Arq Neuropsiquiatr 2011;69(4):699-706

Sporadic amyotrophic lateral sclerosis

New hypothesis regarding its etiology and pathogenesis suggests that astrocytes might be the primary target hosting a still unknown external agent

Roberto E.P. Sica[1], Alejandro F. De Nicola[2], María C. González Deniselle[2], Gabriel Rodriguez[3], Gisella M. Gargiulo Monachelli[3], Liliana Martinez Peralta[4], Mariela Bettini[5]

ABSTRACT

This article briefly describes the already known clinical features and pathogenic mechanisms underlying sporadic amyotrophic lateral sclerosis, namely excitoxicity, oxidative stress, protein damage, inflammation, genetic abnormalities and neuronal death. Thereafter, it puts forward the hypothesis that astrocytes may be the cells which serve as targets for the harmful action of a still unknown environmental agent, while neuronal death may be a secondary event following the initial insult to glial cells. The article also suggests that an emergent virus or a misfolded infectious protein might be potential candidates to accomplish this task.

Key words: ALS, SALS, pathogenesis, astrocytes.

Esclerosis lateral amiotrófica esporádica: nueva hipótesis relacionada con su etiología y patogenia que sugiere que los astrocitos podrían ser el blanco primario alojando un agente nocivo aun desconocido

RESUMEN

El artículo presente describe, brevemente, las características clínicas y los mecanismos patogénicos de la esclerosis lateral amiotrófica esporádica, tales como la excitotoxicidad, el stress oxidativo, el daño proteico, la inflamación, las anormalidades genéticas y la muerte neuronal. Luego de ello, sugiere la posibilidad hipotética de que los astrocitos podrían ser el blanco primario de la acción de una agente ambiental, externo, aún desconocido, y que la muerte neuronal aconteciera secundariamente a ese daño astrocitario inicial. El artículo concluye discutiendo la posibilidad de que un virus ambiental o endógeno o una proteína mal plegada, que adquiriera características de infectividad, puedan ser la causa de la enfermedad.

Palabras-clave: esclerosis lateral amiotrófica, esclerosis lateral amiotrófica esporádica, patogenia, astrocito.

Correspondence
Roberto E.P. Sica
School of Medicine
Buenos Aires University
Pueyrredon 1061 / piso 10, dpto. B
1118 Buenos Aires - Argentina
E-mail: rsica@fmed.uba.ar
srsica@fibertel.com.ar

Support
Funding was provided by a Buenos Aires University research grant (UBACyT M68, 2009-2011)

Received 27 February 2011
Received in final form 18 March 2011
Accepted 5 April 2011

Amyotrophic lateral sclerosis (ALS) is a disease of unknown cause which involves, simultaneously or sequentially, the upper and lower motor neurons. It must be considered as a unique entity in which both types of motor neurons are compromised, either simultaneously or sequentially.

In this article we shall concentrate briefly in the pathophysiology of ALS. Thereafter, we shall put forward a rather new proposal regarding its probable cause.

The remainder of this script will be referred just to the sporadic form of the illness (SALS), avoiding any discussion on the familial form (FALS), even though

School of Medicine, Buenos Aires University: [1]MD, PhD Institute of Cardiological Investigations, Neurological Unit; [2]MD, PhD Laboratory of Neuroendocrine Biochemistry, Instituto de Biologia y Medicina Experimental; [3]MD, Neurological Unit, Ramos Mejía Hospital; [4]MD, PhD Microbiology Department; [5]MD, Microbiology Department.

some facts of FALS will be mentioned when pertinent to SALS' reasoning.

Pathogenesis

Different mechanisms have been recognized so far, leading, all of them, combining their effects, to the death of motor neurons.

Excitotoxicity – Within them, the one which first attracted attention has been excitotoxicity, glutamate mediated. Despite that glutamate may have a role on the development of SALS, its presence in high concentrations within the central nervous system (CNS) is not restricted to SALS; other primary degenerative disorders of the CNS, such as Alzheimer's disease, Parkinson's disease, Huntington's chorea show high values of glutamate in the particular areas where neurons are stressed by those different conditions[1]. Thence, it is possible to assume that the raised concentration of the transmitter constitutes only one step within the ladder of events leading to neuronal failure. Once glutamate is at work, a chain of phenomena develops; excitotoxicity means increased Ca++ flow within the neuron and awakening of the oxidative stress, finally yielding neuronal death.

Glutamate acts at the metabotropic and ionotropic receptors. Although excitotoxicity has been attributed mainly to the sustained activation of the highly Ca++ permeable NMDA receptors, AMPA receptors are of crucial importance contributing to the excess of Ca++ going inside of the motor neuron due to the lack of gluR2 subunit, which is responsible for blocking Ca++ entry within the cell, in most of those SALS patients' motor neuron receptors[2].

The maintained depolarization of the motor neuron membrane allows the opening of the voltage-dependent Ca++ ion channels, increasing further the concentration of this ion into the cell.

The capacity of the neuron for buffering the excess of Ca++ mainly relies upon the ability of certain cytosolic proteins to bind the ion, such as parvalbumin and calmodulin, which, in these circumstances, are downregulated due to the development of endoplasmic reticulum stress[3], further reducing the ability of the cell to handle Ca++ .

