Changes in gene expression that occur across the central nervous system (CNS) during neurological diseases do not address the heterogeneity of cell types from one CNS region to another and are complicated by alterations in cellular composition during disease. Multiple sclerosis (MS) is multifocal by definition. Here, a cell-specific and region-specific transcriptomics approach was used to determine gene expression changes in astrocytes in the most widely used MS model, experimental autoimmune encephalomyelitis (EAE). Astrocyte-specific RNAs from various neuroanatomic regions were attained using RiboTag technology. Sequencing and bioinformatics analyses showed that EAE-induced gene expression changes differed between neuroanatomic regions when comparing astrocytes from spinal cord, cerebellum, cerebral cortex, and hippocampus. The top gene pathways that were changed in astrocytes from spinal cord during chronic EAE involved decreases in expression of cholesterol synthesis genes while immune pathway gene expression in astrocytes was increased. Optic nerve from EAE and optic chiasm from MS also showed decreased cholesterol synthesis gene expression. The potential role of cholesterol synthesized by astrocytes during EAE and MS is discussed. Together, this provides proof-of-concept that a cell-specific and region-specific gene expression approach can provide potential treatment targets in distinct neuroanatomic regions during multifocal neurological diseases.

The biology of cell types within the central nervous system (CNS) differs between neuroanatomic regions serving different functional neurological pathways (1–3). Channel distribution, neurotransmitters, and neurotrophic factors all differ by functional neuroanatomic pathway (2), and regional differences in astrocytes (1) and oligodendrocytes (3) have been shown. Thus, molecular mechanisms underlying distinct disabilities may differ based on the neurological pathway involved. Multiple sclerosis (MS) is by definition multifocal, characterized by a variety of disabilities affecting walking, vision, cognition, and fatigue, to name a few. The incidence and severity of each disability varies from patient to patient. Accordingly, we hypothesized that a “one size fits all” treatment may not be optimal to achieve neuroprotection in MS. Rather, treatments tailored for each specific disability may be more effective. This approach is challenging since elucidation of gene expression changes in neuroanatomic regions associated with each disability would be needed.

A neuroprotective treatment optimally designed for one disability may have maximal effects on one or two disabilities but have only modest effects on other disabilities, thereby diluting out effects on commonly used standard composites of all disabilities in MS clinical trials. Accordingly, the discovery of disability-specific targets should be accompanied by disability-specific biomarkers. To this end, our group has recently used MRI to create disability-specific atlases of gray matter loss in MS, which revealed distinct localized gray matter atrophy in clinically eloquent areas aligned with different disabilities (ref. 4; see comment in ref. 5).

Previous methods of identifying new molecular targets in neurological diseases have largely entailed examining gene expression in whole CNS tissues from disease versus normal controls in preclinical models of disease. Genes overexpressed or underexpressed in disease models were then investigated in humans with disease as potential targets for new treatments. A major limitation of this approach is that CNS tissues contain multiple different cell types and that the composition of these cell types is altered during disease. For example, in MS and its preclinical model EAE, there is infiltration of immune cells, proliferation and migration of progenitors, and damage or death of oligodendrocytes and neurons. Thus, a difference in gene expression during disease may reflect in part the difference in cellular composition, confounding mechanistic insights into the effect of disease on the biology of any given CNS cell. Examination of gene expression changes in whole CNS tissue may also

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Data deposition: Datasets generated during this study have been deposited in the Gene Expression Omnibus (GEO) database, https://www.ncbi.nlm.nih.gov/geo/ [SuperSeries accession no. GSE100330] and accession nos. GSE100329 (astrocyte-specific and region-specific transcriptomes in control and EAE mice; 45 samples; paired end), GSE100294 (optic nerve astrocyte-specific transcriptomes in control and EAE mice; 8 samples; single end), and GSE100297 (human optic chiasm from healthy controls and multiple sclerosis patients; 10 samples; single end).

