Regulatory T Lymphocytes in Amyotrophic Lateral Sclerosis: Emerging Evidence to Support Adaptive Immunotherapy?

Sara Rashid Chehreh Bragh[1,2], Farzaneh Rahmani[3,4], and Nima Rezaei[1,4,5]

[1] Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[2] NeuroImmunology Research Association (NIRA), Universal Scientific Education and Research Network (USERN), Tehran, Iran

[3] NeuroImaging Network (NIN), Universal Scientific Education and Research Network (USERN), Tehran, Iran

[4] Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

[5] Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran

Received: 10 Dec. 2017; Accepted: 25 Jan. 2018

Amyotrophic lateral sclerosis (ALS) remains to be one poorly understood neurodegenerative disorder, both as a result of the significant delay between onset of symptoms and establishment of diagnosis, impeding validation of proposed pathomechanisms in early preclinical stages of ALS, and as a result of the seemingly complex constellation of factors that have a part in ALS pathogenesis (1). Hall mark pathological feature of ALS is death of upper, i.e. cortical, and lower i.e. spinal motor, neurons. Chronological spread of neuronal death form cervical/bulbar motor neurons to lumbar spinal motor neuros to upper motor neurons, closely follows propagation of disease pathology through different stages of ALS (2).

There is a strong role suggested for mitochondrial dysfunction and oxidative stress, in ALS pathology, supported by in vitro evidence from copper/zinc superoxide dismutase 1 (SOD-1) knockout mice models of the disease (3), and for glutamate excitotoxicity due to astrocyte dysfunction. Ca2+ ions accumulate in synaptic space as a result of excitotoxicity and reduced uptake due to mitochondrial dysfunction, commencing Ca2+ mediated apoptotic pathways and aggravating intracellular oxidative stress (4). This is superimposed by oxidative stress induced by misfolded protein response to intracellular aggregates of mutant SOD1. These all culminate in axonal accumulation of neurofilaments, impeding axonal transport and perpetuating neuronal apoptosis.

Besides the above evidence, supporting a role for neuron-neuron and intraneuronal events as primary culprit for motor neuron death in ALS, emerging evidence also support "non-cell autonomous mechanisms" of ALS pathogenesis mediated by astrocytes, microglia and other glial cells. Studies using mouse model of mutant SOD1 (mSOD1) have deciphered important findings that comprise basic concepts of this model. SOD1 mutation in neither motor neurons, nor astrocytes or microglia alone is not sufficient to reproduce ALS, while selective silencing of expression of mutated SOD1 in microglia only, is able to delay the onset and impede progression of ALS in mice expressing mutant SOD1 in all other cell types (5). Cornerstone notion of this model is that ALS is a neurodegenerative disorder of "primary glial origin" with secondary involvement of motor neurons in neuronal toxicity.

Activated microglia, predominantly in M2 phenotype, infiltrate lumbar spinal cord along with infiltration of T-cells with dominant Th2 response resulting in a local cytokine network in favor of production of nerve growth factors like BDNF, reduced levels of oxidative compounds and increased levels of IL-4 expression. These events precede the emergence of first motor symptoms and are identified in the absence of any pathological evidence of motor neuron death in mSOD1 mice (6). Along with cranial progression of the pathology, CD3+ lymphocyte staining significantly increases in the lumbar cord, with most lymphocytes adopting a Th1 phenotype, microglia transform into M1 phenotype, and local production of neuroprotective and anti-inflammatory factors drop in lumbar spinal cord, concordant with the onset of muscle weakness in hind limbs and motor neuron death. This is when footsteps of neuronal insult first appear in cervical spinal cord, demonstrated by CD68 and CD11c expression i.e. microglial activation, expression of transcription factor gata-3 mRNA, i.e. activation of Th2 response and the balance of overall local immune response in favor of neuroprotection over neurotoxicity (6). In consort with

Corresponding Author: N. Rezaei
Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 6657 6573, Fax: +98 21 6692 3054, E-mail address: rezaei_nima@tums.ac.ir

this, FOXP3, the crucial transcription factor of regulatory T-cells reaches highest level of local expression along with peak levels of neuroprotective factors in stable/early phase of ALS and reciprocally declines during rapidly progressive stage of ALS (7).

These evidences are compatible with the two-phase theory for progression of ALS, supporting the protective role of Th2 lymphocytes, regulatory T cells, IL-4, and M2 microglia in the slowly progressive stage of ALS.

Having known that expression of FOXP3 mirrors progression of disease pathology and that lower baseline FOXP3 expression predicts more rapid progression of ALS, researchers have tried to restore the neuroprotective milieu of stable phase of ALS through passive transfer of CD4+and regulatory T cells from mice in early phase of ALS, to mice inherently lacking T lymphocytes i.e. mSOD1/RAG2 -/-, prior to onset of ALS symptoms (8). Passive vaccination of mice with endogenous CD4+ helper and regulatory T-cells significantly delayed the onset of ALS and prolonged animals survival. Interestingly, this effect was more significant for adaptive transfer of regulatory T-cells and led the same group to perform the primary research to prove the efficacy of the ex-vivo use of regulatory T-cells from ALS patients to suppress effector T-cells and reproduce sustained IL-4 and Th2 activity as observed in the early phase of ALS. Beers and his colleagues reported successful ex-vivo activation of regulatory T-cells using IL-2 and rapamycin, restoring their ability to suppress endogenous effector T-cells from patients with ALS (9).