Nevertheless, enhanced glutamate concentration levels, as measured in the spinal fluid, seems to be related neither with the intensity of the neuronal damage nor with the extension of the clinical compromise observed in SALS; a study by our group[4] found that glutamate levels in the spinal fluid of SALS patients showed no relationship with the rate of cultured murine cortical neuronal death it produces, nor with the level of clinical diagnostic certainty, according to El Escorial criteria.

Based on the glutamate hypothesis, Rothstein et al. advanced a further step towards the identification of the cause of the disease when they added the astrocyte within the pathogenesis of the illness[5]. They found that the main transporter of the glutamate at the spinal cord and one of those acting at cerebral cortex, the EAAT2 (named GLT-1 in mice), which is selective for astroglia, was severely decreased in both structures of SALS patients. Transporter activity can be regulated by gene expression, transporter protein targeting and trafficking and through post-translational modifications of the transporter protein[6]. The loss of EAAT2 in SALS may be due to aberrant mRNA, resulting from RNA processing errors[7] or from mistakes in translational or post-translational processes. The same group found that epigenetic factors, such as low methylation levels on DNA CpG sites of the promoter at the astrocytes, may play a role disrupting the EAAT2 mRNA[8]. Therefore, it is apparent that the transporter EAAT2 is unable to remove enough glutamate, per unit time, at the synapses, conceding the accumulation of the aminoacid at the site.

Other processes develop within the neuron after the increased Ca++ entry.

Recently attention has been paid to the so-called endoplasmatic reticulum stress, triggered by excitotoxicity and characterized by augmented production of chaperone proteins and increased ubiquitin reactivity, which become almost useless due to the progressive proteasome impairment which develops in SALS.

All those changes appear along with enhanced protein lipoxidative, glycoxidative and primary oxidative damage[9]. Therefore, oxidative stress seems to play a major role in the harm to motor neurons.

Oxidative stress – Oxidative stress is one of the mechanisms by which motor neuron death occurs. Mutations of the anti-oxidant enzyme superoxide dismutase 1 (SOD1) cause disease in a minority of familial cases[10]; notwithstanding that the abnormal behaviour of this enzyme may contribute, as well, to the derangement of the motor neurons in SALS patients[11]. In this regard, we could recognized 2 groups of SALS patients which were individualized according to the activity of the enzyme in their erythrocytes; the larger showing an age-related decreased function, while the smaller depicting increased activity of the enzyme[12]. Recently, Gagliardi et al.[13] found abnormally high levels of SOD1 transcript in the spinal cord, brain stem and lymphocytes of SALS patients.

Different enzymatic pathways may contribute to the generation of reactive oxygen species (ROS) in SALS.

Most probably, in SALS the increment of the intracytoplasmatic Ca++ concentration, due to excitotoxicity, enhances the activity of phospholipase A, neuronal nitric oxide synthase (nNOS) and xantine oxidase enzymes[14].

In this realm, the cells' energy-producing organelles, the mitochondria, are arguably the most crucial structures which may be affected by the oxidative stress. These organelles, the mitochondria, are themselves a major source of ROS when excitotoxicity injures the cell; within these circumstances decreased activity of respiratory complexes I and III boosts ROS production. In SALS, free radicals may build up to toxic levels and damage cells of different tissues. Within this family of molecules, superoxide anion (O2), hydroxyl radicals (HO) and nitric oxide (NO) are the most relevant.

Under oxidative stress the mitochondria morphology changes; they become smaller, their crests disrupted; edema, crystolysis and vacuolization abrade them, while their membranes break down[15]. All these features are not limited to the motor neurons; similar changes were found in liver[16] and muscle[17] cells and in lymphocytes[18] of SALS patients. More recently, we have described similar mitochondrial changes in skin cells from these patients[19]. These observations suggest that the oxidative stress is not just a phenomenon constrained to the motor neurons, but a widespread disturbance afflicting quite different organs within the body. Supporting this notion is the increased level of oxidative markers in biological fluids found by different authors[20]; we also could observe increased serum levels of thiobarbituric acid reactive substances (Tbars) in SALS patients studied at our laboratory (unpublished results). Remarkably, our group has recently found that a normal biological molecule, progesterone, endowed with anti-oxidant capacities, is increased in the sera of SALS patients, its value correlating with the patient's life-time expectancy[21].

Proteins – One of the consequences of the already discussed factors involved in ALS pathogenesis is the chemical inhibition of the proteasome[22]. Its down-regulation accounts for the appearance of progressive accumulation of ubiquinated and poli-ubiquinated proteins which, ultimately, collapse into proteinaceus aggregates forming inclusion bodies within the cytoplasm and the nucleus of the neuron.

The misbehaviour of two others proteins seems to be intimately related with the disease's development. These are the TAR (transactive response) DNA binding protein (TDP-43 or ALS 10) and the fused in sarcoma protein (FUS or ALS 6). Both of them appear to participate in SALS[23,24]; nevertheless, it is worth to note that descriptions of their abnormal function have been done in FALS, transgenic mice (TgALS) and in the Wobbler genetic model[25,26] as well.

TDP-43 is a DNA-binding protein found in the cell's nucleus, it has a role in the translation of DNA into RNA regulating transcription and splicing. It binds specifically to pyrimidine-rich motifs of TAR DNA and to single stranded TG repeated sequences. It also binds to RNA, specifically to UG repeat sequences. Its gene location is at chromosome 1p36.22.

