been implicated in motoneuron death in ALS via activation of apoptosis[22].

A trophic disturbance certainly rather than a direct toxic action of chemical reactants as for example by oxygen superradicals would appear primarily implicated in such disease progressiveness.

What would link a postulated mechanism have various features of distal neurofilament degeneration associated with mutations of superoxide dismutase, in familial cases of the disease and also in transgenic mutant mice[23]. Certainly, a tendency for neurofilament subcomponent accumulation and aggregation might evolve as a consequence of impaired metabolic phenomena inherently arising from oxidative stress. The generation of increased amounts of reactive oxygen radicals would perhaps evolve as an imbalance developing in terms of abnormalities of oxygen utilization within a context of overall increased metabolic activity, including in particular bicarbonate levels in biological fluids[24].

Hence, for example, it might very well be true that an uncoupling of metabolic utilization of oxygen would occur, subsequently promoting increased metabolic activity. The end-result would be a self-enhancing cascade -like series of events ultimately precipitating marked metabolic disturbances, as with greatly increased oxygen radical availability. Indeed, inability to effectively utilize oxygen in metabolic pathways, would assume pathogenic roles in inducing characteristics in progression of neuronal nonviability in ALS.

In this connection, Cu,Zn SOD might assume neuroprotective roles against a wide variety of potential injurious agents[25]. In addition, minimal SOD activity in Drosophila would tend to limit the time in which a viable redox environment could be maintained rather than chronically increase oxidative stress[26].

What phenomena of progression as energy-providing metabolic pathways would particularly enhance the accumulation of oxygen radicals due to an essential inability to utilize such oxygen? Generation of ATP itself would be expected to act as an essential metabolic mechanism of utilization of oxygen, and it might very well be true that inability to effectively generate ATP would tend to promote the generation of large amounts of free oxygen radicals. In this regard, for example, misfolded Cu,ZnSOD molecules might oligomerize into increasingly high molecular weight species with consequent oxidative stress[27]. Na,K-ATPase would appear vulnerable to aberrant SOD1 activity, with loss of activity in energy metabolism and in ion/fluxes thus contributing in a potentially significant manner to ALS pathology[28].

Mitochondrial implication in motor neuron disease might underlie the denervation atrophy of the skeletal myofibers; myofibers themselves might very well be considered integral extensions of the axon that pathobiologically determine progression of the ALS disease process.

Hence, the terminal axon together with the innervated myofibers would be central to pathologic involvement in motor neuron disease, subsequently inducing a nonresponse to trophic influences. Disrupted trophic effect distally might itself effectively evolve as a

breakdown in functional/anatomic integrity of neurofilaments.

Hence, a disconnection phenomenon of neurofilament continuity would appear an essential mechanism of progression in motor neuron disease, as a phenomenon analogous to the denervation axotomy of nerves causing muscle atrophy after nerve transection.

However, the main difference between these two generic disorders might implicate motor neuron disease primarily as individual axons implicating axonal discontinuity as perhaps an induced dysfunction subsequently evolving as an anatomic disruption as entire groups of axons are affected in later stages of the ALS disease.

In a sense, perhaps, the basic features of motor neuron disease are those of neurofilament degeneration or loss of integrity, leading to dysfunctional axonal involvement with discontinuity primarily on an individual axonal basis with evolving insufficiency of trophic effect and of increased compensatory metabolic activity as evidenced in the parent neuronal cell body.

What would cause primary damage to neurofilament integrity on an individual axonal basis? Perhaps the very existence and particularly the rate of progression of increased compensatory attempts on the part of the neuronal cell body would attest to fundamental defects in neurofilament synthesis and maintenance as reflected also in increased breakdown of integrally formed neurofilament—in fact, an essential difference between familial cases of motor neuron disease and sporadic cases of motor neuron disease might specifically revolve around such a dual pathway system of impaired synthesis versus impaired maintenance of neurofilament as primordial subcomponents of the individual axon.

Sporadic cases of motor neuron disease, as perhaps essential systems associated with trans-synaptic degeneration, might actually incorporate such trans-synaptic degeneration that is centered to both origin and progression of the disease process in ALS.

Such a situation perhaps might be suggestive of a fundamental phenomenon of trans-synaptic degeneration at the motor endplate that evolves specifically as a manifested myofiber atrophy, a phenomenon central to pathogenesis of a relentless disease process of ALS type.