Use of mutated SOD1 as a target for adaptive immunotherapy or target for ALS vaccination has gained momentum in the past decade. Promising evidence was produced after identification of a homology between the P2X4 immunizing peptide and part of the mutant motif of mSOD1 (10). Next, AJ10, an antibody against misfolded mSOD1, was used for passive immunization of mice with stable ALS (11). Nonetheless, this trial failed to produce evidence of disease regression, and the introduction of AJ10 even exacerbated mSOD1 accumulation in the spinal cord, conferring more rapid clinical evolution of mice receiving vaccine compared to their peers. This was while microglial activation was enhanced with vaccination, but apparently in a neurotoxic manner. The authors suggested failure of co-activation of T-cell regulatory response by AJ10 as the possible mechanism of this failure, further stating that co-activation of regulatory T-cells by antigen copolymer-1 might serve as game changer to induce regulatory properties in microglia, during passive immunization. Cop-1, Copaxone, vaccination has already proved useful in mSOD1 mouse models, prolonging animals life span and boosting local T-cell mediated immune response in favor of self-reacting T cells (12). Copaxone is believed to expand regulatory T-cells population following immunization in patients with multiple sclerosis, even maintaining the immunomodulatory effects years after termination of treatment (13). Meanwhile, Cop-1 exerts its immunoreuglatory activity in ALS, in a more complicated manner than mere activation of regulatory T lymphocytes, given the fact that Cop-1-reactive T cells are more likely to expand with high IFN-γ levels. Instead, it is believed result in activation of a wide array of T-cell populations, namely nonencephalitogenic self-reacting T cells. This results in an increase in the number of resident self-reactive T-cells is in favor of tissue maintenance conferring neuroprotection in the early phase of mouse models of ALS.

Enhancing the number of regulatory T cells and their anti-inflammatory traits might bear therapeutic benefits in terms of slowing the rate of disease progression in ALS. The above findings, although promising, have hardly reached a consensus of the efficacy of immunotherapy and vaccination in ALS. Adaptive transfer of activated regulatory T-cell appears to be promising, while failure to recruit patients in advanced stage ALS, questions reliability of the above mentioned clinical trials.

Considering the aforementioned health measures in the future studies may well lead to the establishment of new modalities to delay the pace of aging. It is also noticeable that the physicalistic, comparative interpretations of Shifa (the Book of Healing) be born in mind when trying to in detail elucidate Avicenna's strategies of how to deal with body weariness specifically in the context of old age.

References

1. de Carvalho M, Swash M. Can selection of rapidly progressing patients shorten clinical trials in amyotrophic lateral sclerosis? Arch Neurol 2006;63:557-60.
2. Kanouchi T, Ohkubo T, Yokota T. Can regional spreading of amyotrophic lateral sclerosis motor symptoms be explained by prion-like propagation? J Neurol Neurosurg Psychiatry 2012;83:739-45.
3. Bonafede R, Mariotti R. ALS Pathogenesis and Therapeutic Approaches: The Role of Mesenchymal Stem Cells and Extracellular Vesicles. Front Cell Neurosci 2017;11:80.

4. Martin LJ. Mitochondrial pathobiology in ALS. J Bioenerg Biomembr 2011;43:569-79.
5. Lobsiger CS, Cleveland DW. Glial cells as intrinsic components of non-cell autonomous neurodegenerative disease. Nat Neurosci 2007;10:1355-60.
6. Lewis KE, Rasmussen AL, Bennett W, King A, West AK, Chung RS, et al. Microglia and motor neurons during disease progression in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis: changes in arginase1 and inducible nitric oxide synthase. J Neuroinflammation 2014;11:55.
7. Beers DR, Henkel JS, Zhao W, Wang J, Appel SH. CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. Proc Natl Acad Sci U S A 2008;105:15558-63.
8. Beers DR, Henkel JS, Zhao W, Wang J, Huang A, Wen S, et al. Endogenous regulatory T lymphocytes ameliorate amyotrophic lateral sclerosis in mice and correlate with disease progression in patients with amyotrophic lateral sclerosis. Brain 2011;134:1293-314.
9. Beers DR, Zhao W, Wang J, Zhang X, Wen S, Neal D, et al. ALS patients' regulatory T lymphocytes are dysfunctional, and correlate with disease progression rate and severity. JCI Insight. 2017;2:e89530.
10. Sabado J, Casanovas A, Hernandez S, Piedrafita L, Hereu M, Esquerda JE. Immunodetection of disease-associated conformers of mutant cu/zn superoxide dismutase 1 selectively expressed in degenerating neurons in amyotrophic lateral sclerosis. J Neuropathol Exp Neurol 2013;72:646-61.
11. Sabado J, Casanovas A, Rodrigo H, Arque G, Esquerda JE. Adverse effects of a SOD1-peptide immunotherapy on SOD1 G93A mouse slow model of amyotrophic lateral sclerosis. Neuroscience 2015;310:38-50.
12. Angelov DN, Waibel S, Guntinas-Lichius O, Lenzen M, Neiss WF, Tomov TL, et al. Therapeutic vaccine for acute and chronic motor neuron diseases: Implications for amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 2003;100:4790-5.
13. Spadaro M, Montarolo F, Perga S, Martire S, Brescia F, Malucchi S, et al. Biological activity of glatiramer acetate on Treg and anti-inflammatory monocytes persists for more than 10years in responder multiple sclerosis patients. Clin Immunol 2017;181:83-88.