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EAE was induced in astrocyte RiboTag mice. Clinical 0.1 threshold for differ-
ences in gene expression in a cell-specific and region-specific manner during a multifocal neurological disease, we applied RiboTag technology (9) to the MS preclinical model, experimental autoimmune encephalomyelitis (EAE). Astrocytes were the cell of focus since the hallmark multiple sclerotic lesions are based on astrocytic scarring, and both beneficial and deleterious roles of astrocytes have been shown in neuroinflammatory diseases, including EAE and MS (1, 10–12). The RiboTag approach yields ribosomes with a tag [hemagglutinin (HA)] in a specific cell type using Cre-loxP re-
combination (9). RiboTag mice were crossed with mice of the mGFAP-Cre line 73.12 (13) to express HA-tagged ribosomal protein only in astrocytes, and then astrocyte-specific RNAs from different neuroanatomic regions were isolated and examined for changes in gene expression in astrocytes during EAE using RNA sequencing and bioinformatics analyses. Regional differences in the disease-related astrocyte transcriptome were observed, and treatment targets were discovered in EAE and confirmed in MS. Together, this represents an approach to neuroprotective treatment development in MS that is based on cell-specific and region-specific gene expression analyses.

Results

Specificity of Astrocyte mRNA Isolation. Colocalization of the HA tag specifically with astrocytes in astrocyte RiboTag mice was first shown at the protein level by immunohistochemistry (Fig. 1 A–D). Efficiency of HA colocalization to astrocytes in spinal cord at the protein level was 91.2%, as determined by the percent area of HA staining that colocalized with GFAP staining. RNA-seq analysis of astrocyte-enriched RNAs (immunoprecipi-
tated by anti-HA antibody) confirmed enrichment of astrocyte-specific gene expression, and deenrichment of neuronal, oligo-
dendrogial, and microglia-specific gene expression, shown as the log fold change calculated between spinal cord astrocyte RNA versus spinal cord total cell RNA (immunoprecipitated with control antibody, anti-RPL22) (13) (Fig. 1E).

Regional Differences in Astrocyte-Specific Gene Expression During EAE. EAE was induced in astrocyte RiboTag mice. Clinical signs of EAE began at day 11 after disease induction, and mice were killed at day 45 (mean EAE score = 3.1), as were age- and sex-matched normal controls. Comparison was made between RNAs from spinal cord astrocytes versus RNAs from spinal cord all cell types using principal component analysis (PCA) graphed three-dimensionally. Separation between spinal cord astrocyte-specific RNAs versus spinal cord total cell RNAs was observed, underscoring the difference between a cell-specific versus a whole tissue gene expression approach (Fig. 1F). This was consistent with the difference in the number of genes with signif-
ificantly [false discovery rate (FDR) < 0.1] different expression when comparing EAE versus healthy controls, with approxi-
ately twice as many significant genes from analysis of spinal cord astrocytes (n = 2,015) compared with spinal cord all cells (n = 1,168). Together, this demonstrated that many gene differ-
ces in astrocytes in spinal cord during EAE are missed using a whole tissue gene expression approach.

EAE-induced gene expression changes in astrocytes differed between neuroanatomic regions when comparing astrocytes from spinal cord, cerebellum, cerebral cortex, and hippocampus. Compared with spinal cord astrocytes during EAE, gene expression in cerebellar astrocytes was distinct, but more similar to spinal cord than cortical astrocytes and hippocampal astrocytes (Fig. 1F). This separation between astrocytes derived from various regions of the CNS was observed in both EAE and normal controls, consistent with the concept of regional heterogeneity of astrocytes in normals (1), with this being an observation of regi-
Onal differences in gene expression in astrocytes during EAE. The number of genes with significantly (FDR < 0.1) different expression was highest in spinal cord astrocytes (n = 2,015) compared with the other brain regions (cerebellum, n = 197; hippocampus, n = 49; cerebral cortex, n = 20). See Dataset S1 for the list of significant gene differences with (FDR < 0.1) in astrocytes of each region. The FDR < 0.1 threshold for differ-
entially expressed genes was based on previously published analyses of RNA-seq data from astrocyte RiboTag mice (13).

