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Is Pre-Symptomatic Immunosuppression Protective in *CSF1R*-Related Leukoencephalopathy?

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Adult-onset leukoencephalopathy with spheroids and pigmented glia (ALSP) is the collective disease referring to patients previously diagnosed with hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS) or pigmented orthochromatic leukodystrophy (POLD). ALSP is a white matter disease with a complex neurological phenotype consisting of pyramidal tract dysfunction, parkinsonism, and frontal-predominant cognitive impairment.¹ This autosomal-dominant disease typically affects females earlier than males with symptom onset in the fourth decade of life and average disease duration of 7 years.² In 2011, a mutation in the colony-stimulating factor 1 receptor (*CSF1R*) gene was first identified in the initial collection of HDLS families.³ Two separate POLD families were later found to carry *CSF1R* mutations, therefore confirming that HDLS and POLD were actually a single *CSF1R*-related leukoencephalopathy.¹ There are now more than 70 different pathogenic mutations, most of which occur in the tyrosine kinase domain resulting in disruption of protein function.⁴ The disease is currently known as *CSF1R*-related leukoencephalopathy to distinguish it from a somewhat similar condition produced by mutations in the *AARS2* gene⁵ or other unknown genetic causes. For the genetic or sporadic cases of unknown cause, the nomenclature of ALSP-gene negative seems to be appropriate for now. The CSF-1R protein is mainly expressed in microglia leading to the disease designation of microgliopathy. Recent studies from a mouse model of ALSP strongly support this designation.⁶

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(1) Research Project: A. Conception, B. Organization, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript Preparation: A. Writing the First Draft, B. Review and Critique.

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Role of the CSF-1R in Microglia

The CSF-1R protein is a class III receptor tyrosine kinase that is primarily expressed in mononuclear phagocytic cells, including all microglia.⁷ The receptor is activated by the binding of either of its ligands, the homodimeric growth factors, CSF-1 and interleukin-34.⁸ CSF-1R activation leads to autophosphorylation of intracellular domain tyrosines, creating binding sites for downstream intermediates that regulate the survival, proliferation, differentiation, and function of mononuclear phagocytes.^{8–10} CSF-1R expression is absolutely required for the development and maintenance of all microglia.¹¹ *Csf1r*^{−/−} nullizygous mice and humans bearing homozygous *CSF1R* mutations exhibit perinatal lethality and their brains have gross anatomical and histological abnormalities in the same areas (eg, the olfactory bulb, cortex, and corpus callosum).^{3,12–15} CSF1R inhibition in adult mice virtually eliminates rodent central nervous system (CNS) microglial populations.¹⁶ Activation of the CSF1R also promotes trophic functions of microglia¹⁷ and macrophages.¹⁸ This effect is balanced by the CSF-2 biochemical cascade, which facilitates pro-inflammatory responses in macrophages.¹⁰ Similarly, in microglia, CSF-2 increases phagocytosis of unopsonized myelin,¹⁹ production of interleukin (IL)-6, and the ability to present antigen in the absence of additional inflammatory stimulation.²⁰ Therefore, unlike CSF-1R and its ligands that promote trophic functions, elevation of CSF-2 in the brain may trigger demyelination and neurotoxicity.

Mechanistic Insights from the *Csf1r*^{+/−} Mouse Model of ALSP

Consistent with a case of ALSP in a patient lacking expression of one *CSF1R* allele,⁷ *Csf1r*^{+/−} mice develop an ALSP-like disease.²¹ Similar to ALSP patients, these mice exhibit behavioral, radiological, histopathological, and ultrastructural alterations associated with microgliosis and demyelination. Unlike mouse models of several other neurodegenerative diseases, there is no increase in leukocyte infiltration in the brains of affected *Csf1r*^{+/−} mice compared to wild-type controls²² and microglial lineage-specific *Csf1r* heterozygosity is sufficient for disease development.⁶ These findings support the notion that ALSP is a primary microgliopathy.⁴

Gene expression studies in the mouse model indicate that *Csf1r*^{+/−} microglia do not possess an overt inflammatory phenotype, but rather a maladaptive state indicative of a pro-oxidant and demyelinating phenotype.²² The expression of *Csf2*, encoding a pro-inflammatory microglial mitogen²³ is elevated in the brains of both human ALSP patients and *Csf1r*^{+/−} mice²² and studies in mice suggest a significant contribution of CSF-2 to microglial dyshomeostasis and ensuing pathology.^{6,21,22} Therefore, attenuation of CSF-2 signaling might be beneficial in ALSP.