Physiologically, normal TDP-43 is a nuclear protein; however, when it adopts a pathological state it can be found, as insoluble clumps, in neuronal nuclei, perikarya, and neurites[27].

The findings of TDP-43 mutations in some ALS patients suggested that TDP-43 abnormalities might be the cause of the disease, but only rarely, because recent studies, searching several hundred SALS patients, did find just a very low proportion of TDP-43 mutations, a fact which signals that such mutations are not common[28]. Therefore, it may be that factors, other than TDP-43 mutations, may alter the TDP-43 proteins disrupting their normal RNA-handling capacities in the nucleus.

The other protein, first described in myxoid liposarcoma and acute myeloid leukemia, known as FUS, is a nuclear ribonucleoprotein that plays a role in homologous DNA pairing, recombination and repair. Normally, FUS protein molecules stay in the nucleus. Its gene location is at chromosome 16p11.2. It is involved in pre-mRNA splicing and the export of fully processed mRNA to the cytoplasm.

It has been identified a missense mutation in the gene encoding FUS, linked to ALS 6. Post-mortem analyse of some patients with FUS mutations has shown FUS-immunoreactive cytoplasmic inclusions and predominantly lower motor neuron degeneration[29]. Recently, other authors have found FUS mutations in SALS as well[30]. FUS protein molecules made from mutated FUS genes are more likely to be located in the cytoplasm, where they tend to clump together.

Interestingly, in fronto-temporal dementia with ubiquitinated bodies increased levels of insoluble FUS were found, despite that no mutations in the FUS gene were observed in any patient[31], suggesting that FUS mutations are not mandatory for altering the protein. Therefore, as has been mentioned previously with regard to TDP-43, possibly, other factors different from FUS mutations, may alter the FUS proteins and disrupt their normal functions in the cell's nucleus.

It is worth mentioning that the integrity of the neuronal cytoskeleton is needed for the health of the cell. In this regard, in SALS patients, neurotubules and neurofilaments in motor neurons are depleted, impairing normal transport along neurites and the availability of molecules able to buffer abnormal enzymatic activity[3].

Inflammation – Few authors have addressed this subject; recently Keizman et al.[32] described the presence of systematic low grade inflammation in those patients,

which appears to modestly correlate with their level of disability. They found increased serum concentration of fibrinogen and c-reactive protein, elevated erytrosedimentation rate and enhancement of the neutrophils to lymphocytes ratio as compared to control subjects.

Others results supporting inflammation contributing to SALS pathogenesis stem from observations in the spinal cord and brain of SALS patients, which show activated microglia coupled to the presence of some lymphocytes, macrophages and increased levels of TNFα, IL6 and IL-17 molecules; these last molecules were increased, as well, in the sera of those patients[33].

The meaning of these findings remains unresolved but, somehow, it seems to be an attempted neuroprotective response within the affected tissues, translated in subtle systemic and local changes[32].

Neuronal death – When discussing the type of neuronal death in ALS, controversies still exist regarding its type. Most of the findings favour apoptosis as the usual final step of the motor neuron life[34]. Nevertheless, the pathways leading to this final output may vary. Some authors have suggested that a particular apoptosis occurs in SALS which has been named "cytoplasmic apoptosis", mainly consisting of cell shrinkage, cytoplasmatic blebbing, nuclear chromatin condensation and DNA degradation, conducted by the Ca++ activation of certain enzymes such as protein kinases, endonucleases, proteases and phospholipases[35], which constitutes a different pathway from the one observed in the TgALS mode, in which the classical mitochondrial apoptotic pathway has been postulated. However, it is worth to note that in the Wobbler mice, neuronal death is accomplished by vacuolization of the motor neuron, instead of the classical apoptosis[36].

Autophagy is another process observed in SALS patients' motor neurons[37]. Nevertheless, its role in cell death is unclear. Autophagy is mainly considered a survival mechanism which provides the cell with its own sources of nutrients when importing nutrients from outside is limited or impaired. It may have a protecting role by eliminating misfolded proteins, which may be toxic for the cell, and damaged mitochondria able to trigger apoptosis. Nonetheless, autophagy can be found in dying cells as well. In these circumstances, it is unclear whether it constitutes a cell's attempt to stabilize its metabolism by sacrificing its own components or whether it contributes to the cell's death.

Genes associated with SALS – Currently, in various diseases the genetic-epigenetic interactions are being considered to strongly influence the course of the illness.

In SALS gene methylation may block the expression of genes, otherwise needed for neuronal survival. Alternatively, lack of methylation can put at work genes that normally are kept silent. In a recent study Morahan et al.[38] reported a detailed analysis of the methylation status of the whole genome in brains of SALS patients. They found a large number of methylation changes in patients' genes proved to be involved in Ca++ dynamics, excitotoxicity, oxidative stress, neurotrophic factors receptors, ubiquitin protein, xenobiotic detoxification and DNA repair. The exact importance of these observations is still unknown.

SOD1, TDP-43, FUS and optineurin genes may be, as well, compromised in SALS. The three first genes were previously mentioned. Regarding the optineurin gene, three types of mutations have been described in ALS, either FALS or SALS[39]; the gene is mainly involved in the inhibition of activation of the nuclear factor kappa B. Its locus is at chromosome 10 p13.