Up-Regulation of Immune Pathway Gene Expression in EAE. Canonical pathway analysis (Fig. 2 A–C and Table S1) of astrocyte-specific RNAs differentially expressed in EAE versus normal (FDR < 0.1) showed that genes involved in the antigen pre-
sentation pathway and the IFN signaling pathway were signific-
antly different in astrocytes from spinal cord (Fig. 2A) and cerebellum (Fig. 2B). The change was in the direction of an increase in gene expression in EAE compared with normal (Fig. 2D). Since antigen presentation by astrocytes in spinal cords of EAE mice has been previously characterized (14, 15), this find-
ing validated the RiboTag approach.

Down-Regulation of Cholesterol Synthesis Pathway Gene Expression in EAE. In spinal cord astrocytes, several cholesterol synthesis pathways were significantly different in EAE compared with normal, with these pathways dominating the profile of top pathways in this cell type (Fig. 2A and Table S1). In contrast to an increase in immune pathway gene expression in spinal cord astrocytes, the direction of change for cholesterol synthesis pathway gene expression was a decrease in EAE compared with normal (Fig. 2D and Table S2). Cerebellar astrocytes also showed numerous cholesterol synthesis pathways that were signif-
ically different during EAE compared with normal (Fig. 2B),
Expression of cholesterol synthesis pathway genes is decreased and of immune pathway genes is increased in spinal cord astrocytes during EAE.

Fig. 2. Expression of cholesterol synthesis pathway genes is decreased and of immune pathway genes is increased in spinal cord astrocytes during EAE. (A) Top canonical pathways are shown in spinal cord astrocytes (Sc-astr), (B) cerebellar astrocytes (Cb-astr), and (C) spinal cord all cells (Sc-all), each in descending order of significance. Astrocyte-specific RNAs differentially expressed in EAE versus normal (FDR < 0.1) showed that genes involved in several cholesterol synthesis pathways were significantly enriched in spinal cord and cerebellum (Table S1), with gene expression decreased in EAE (Tables S2 and S4). The antigen presentation pathway and IFN signaling pathway were also significantly enriched in both regions (Table S1), with gene expression increased in EAE (Table S4). Several pathways enriched in spinal cord astrocytes (Sc-astr) were not detectable in spinal cord all cells (Sc-all), n = 9 mice (5 EAE, 4 normal). Full names of pathways are listed in Table S1. (D) Heat map of direction of change of genes in pathways. A heat map of cholesterol synthesis and immune pathway genes that were significantly different between EAE and normal (NL) in astrocyte-specific RNAs (FDR < 0.1) revealed that cholesterol synthesis pathway genes were decreased in EAE (green) while immune pathway genes were increased (red). Green color represents lower expression, and red color represents higher expression. n = 9 mice (5 EAE, 4 normal). Significant differences in gene expression in spinal cord astrocytes were confirmed in another independent set of astrocyte RiboTag mice using RNA-seq analysis with n = 10 mice (5 EAE, 5 normal).

Focusing on White Matter Astrocytes: Optic Neuritis. Regional differences in astrocyte gene expression during disease revealed that genes in cholesterol synthesis pathways were decreased more in spinal cord and cerebellum than in hippocampus and cerebral cortex (Table S2), despite the fact that hippocampus and cerebral cortex each have significant pathology during chronic EAE, including an increase in reactive astrocytes (16–21). The relative abundance of white matter in the first two regions compared with the latter two regions suggested that astrocytes from white matter might drive the observed changes in cholesterol synthesis gene expression during EAE. Since the anterior visual pathway is known to be affected by optic neuritis during EAE and contains only white matter, the astrocyte transcriptome of optic nerve during EAE was determined. First, colocalization of the HA label with GFAP in astrocytes of optic nerve during EAE was determined. Then, to assess the enrichment of astrocyte-specific mRNA transcripts from optic nerve, qPCR analyses of cell-specific gene expression confirmed enrichment of astrocyte-specific gene expression and deenrichment of oligodendroglial, microglial, and axonal-specific gene expression (Fig. S4E). Then, to assess the enrichment of astrocyte-specific mRNA transcripts from optic nerve, qPCR analyses of cell-specific gene expression confirmed enrichment of astrocyte-specific gene expression and deenrichment of oligodendroglial, microglial, and axonal-specific gene expression (Fig. S4E).