Effect of Steroids on Microglial Function

A properly functioning inflammatory system is critically important for maintaining and defending the CNS. Maladaptive immune-mediated processes may result in a plethora of inflammatory and autoimmune diseases. Glucocorticoids (GC) are an effective means of treating these diseases, such as multiple sclerosis (MS) or any number of antibody-

mediated autoimmune limbic encephalidities. GCs appear to exert their anti-inflammatory effect through a number of different mechanisms. GCs downregulate pro-inflammatory cytokines and adhesion molecules necessary for T cells to penetrate the blood-brain-barrier, promote immune cell apoptosis, and directly inhibit T cell activation and other inflammatory mediators.²⁴

Although T cells are the target of most immunotherapy in MS, macrophages play an important role for innate immune responses and facilitate adaptive immune responses as antigen-presenting cells.²⁵ This contributing role of macrophages prompted questions regarding a potential effect of GC on monocyte-derived macrophages, which are a major cell type involved in reactivation of infiltrating T cells.²⁶ Recent work demonstrated the effects of methylprednisolone in patients with MS. The investigators found that methylprednisolone not only caused a shift in macrophage gene expression profiles toward an anti-inflammatory phenotype, but also enhanced monocyte chemotaxis toward CCL2, CCL5, and CX3CL1.²⁷ Together, these findings suggest that GC exposure polarizes monocytes toward an anti-inflammatory phenotype and promote their migration into the area of active CNS inflammation. This effect may also partly explain why GCs are also effective at treating other CNS immune-mediated conditions.

Although evidence from the mouse ALSP model indicates an absence of leukocytic infiltration, studies in mouse models indicate that GC have anti-inflammatory effects on microglia. Early in vitro studies have shown that the microglia express GC receptors, and that GC and CSF-2 act in an antagonistic fashion in microglia. CSF-2 lowers the expression of the GC receptor and GC, acting via the GC receptor, inhibit the action of CSF-2 in increasing microglial ramification.²⁸ Furthermore, studies in Parkinson's disease models indicate that microglial GC receptor expression plays an important anti-inflammatory role.^{29,30} These studies raise the possibility that GC might counteract the contribution of CSF-2 to microglia dyshomeostasis in ALSP.

Illustrative Case

We evaluated a 43-year-old woman with progressive imbalance and memory difficulties of 3 years duration. Despite no family history of neurologic disease, genetic testing revealed a mutation in the *CSF1R* gene (p.M875I, c.2625G > A). A MRI of the brain showed frontal predominant white matter T₂ sequence hyper-intensities suggestive of leukoencephalopathy accompanied by mild bifrontal cortical atrophy (Fig. 1). She was diagnosed with *CSF1R*-related leukoencephalopathy.

Genetic testing of the patient's parents revealed that her mother is a carrier of the same *CSF1R* mutation. The patient's mother had a history of surgically treated right carpal tunnel syndrome, right ulnar nerve transposition surgery and rheumatoid arthritis but no other neurologic disease. From age 46 to the time of our evaluation at age 71 years, her rheumatoid arthritis was treated with various immunosuppressive combinations of prednisone, hydroxychloroquine, methotrexate, and adalimumab (Fig. 2). Our examination revealed atrophy of bilateral intrinsic hand muscles with symmetric hyperreflexia involving lower more than upper extremities without extensor plantar responses or ankle clonus. She

was unable to perform tandem walking and postural reflexes were impaired. Sensation was normal to all modalities, ataxia was absent, and she had subtle hypertonia in the legs. Neuropsychological testing revealed a normal cognitive profile with subtle visuospatial inefficiency. She also had skin atrophy with several bruises and at least one small site of active bleeding. This was felt to be related to prolonged steroid use. At age 71, her brain MRI revealed no features of leukoencephalopathy (Fig. 1).