Likewise, it may be possible that abnormalities of genes related to DNA repair hold functions in the progression of the disease as well[40].

Basis for a new hypothesis enunciation

So far, all the features described above are related with the pathogenesis of the disease, but actually, none of them appears to be the cause of the illness.

In this regard, some clinical clues may shed some light on the subject. In this realm, two reports by Ravits et al.[41,42] and one from our group[43] are pertinent.

Ravits et al.[41,42] described, by employing clinical observations and histological examination of nervous tissues, that SALS starts by affecting one neuronal territory, following its course by involving another area in the vicinity of the former; this pattern revering the somatotopic organization of the motor cortex when the upper motor neurons show signs of compromise. However, in the territory of lower motor neurons the progression of the disease, expressed by muscle weakness and atrophy, is usually lineal, spreading from one metamerae to another in its neighbourhood. Recently, our group[43] has distinguished 8 spread patterns (SP) characterizing the clinical behaviour of the illness; interestingly, the identification of the type of SP allows to attain a sharper prognosis in regard to the patients' survival time.

Together, these results allow a preliminary and plausible conclusion suggesting that axons and dendrite processes belonging to upper and lower motor neurons might play a role in organizing the concurrent involvement of both types of cells.

The described behaviour would make rational to suggest that a still undefined agent, which could be an environmental one, spreads freely from one neuron to another among pyramidal neurons, either by cells contiguity or through their synaptic contacts. Also it would

have the ability to be transported along the pyramidal axons until reaching the spinal motor neurons. This last viewpoint necessarily asserts that the origin of the disease should be located at the upper motor neuron; however, this is not mandatory, because the environmental agent may involve first the lower motor neuron, later on travelling to the upper motor neuron by taking advantage of the retrograde flow of the pyramidal axons. This hypothetical behaviour would explain, as well, the frontal lobes impairment, often seen in these patients[44], and the somatosensory cortical disturbances, depicted by electrophysiological tests[45], because the travelling agent might be taken up by axons connecting the pyramidal neurons to the prefrontal and somatic-sensory receiving areas. In this respect it is worth mentioning a recent observation made by our group which has shown that patients who started with pyramidal signs at the lower limbs developed frontal lobe disorders sooner than those whose initial manifestations were outside of the hind limbs. In this regard, it is worth noting that cortical motor representation of the lower limbs is closer to the prefrontal regions than other body motor representations situated in the motor cortical strip (Abel C, Sica REP. unpublished results).

This concept would also explain the different phenotypes of patients affected by this condition which embraces dissimilar possibilities, namely a start up involving the upper or the lower motor neurons, the predominance of pyramidal or spinal motor neurons signs, plus other accompanying clinical features. The rate of progression would depend on the rate of production of the putative external agent and on its capacity to swiftly migrate from one cell to another.

Based on aforesaid features, it would be possible to accept that the agent insulting the neurone has to have a nature which allows it to travel from one neurone to another through their synapses or membrane contacts. Another property that this agent has to accomplish is to gain "strength" once it has invaded the neighbouring neurons, with the aim to travel, once more, to the nearest neuronal station, at the time that it makes sick its actual hosting neuron.

By introducing this sort of reasoning, we ought to speculate now about the nature which should exhibit the theoretical environmental agent behaving as suggested above.

It is difficult to blame an ambient toxin, because such a substance will exhaust itself after a time, due to its metabolism, progressively slowing down its spreading and leaving a population of death neurones, but a limited one. Within this scenario, it is possible to foresee that once the toxin has exhausted itself, the disease would stop its development. This would be the case for an acute or short time exposition to any given toxin. However, it would be possible to expect that chronic exposure to an environmental toxic agent would be able to sustain the disease; whether this were the case, it might be anticipated that the whole neuronal pool which will be involved in the full developed disease, at its later stage, would show signs of compromise, albeit subtle perhaps, from its very beginning, a circumstance not seen in SALS, even when on top of a careful neurological examination, a comprehensive electromyographical search is carried on at the same time. Clinically or electrophysiologically, the usual observations are that clinical normal and involved regions coexist; besides, active denervated and normally functioning muscles can be depicted by the electrophysiological exam.

What has been said above would lead to suspect that a living agent, able to reproduce itself, might underlie and be the cause of the disease.

A virus can invade the motor neuron nucleus' cell, can reproduce itself taking advantage of the host DNA, can travel through the cell's cytoplasm and the axons introducing itself into the flow, can be release at the synaptic cleft, can be in the extracellular medium once the cell has been destroyed, and can enter a neighbouring cell, neurone or astrocyte, provided that this receiving cell has receptors though which a virus can make its way into it, either directly or with the help of a transporter protein.

If this concept is accepted, we can go a step further asking: what type of virus can fill up all those requisites? The answer is: several.

There is an extensive literature devoted to the search of a virus as the ultimate cause of this illness. Several authors, including ourselves, tried to find virus molecules in the motor neurons or other involved tissues, such as muscle, of SALS patients, but failed.

Among the most suspected virus were enterovirus[46], herpes virus[47,48], Borna virus[49], retro-viruses[50] and hepatitis C virus[51].