Similar to astrocytes from spinal cord (Fig. S4A), astrocytes from optic nerve during EAE showed a difference compared with normal in several cholesterol synthesis pathways (Fig. 4A).
and Table S3). The direction of the change in cholesterol synthesis pathway genes was decreased in EAE compared with normal as shown in the heat map (Fig. 4B). This change in gene expression in astrocytes of optic nerve by RNA-seq was then confirmed in an independent set of RiboTag EAE mice where gene expression was assessed by qPCR. Hmgcs1 and Fdft1, two cholesterol synthesis genes in optic nerve that were found to be significantly down-regulated during EAE by RNA-seq, were confirmed to be decreased in EAE by qPCR (Fig. 5A). Quantification of expression in astrocytes in WT mice at the protein level also showed a decrease during EAE (Fig. 5 B and C). Immunohistochemistry showed colocalization of HMGCS1 and FDFT1 with astrocytes in optic nerve (Fig. 5D and Fig. S5). FDDS was not decreased in optic nerve during EAE as shown by RNA-seq analysis and thus was not further assessed by qPCR and immunohistochemistry.

Regarding inflammatory pathways in astrocytes in optic nerve during EAE, astrocytes from optic nerve showed an increase in cholesterol synthesis gene expression in astrocytes in spinal cord and cerebellum, and optic nerve suggested a novel target for treatment in EAE, namely cholesterol homeostasis. During adulthood, astrocytes are the main CNS cells producing cholesterol, with transport via apolipoprotein E (apoE) to neurons to make membranes and synapses and to oligodendrocytes to make myelin (25). We hypothesized that decreased cholesterol synthesis in astrocytes during EAE could lead to decreased cholesterol transport. Thus, we investigated CS-6253, an agonist for ATP-binding cassette transporter A1 (ABCA1) that is known to increase efflux of cholesterol to extracellular apoE (26, 27). Since it was not known whether treatment with CS-6253 could affect cholesterol synthesis gene expression, astrocyte-RiboTag mice that either had EAE or were normal were treated with either CS-6253 or vehicle, and qPCR determined if CS-6253 treatment affected cholesterol synthesis gene expression in astrocytes in spinal cords. Significant increases in Hmgcs1, Fdps, and Fdft1 were observed in spinal cords of CS-6253–treated EAE mice and also in normal controls (Fig. 6A). Immunohistochemistry showed increased expression of HMGCS1, FDFS, and FDFT1 at the protein level in CS-6253–treated versus vehicle-treated WT EAE mice, with CS-6253–treated EAE mice demonstrating levels as high as those in normal mice (Fig. 6B). Expression was principally in astrocyte cell bodies, but also in astrocyte processes (Fig. 6C).

Changes in Astrocyte Gene Expression in Early Versus Late EAE. While our focus was on astrocytes during late EAE, we next asked whether gene expression changes in astrocytes that were observed late were also observed earlier during EAE. Using qPCR for mRNAs from an additional set of astrocyte RiboTag mice, we found that cholesterol synthesis gene expression was not significantly decreased early during EAE (day 17) but was decreased late during EAE (day 50), in both spinal cord and optic nerve, with each confirmed at the protein level by immunohistochemistry in WT mice (Fig. S6A, B, D, and E). Expression of MHC class II of the antigen presentation pathway in astrocytes was markedly increased in early EAE and then decreased late, albeit still higher than normal (Fig. S6C). Early immune activation, with some persistence late, is consistent with previous literature showing that immune responses in the CNS during EAE are not quiescent in chronic EAE in the C57BL/6 model (22–24). Interestingly, optic nerve astrocytes showed an increase in MHC class II early, and this did not decrease late (Fig. S6F).