Is Early Chronic Steroid Use Protective in Asymptomatic Carriers of *CSF1R* Mutations?

ALSP is caused by autosomal dominant mutations in *CSF1R* resulting in a partial loss of function in CSF-1R signaling. Analysis of the gene expression in microglia isolated from affected *Csf1r*^{+/-} mice indicate a maladaptive phenotype.²² Similarly, alterations in the molecular signature of microglia in ALSP patients are evidenced by reduced expression of ionized calcium-binding adaptor molecule 1 (IBA-1) and P2RY12 in white and gray matter, increased expression of CD68 and CD163 in affected white matter and reduced expression of P2RY12, CX3CR1 and CSF1R in affected white matter.³¹ Furthermore, the elevation of *CSF-2* and expression of several markers of oxidative stress, altered cholesterol metabolism and demyelination observed in the mouse model also occur in ALSP patients.²² These results indicate that decreased CSF-1R function combined with increased CSF-2 signaling contribute to the loss of homeostatic functions in both human and mouse microglia.

Although asymptomatic carriers have been reported,^{32,33} the disease penetrance of *CSF1R* mutation carriers is age dependent with 95% penetrance by age 60.² We hypothesize that the clinical manifestations of *CSF1R*-related leukoencephalopathy were minimized by chronic immunosuppression in our patient's mother. Adalimumab acts by neutralizing tumor necrosis factor alpha (TNF- α), hydroxychloroquine inhibits lysosomal function and methotrexate inhibits dihydrofolate reductase to disrupt nucleic acid synthesis. These medications may have played a role in reduction of inflammatory mediators in the process, but the broader effects of glucocorticoid activity and the continuous administration may be of more importance.

Prednisone is a glucocorticoid, similar to methylprednisolone and is expected to interfere with immune function, as described earlier. In particular, the studies summarized above, indicating GC-attenuated CSF-2 signaling in microglia,²⁸ are consistent with a beneficial effect of GC in ALSP. Therefore, we postulate that the long-term prednisone treatment of our patient's mother resulted in significant correction of the maladaptive microglial phenotype and reapproximation of the normal physiologic balance between CSF-1R and CSF-2R signaling.

Our findings merit study of this mechanism in mouse models, which are currently in development. Proof of this mechanism could significantly reduce, or even prevent symptoms related to *CSF1R*-related leukoencephalopathy, which is a rapidly fatal disease for which there are no proven therapies. There are several reports of symptomatic patients who have experienced disease stabilization with hematopoietic stem cell transplantation (HSCT).³⁴⁻³⁶ Unfortunately, many of these patients already have significant impairment at the time of

transplantation. It has been suggested that HSCT in pre-symptomatic *CSF1R* mutation carriers could prevent symptoms from developing. However, the potential for complications related to HSCT raises questions as to whether this should be attempted particularly in more advanced stages of the illness. Based on previous study, hematopoietic stem cells may gain access to the CNS only when the blood-brain-barrier is disrupted because of stress.^{37,38} This may mean that HSCT effectiveness in the symptomatic disease stage does not translate into effectiveness in the preclinical stage. Presymptomatic immunotherapy is certainly not free of the potential for adverse events, however, steroid-related side effects may prove relatively mild compared to the certainties of *CSF1R*-related leukoencephalopathy. Moreover, proof of this mechanism could help inform steroid-sparing therapy options that would minimize side-effects related to chronic steroid therapy.

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FIG. 1.

Top row: MRI (fluid attenuated inversion recovery) FLAIR sequence axial (**A**), T₁-weighted sagittal (**B**) and coronal (**C**) images of a 43-year-old patient with CSF1R-related leukoencephalopathy. Bottom row: FLAIR sequence axial (**D**), T₁-weighted sagittal (**E**) and coronal (**F**) images of the patient's mother at 71 years of age. There is mild biparietal, and to a lesser extent, bifrontal cortical atrophy.

FIG. 2.

Illustration of immunosuppressive therapy administered to a 71-year-old carrier of a pathogenic mutation in the *CSF1R* gene.