Perhaps, retro-viruses were the most likely candidates for doing the type of damage seen in SALS. Favouring this suspicion there are some previous observations in the literature which help to sustain this position. McCormick et al. and MacGowan et al.[52,53] have detected the presence of reverse transcriptase in the spinal fluid of patients afflicted with the disease. Some authors reported patients with SALS who, simultaneously, acquired the human immune-deficiency syndrome due to the action of the HIV1 virus; interestingly, some of them were able to control and improve their neurological condition once the HIV1 infection was aggressively treated[54]. However, in this regard an old paper by Zil'ber et al.[55] has shed further light supporting this possibility; the authors were successful for reproducing the illness in mon-

keys by administering extracts obtained from the spinal cord of people who have died from SALS. The disease in monkeys was characterized by a very long incubation period, which lasted five years or more, and which was very similar, clinically and pathologically, to the human disease; the agent producing the disease could be transferred twice in those animals without attenuation. Interesting enough, the authors were unable to reproduce the illness in mice and guinea pigs following the same protocol; they have concluded that the responsible agent might be a virus, a lenti-virus, that they could not individualize at that time. Another interesting conclusion from this report, not mentioned by the authors, is that the agent was quite species-specific, because transmission was only successful between humans and monkeys.

Very recently Douville et al.[56] gave new impulse to the idea that, somehow, retroviruses might be involved in the generation of the disease; they found increased transcripts of human endogenous retrovirus HERV-K subtypes, along with the presence of reverse transcriptase enzyme proteins, in the frontal lobes neurons of SALS and FALS patients, mainly at the pre-frontal areas, allowing them to suggest that these new observations may explain previous findings which have shown enhanced reverse transcriptase activity in serum and cerebrospinal fluids of these patients, as has been mentioned above. Whether these genomic HERV insertions represent just a marker of the disease or play a role in the aetiology and pathogenesis of the illness is a matter of further discussion yet.

Despite all the efforts done in this direction, nobody could detect viral particles within motor neurons, either cortical or spinal, nor within skeletal muscle tissues. Furthermore, when one looks at the motor neuron pool of these patients within the afflicted region, what is found is a paucity of neurons, not a hint of any fight of the neuron against a putative invading agent able to destroy it, just a mild inflammatory response in the surrounding tissues, mainly based on the presence of reactive microglia. The neurons fade away silently, mainly due to apoptosis.

The primary astrocytic damage hypothesis of SALS

Acknowledging the facts mentioned above, it appears reasonable to look outside the motor neurons, searching its environment. When doing this the obvious finding is the presence of the astrocyte.

Astrocytes have been initially though to act just as glue-like structures bringing neurons together. This conception persevered during almost 80 years since the original descriptions of Virchow and Golgi. However, this notion have changed along the last 20 years or so, when it became clear that their stellate processes elaborate end-feet structures which contact microvessel walls and are in intimate relation with neuronal synapses.

The functional unit composed by the microvessel, the astrocyte and the neuron has the goal of providing oxygen and glucose to the neuron according to its functional needs. Therefore, one of the most important tasks these cells have is to sense the state of the synaptic activity. In regard to this, the glutamate delivery at the motor neurons synapses wakes up several changes within the astrocytes which are transmitted to their vascular feet in order to obtain substrates and to accommodate the blood flow according to the metabolic requirements of the neuron[57].

Furthermore, the concept that astrocytes play a role in the information processing within the nervous system has gained acceptance in the last years, acknowledging that these cells are active partners of the neurons, either at the pre and post-synaptic levels, able to integrate or modify converging information and having a bi-directional reporting flow between them and the neurons through their contacts with the neuronal synapses. This sort of network leaded to the concept of the "tripartite synapses", involving the pre and postsynaptic neuron and the accompanying astrocytes.

Accounting to these observations and knowing the different functions play by these glial cells, it has been recognized that astrocytes comprise a heterogeneous population. The most conspicuous difference is found between astrocytes residing in the grey matter, named protoplasmic, and those situated at the white matter, called fibrous. Nevertheless, complexity goes further; different populations of astrocytes, within the grey matter, can be recognized, their structure and activity are suited to the type of neuron with which they contact[58]. These particularities provide a great plasticity to astrocytes, capable to rapidly adapt to the changing situations in their environment. These properties allow to suppose that astrocytes which are related to the motor neurones are unique in terms of their capabilities to furnish the particular needs of that type of neurons.

Another anatomical feature is worth to bear in mind. Astrocytes form a net amongst them, neurons, dendrites and axons by means of the extension of their numerous processes radiating outwards of their cell bodies and creating functional units in such a manner that a single astrocyte can contact several neurone bodies, hundreds of dendrites and thousands of synapses. Interesting enough, as well, is their capacity for swift communication among them in response to stimuli, thanks to the existence of gap-junctions at their surface; an example of this capability is their handling of calcium waves, which are rapidly transmitted throughout their population by means of those gap-junctions.

After considering the importance of the astrocytes within the CNS, let us go back to the ladder of events which occurs in SALS.