Such a situation would perhaps be consistent with a phenomenon of dysfunctional trophic effect, as for example in terms of decreased free insulin-like growth factor-I[29], at the neuromuscular junction, an effect sequentially or concomitantly associated with trans-synaptic degeneration involving in direct manner the innervated myofiber or myofibers.

Is it conceivable that, in some way, innervation of myofibers actively exerts significant trophic effects within a context of possible degeneration of the myofibers when adversely affected by a distinct pathway mechanism of individual axonal denervation? Certainly, it would seem that a fundamental retrograde phenomenon of trans-synaptic degeneration with breakdown of neurofilament integrity might implicate central pathogenic mechanisms in a manner leading to evolving myofiber paralysis as systems of individual axonal injury manifested as myofiber atrophy.

The apparently overcompensatory activities of the neuronal cell body would in a sense perhaps contribute to self-propagation of a disease process that would actively contribute to the pathophysiologic basis of the active trans-synaptic degeneration. For example, overcompensatory attempts on the part of the cell body could possibly imply an increased synthesis of neurofilaments that specifically promotes neurofilament aggregation as skeins and axonal spheroids in ALS..

Indeed, accumulation of neurofilaments in the neuronal perikaryon might operatively constitute an overcompensatory series of pathway effects specifically related to individual axonal degeneration in inducing further progressive myofiber degeneration.

Such dysfunctional compensatory production of neurofilaments might result somehow in abundant neurofilament accumulation of subcomponents—hence, the endresult of dysfunctional attempts at part of neurofilament synthesis might lead to the progressive production of defective neurofilaments.

Hence, a spiral form of pathologic involvement might actively evolve in motor neuron disease, in a manner specifically implicating a central neurofilament disintegration implicating combined neurofilament breakdown and defective neurofilament synthesis as systems of dysfuncitonal discontinuity of axons subsequently resulting in anatomic disruption of axonal integrity and neuronal cell death.

Such an integrated series of mechanisms would perhaps directly implicate trans-synaptic degeneration, a phenomenon possibly consistent with a primary physiologic role for active trophic effects determining myofiber viability as induced by the supplying axon across the neuromuscular junction.

Indeed, in the central nervous system a basic central concept of synaptically determined viability of neurons

might incorporate also systems of synaptically determined selective vulnerability determining also dynamics of evolution and progression of a disease process of ALS type that is both peripheral and central nervous system in distribution.

CONCLUSIONS

Systems of specifically individual axonal disruption as one integral pathway conducive to skeletal myofiber degeneration in amyotrophic lateral sclerosis might evolve as neuronal overcompensatory or dysfunctional mechanisms of injury. Such systems might relate to inherent susceptibility of neurons that undergo increasing metabolic activity and increased oxygen availability in the face of impaired ability to effectively utilize such increased oxygen.

In simple terms, perhaps, one might speak not only of trans-synaptic degeneration of skeletal myofibers, but particularly of how individual axonal disruption of neurofilaments would itself constitute an inherent component of active skeletal myofiber atrophy as systems of increased susceptibility to various pathways of progressiveness of the disease process.

Indeed, one might simply equate amyotrophic lateral sclerosis to different modes of effective influence inducing heightened neuronal and skeletal myofiber susceptibility as integral pathways of evolving neuronal cell death beyond simple neuronal networks of innervation or denervation.

Indeed, in terms of skeletal muscle biopsy in patients with active amyotrophic lateral sclerosis, one might recognize features of denervation atrophy simply as modes of both direct and indirect myofiber involvement inherently arising as dysfunctional attempts at neuronal recovery.

Indeed, much of the progressive course of the skeletal muscle atrophy as a denervation phenomenon in amyotrophic lateral sclerosis might simply evolve as modes of neuronal attempts at recovery of such innervation in terms of neurofilament accumulation and aggregation. Furthermore, the spectrum of selectivity of motor neuronal involvement in amyotrophic lateral sclerosis might specifically relate to how the supplied skeletal myofiber is itself a main determinant in inducing further injury to the supplying neuron in possibly inducing further overcompensatory or dysfunctional attempts at recovery of innervation or reinnervation.

A spiral progression pathway of increasing severity would specifically evolve as an individual axon of disruption that implicates myofiber atrophy in terms also of subsequent further individual axonal injury as such myofiber denervation atrophy itself evolves.

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