Targeting Cholesterol Homeostasis as a Treatment for EAE. The astrocyte-specific molecular signature of decreased cholesterol synthesis pathways in EAE spinal cord, cerebellum, and optic nerve suggested a novel target for treatment in EAE, namely cholesterol homeostasis. During adulthood, astrocytes are the main CNS cells producing cholesterol, with transport via apolipoprotein E (apoE) to neurons to make membranes and synapses and to oligodendrocytes to make myelin (25). We hypothesized that decreased cholesterol synthesis in astrocytes during EAE could lead to decreased cholesterol transport. Thus, we investigated CS-6253, an agonist for ATP-binding cassette transporter A1 (ABCA1) that is known to increase efflux of cholesterol to extracellular apoE (26, 27). Since it was not known whether treatment with CS-6253 could affect cholesterol synthesis gene expression, astrocyte-RiboTag mice that either had EAE or were normal were treated with either CS-6253 or vehicle, and qPCR determined if CS-6253 treatment affected cholesterol synthesis gene expression in astrocytes in spinal cords. Significant increases in Hmgcs1, Fdps, and Fdft1 were observed in spinal cords of CS-6253–treated EAE mice and also in normal controls (Fig. 6A). Immunohistochemistry showed increased expression of HMGCS1, FDFS, and FDFT1 at the protein level in CS-6253–treated versus vehicle-treated WT EAE mice, with CS-6253–treated EAE mice demonstrating levels as high as those in normal mice (Fig. 6B). Expression was principally in astrocyte cell bodies, but also in astrocyte processes (Fig. 6C).

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Finally, CS-6253–treated EAE mice had less severe EAE disability compared with vehicle-treated, as shown by motor function using standard EAE walking scores (Fig. 6D) and rotarod testing performance (Fig. 6E).

**Translation to Multiple Sclerosis.** Translating our findings in optic neuritis in EAE to humans, optic neuritis is common in MS. Therefore, gene expression analyses of white matter tracts of the anterior visual pathway in MS optic chiasm tissues were examined using MS brain autopsy tissues. We compared gene expression in five MS and five healthy age-matched controls (MS mean age = 57.6 y, healthy controls mean age = 56.2 y; MS disease duration mean = 20.4 y; MS and healthy control all female; see **SI Materials and Methods** for additional subject characteristics). Cholesterol synthesis pathways were significantly different in MS optic chiasm compared with healthy control optic chiasm (Fig. 7A and Table S3), with cholesterol synthesis gene expression decreased in MS compared with healthy controls (Fig. 7B).}

**Discussion**

RiboTag technology was applied to EAE to determine astrocyte-specific gene expression networks in multiple regions of the CNS. Our results provide proof of concept that unbiased bioinformatics analyses of the molecular signature of gene expression during disease in a cell-specific and region-specific manner can provide unique insights. Here, the study of astrocytes in EAE unexpectedly pointed to cholesterol homeostasis. To explore how a decrease in cholesterol synthesis in astrocytes might affect demyelinating autoimmune disease, we reviewed known cholesterol changes during development, with synthesis by neurons at the embryonic stage, oligodendrocytes at the postnatal stage, and astrocytes at the adult stage (25). In adult brain, cholesterol synthesized in astrocytes are transported via ATP-binding cassette transporter (ABCA1) to apolipoprotein E (ApoE) to neurons and oligodendrocytes. Neurons use cholesterol to make cell membranes and synapses (28, 29) while oligodendrocytes use cholesterol to make myelin (25, 30) (Fig. 8A). Thus, we hypothesize that reduced cholesterol synthesis in astrocytes that synthesizes cholesterol changes during development, with synthesis by neurons at the embryonic stage, oligodendrocytes at the postnatal stage, and astrocytes at the adult stage (25). In adults, cholesterol synthesized by astrocytes is transported via ATP-binding cassette transporter (ABCA1) to apolipoprotein E (ApoE) to neurons and oligodendrocytes. Neurons use cholesterol to make cell membranes and synapses (28, 29) while oligodendrocytes use cholesterol to make myelin (25, 30) (Fig. 8A). Thus, we hypothesize that reduced cholesterol synthesis in astrocytes