As far as our current knowledge of the disease allows, the first step in SALS development is the accumulation of glutamate within the synaptic cleft of the motor neurons. After Rothstein's group papers[5-8] it has been acknowledged that an impairment of the glutamate transporter EAAT2, expressed in astrocytes, seems to be the culprit of such aminoacid accumulation. Whether this is the initial event occurring in the disease, although not absolutely proven, it is a reasonable possibility. Thence, the obvious question arising is: which is the cause of the EAAT2 impairment?

Attempts have been made to obtain the answer. Some features are known; in SALS the EAAT2 transporter mRNA is down-regulated and the excess of TNFα contributes to reduce the density of the transporter protein. However, these features might be the result of the insult harming the astrocyte by a still unknown agent.

From the pathological view point SALS keeps some similarities with diseases in which the actor bringing forth the illness is known. In this regard, the complex AIDS-dementia is pertinent; in this disease there is a progressive loss of cortical neurons, leading to dementia, but the infected cell is only the astrocyte and the inflammatory response is constrained to activated microglia joined to the presence of scattered mononuclear cells[59]. Other illness which keeps resemblance with SALS is the Creutzfeldt-Jakob dementia, a well known prion disease where cortical neurons are abated, but the main changes are localized in astrocytes[60].

Therefore, it sounds reasonable to switch the current efforts in searching for the cause of SALS to the study of the behaviour of the astrocytes, looking for, within them, the presence of a putative virus, which might be an emergent human new virus or a human endogenous retrovirus or the structural abnormality of a protein able to become infectious, once misfolded, in a way mostly resembling the prion protein described by Prusiner years ago. Already, some authors have stepped into that trail by looking preferentially to changes occurring within the astrocytes, describing abnormalities of their cytoskeleton proteins as a characteristic feature of both sporadic and familial forms of the disease[61]. Even more, murine models of the disease, of either transgenic or genetic origin, also show strongly reactive astrocytes. In the Wobbler mouse, spinal cord astrogliosis is an early finding during the course of the disease[62]. Recently it has been shown that astrocyte density increases in the spinal cord of newborn Wobblers before the appearance of neuronal abnormalities (Meyer et al., unpublished results).

Therefore, in SALS, the putative aethiologic factor may primarily harm the astrocytes particularly associated with motor neurons, affecting the EAAT2 transporter characteristics and properties, triggering the chain of events which will lead to the motor neurons injury occurring in this disease.

Most of the current research in ALS is devoted to the search of genes which changes would underlie the pathogenesis of the disease. However, the suspected genes, despite of being their changes or mutations the cause of serious mistakes in the handling of several proteins, cannot be accused of being the original cause for the SALS form of the illness; their changes may be the result of the insult produced by an environmental agent acting primarily onto the particular astrocytes closely related with the motor neurons; even though that the genes alterations, once produced, will facilitate the appearance of the illness. But, this is a quite extended mechanism in Nature, where the activity of the different genes, constituting an individual genome, will vary, for good or bad, according to the circumstances; this conception applies almost to any acquired disease due to non-hospitable environments, including most infections.

If somebody would wish to put together the whole ALS pathology, embracing SALS and FALS, we would decide to look for a misfolded protein with the capacity of being infectious and able, giving the circumstances, to be transmitted from generation to generation. This is based on the observed conduct of the prion protein, which can yield sporadic and familial forms of spongiform dementia and other related diseases; notwithstanding that the recent observation of Douville et al.[56], who found human endogenous retrovirus inserts within the genome of the brains of ALS patients, opens another door for searching the cause of this devastating illness.

REFERENCES

1. Dong XX, Wang Y, Qin ZH. Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. Acta Pharmacol Sin 2009;30:379-387.
2. Kawashara Y, Kwak S. Excitotoxity and ALS: what is unique about the AMPA receptors expressed on spinal motor neurons? Amyotroph Lateral Scler Other Motor Neuron Disord 2005;6:131-144.
3. Ilieva EV, Ayala V, Jové M, et al. Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. Brain 2007;130: 3111-3123.
4. Fiszman ML, Ricart KC, Latini A, Rodriguez G, Sica RE. In vitro neurotoxic properties and excitatory aminoacid concentration in the cerebrospinal fluid of amyotrophic lateral sclerosis patients. Relationship with the degree of certainty of diagnosis. Acta Neurol Scand 2010;121:120-126.
5. Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann Neurol 1995;38:73-84.
6. Sattler R, Rothstein JD. Regulation and dysregulation of glutamate transporters. Handb Exp Pharmacol 2006;175:277-303.
7. Lin CL, Bristol LA, Jin L, et al. Aberrant RNA processing in a neurodegenerative disease: the cause of absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. Neuron 1998;20:589:602.
8. Yang Y, Gozen O, Vidensky S, Robisnon MB, Rothstein JD. Epigenetic regulation of neuron dependent induction of astroglial synaptic protein GLT1. Glia 2010;58:277-286.
9. Barber SC, Shaw PJ. Oxidative stress in ALS: key role in motor neuron injury and therapeutic target. Free Raic Biol Med 2010 48:629-641.

10. Sreedharan J. Neuronal death in amyotrophic lateral sclerosis (ALS): what can we learn from genetics? CNS Neurol Disord Drug Targets 2010;9: 259-267.
11. Corrado L, D'Alfonso S, Bergamaschi L, et al. SOD1 gene mutations in Italian patients with sporadic amyotrophic lateral sclerosis (ALS). Neuromuscul Disord 2006;16:800-804.
12. Fiszman ML, Borodinsky LN, Ricart KC, Sanz OP, Sica RE. Cu/Zn superoxide dismutase activity at different ages is sporadic amyotrophic lateral sclerosis. J Neurol Sci 1999;162:34-37.
13. Gagliardi S, Cova E, Davin A, et al. SOD1 RNA expression in sporadic amyotrophic lateral sclerosis. Neurobiol Dis 2010;39:198-203.
14. Atlante A, Gagliardi S, Minervine GM, Ciotti MT, Marra E, Calissano P. Glutamate toxicity in rat cerebellar granullae cells: a major role for xantine oxidase in oxigen radical formation. J Neurochem 1997;68:2038-2045.
15. Sasaki S, Iwata M. Mitochondrial alterations in the spinal cord of patients with sporadic amyotrophic lateral sclerosis. J Neuropathol Exp Neurol 2007;66:10-16.
16. Nakano Y, Hirayama K, Terao K. Hepatic ultra structural changes and liver dysfunction in amyotrophic lateral sclerosis. Arch Neurol 1987;44:103-104.
17. Weidemann FR, Winkler K, Kusnetsov AV, et al. Impairment of mitochondria function in skeletal muscle of patients with amyotrophic lateral sclerosis. J Neurol Sci 1998;156:65-72.
18. Curti D, Malaspina A, Facchetti G, et al. Amyotrophic lateral sclerosis: oxidative energy metabolism and calcium homeostasis in peripheral blood lymphocytes. Neurology 1996;47:1060-1064.
19. Rodríguez GE, González Deniselle MC, Gargiulo Monachelli GM, López Costa JJ, De Nicola AF, Sica RE. Morphological abnormalities in mitochondria of the skin of patients with sporadic amyotrophic lateral sclerosis. 19th World Congress of Neurology. Bangkok. 2010. Oral presentation.
20. Ilzecka J. Total antioxidant status is increased in the serum of amyotrophic lateral sclerosis patients. Scand J Clin Lab Invest 2003;63:297-302.
21. Gargiulo Monachelli G, Meyer M, Rodriguez GE, et al. Endogenous progesterone is associated to amyotrophic lateral sclerosis prognostic factors. Acta Neurol Scand 2011;123:60-67.
22. Tsuji S, Kikuchi S, Shimpo K, et al. Proteasome inhibition induces selective motor neuron death in organotypic slice cultures. J Neureosc Res 2005;82: 443-451.
23. Nishihira Y, Tan CF, Onodera O, et al. Sporadic amyotrophic lateral sclerosis: two pathological patterns shown by analysis of distribution of TDP-43 immunoreactive neuronal and glial cytoplasmatic inclusions. Acta Neuropathol 2008;116:169-182.
24. Rademakers R, Stewart H, Dejesus Hernandez M, et al. FUS gene mutation in familial and sporadic amyotrophic lateral sclerosis. Muscle Nerve 2010;42:170-176.
25. Stallings NR, Puttaparthi K, Luther CM, Burns DK, Elliot JL. Progressive motor weakness in transgenic mice expressing human TDP- 43. Neurobiol Dis 2010;40:404-414.
26. Dennis JS, Citron BA. Wobbler mice modeling motor neuron disease display elevated transactive response DNA binding protein. Neuroscience 2009;158:745-750.
27. Mackenzie IR, Rademakers R, Neumann M. TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. Lancet Neurol 2010;9: 995-1007.
28. Kirby J, Goodall EF, Smith W, et al. Broad clinical phenotypes associated with TAR- DNA binding protein (TARDBP) mutations in amyotrophic lateral sclerosis. Neurogenetics 2010;11:217-225.
29. Baumer D, Hilton D, Paine SM, et al. Juvenile ALS with basophilic inclusions in a FUS proteinopathy with FUS mutations. Neurology 2010;75:611-618.
30. Corrado L, Del Bo R, Castellotti B, et al. Mutations of FUS gene in sporadic amyotrophic lateral sclerosis. J Med Genet 2010;47:190-194.
31. Burrel JR, Hodges JR. From FUS to Fibs: what's new in frontotemporal dementia. J Alzheimer's Dis 2010;21:349-360.
32. Keizman D, Rogowski O, Berlines S, et al. Low grade systemic inflammation in patients with amyotrophic lateral sclerosis. Acta Neurol Scand 2009;119:383-389.
33. Henkel JS, Beers DR, Zhao W, Appel SH. Microglia in ALS: the good, the bad and the resting. J Neuroimmune Pharmacol 2009;4:389-398.
34. Hotchkiss RS, Strasser A, Mc Dunn JE, Swandon PE. Mechanisms of disease: cell death. N Eng J Med 2009;361:1570-1583.
35. Bongaerts GP. What of apoptosis is important: the decay process or the causative origin? Med Hypothesis 2008;70:482-487.
36. Gonzalez Deniselle MC, Lopez Costa JJ, Saavedra JP, et al. Progesterone neuroprotection in the wobbler mouse, a genetic model of spinal cord motor neuron disease. Neurobiol Dis 2002;11:457-468.
37. Cherra SJ, Dagda RK, Chu CT. Review: autophagy and neurodegeneration: survival at a cost? Neuropathol Appl Neurobiol 2010;36:125-132.
38. Morahan JM, Yu B, Trent RJ, Panphlett R. A genome wide analysis of brain DNA methylation identifies new candidadte genes for sporadic amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disor 2009;10:418-429.
39. Maruyama H, Morino H, Ito H, et al. Mutations of optineurin in amyotrophic lateral sclerosis. Nature 2010;465:223-226.
40. Coppede F, Migheli F, Lo Gerfo A, et al. Association study between XRCC1 gene polymorphisms and sporadic amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2010;11:122-124.
41. Ravits J, Paul P, Jorg C. Focality of upper and lower motor neuron degeneration at the clinical onset of ALS. Neurology 2007;68:1571-1575.
42. Ravits J, Laurie P, Fan Y, Moore DH. Implacations of ALS focality. Neurology 2007;68:1576-1582.
43. Gargiulo Monachelli GM, Janota F, Bettini M, et al. Regional spread pattern affects prognosis inpatient with amyotrophic lateral sclerosis. Accepted to be presented as poster presention in the 63° AAN (number P 06214) Hawaii, USA, April 9-16, 2011.
44. Havet V. Neuropsychological assessment in amyotrophic lateral sclerosis. Rev Neurol 2006;162(Suppl 4):S151-S157.
45. Ogata K, Tobimatsu F, Furuya H, Kira J. Sporadic amyotrophy lateral sclerosis showing abnormal evoked potentials. Kukuoka Igaku Zasshi 2001;92: 242-250.
46. Vandenberghe N, Leveque N, Corcia P, et al. Cerebrospinal fluid detection of enterovirus genome in ALS: a study of 242 patients and 354 controls. Amyotroph Lateral Scler Other Motor Neuron Disord 2010;11:277-282.
47. Cermelli C, Vinceti M, Beretti F, et al. Risk of sporadic amyotrophic lateral sclerosis associated with seropositivity for herpes viruses and echovirus-7. Eur J Epidemiol 2003;18:123-127.
48. Regueiro M, Bettini M, Godriguez G, et al. Estudio exploratorio de la posible etiología viral de esclerosis lateral amiotrófica. XXX Reunión Científica Anual. Sociedad Argentina de Virología. Villa Giardino, Córdoba, Argentina. December 8-12, 2010.
49. Bode L, Ludwig H. Borna disease virus infection, a human mental health risk. Clin Microbiol Rev 2003;16:534-545.
50. Rarazi A, Nafiss S, Amirzagar AA, Ayatollahi P, Solatandeh A. Association of sporadic amyotrophic lateral sclerosis with human T lymphotropic virus 1, human immunodeficiency virus a human herpes virus 8. Ata Med Iran 2006; 44:256-258.
51. Akhvlediani T, Kvirkvelia N, Shakarishvili R, Tsertsvadze T. ALS like syndrome in a patient with chronic hepatitis C. Georgian Med News 2009;172/173: 70-72.
52. Mac Gowan DJ, Scelsa SN, Imperato TE, Liu KN, Baron P, Polsky B. A controlled study of reverse transcriptase in serum and CSF of HIV-negative patients with ALS. Neurology 2007;68:1944-1946.
53. Mc Cormick AL, Brown RH, Cudkowicz ME, Al Chabali A, Garson JA. Quantification of reverse transcriptase in ALS and elimination of a novel retroviral candidate. Neurology 2008;70:278-283.
54. Moulignier A, Moulonguet A, Pialoux G, Rozenbaum W. Reversible ALS-like disorder in HIV infection. Neurology 2001;57:995-1001.
55. Zil'ber LA, Bajdakova ZL, Gardas'jan AN, Konovalov NV, Bunina TL, Barabadze EM. Study of the etiology of amyotrophic lateral sclerosis. Bull WHO 1963;29:449-456.
56. Douville R, Liu J, Rothstein J, Nath A. Identification of active loci of a human endogenous retrovirus in neurons of patients with amyotrophic lateral sclerosis. Ann Neurol 2011;69:141-151.
57. Haydon PG, Carmignoto G. Astrocyte control of synaptic transmission and neurovascular coupling. Physiol Rev 2005;86:1009-1031.
58. Hewett JA. Determinants of regional and local diversity within the astroglial lineage of normal central nervous system. J Neurochem 2009;110: 1717-1736.
59. Churchill MJ, Wesselingh SL, Cowley D, Mc Arthur JC, Brew BJ. Extensive astrocyte infection is prominent in human immunodeficiency virus associated dementia. Ann Neurol 2009;66:253-258.
60. Kovacs GG, Budka H. Distribution of apoptosis related proteins in sporadic Creutzfeldt-Jakob disease. Brain Res 2010;1323:192-199.
61. Ralowska J, Dziewulska D, Gadamski R, Chrzanowska H, Modrzewska-Leczuk M, Grieb P. Is the spinal cord motoneuron exclusively a target in ALS? Comparison between astroglial reactivity in a rat model of familial ALS and in human sporadic ALS cases. Neurol Res 2011;(Epub ahead of print).
62. González Deniselle MC, Lavista Llanos S, Ferrini M, Lima A, Roldán A, De Nicola AF. In vitro differences between astrocytes of control and Wobbler mice spinal cord. Neurochem Res 1999;24:1535-1541.