![Fig. 5. Validation of the decrease in cholesterol synthesis gene expression in optic nerve astrocytes during EAE. (A) Quantification of cholesterol synthesis gene expression by qPCR using astrocyte-specific RNAs from an additional set of astrocyte RiboTag mice with EAE at day 43 confirmed RNA-seq findings that Hmgcs1 and Fdit1 expression was down-regulated in astrocytes during EAE (P = 0.034 for Hmgcs1; P = 0.0017 for Fdit1). (B and C) Hmgcs1 and Fdit1 were decreased in EAE optic nerve astrocytes of WT mice at the protein level using immunohistochemistry (P = 0.0378 for Hmgcs1; P = 0.0046 for Fdit1). n = 3 to 4 mice in each group. (D) Colocalization of Hmgcs1 (red) with GFAP (green) in astrocytes (yellow) in Merge. Shown are 10x images of coronal optic nerve sections. Data are representative of two independent experiments. (Scale bars: 10x images, 100 μm; Insets, 50 μm). **P < 0.01, *P < 0.05. Error bars indicate SEM between mice.](image1)

![Fig. 6. Treatment targeting cholesterol homeostasis during EAE. EAE (blue) and normal (NL, black) mice were treated with either Vehicle (Veh, solid) or CS-6253 (CS, striped). (A) Quantification of cholesterol synthesis gene expression by qPCR using astrocyte-specific RNAs from CS or vehicle-treated astrocyte RiboTag mice with EAE at day 43 showed that Hmgcs1, Fdps, and Fdit1 expression were up-regulated in astrocytes in CS-treated normal mice (P = 0.0117 for Hmgcs1; P = 0.0324 for Fdps, P = 0.0475 for Fdit1) and in CS-treated EAE mice (P = 0.0059 for Hmgcs1, P = 0.0003 for Fdps, P = 0.005 for Fdit1). n = 5 in each group. (B) Hmgcs1, Fdps, and Fdit1 were also increased in CS-treated EAE spinal cord astrocytes in WT mice at the protein level using immunohistochemistry (P = 0.0008 for Hmgcs1, P = 0.0002 for Fdps, P = 0.00195 for Fdit1). n = 4 in each group. (C) Colocalization of Hmgcs1 (red) with GFAP (green) in astrocytes (yellow) in Merge, showing the CS-mediated increase in Hmgcs1 expression in astrocyte cell bodies and processes. Shown are 10x images of ventral spinal cord white matter, Insets at 40x. (Scale bars: 100 μm.) (D and E) CS treatment resulted in a reduction in clinical EAE severity scores, which assess walking disability (P = 0.005 cumulative disease index after EAE day 30, P = 0.013 all days; CS group n = 6, vehicle group n = 7), and an increase in the number of seconds that mice could stay on the rotarod (P = 0.0077), respectively, each compared with vehicle treatment in EAE. For confirmation, an additional set of EAE mice underwent treatment with CS-6253 or vehicle, again demonstrating improvement in EAE scores (P < 0.0001 cumulative disease index after EAE day 30, P = 0.0006 all days; CS group n = 7, vehicle group n = 9).](image2)
during EAE may lead to reduced cholesterol available for transport to neurons and/or oligodendrocytes. While demyelination and synaptic loss are due to the autoimmune attack in EAE, limited reparative synaptic plasticity and remyelination may be due in part to reduced synthesis of cholesterol by astrocytes (Fig. 8B). Further investigation of this hypothesis is now warranted.

Other effects of decreased cholesterol synthesis in astrocytes during EAE are possible and not mutually exclusive. Another purpose of the de novo synthesis of cholesterol in astrocytes is neurosteroid production. Cholesterol is a precursor for estradiol, progesterone, and testosterone. Neurosteroid production in the CNS is known to be a neuroprotective response to brain injury (31–34), and expression of neurosteroid receptors is increased in MS brain (35). Sex steroids delivered systemically are lipophilic, cross the blood–brain barrier, and are neuroprotective in MS models (36–38). Thus, exogenous sex hormone treatments may compensate for decreased neurosteroid production from cholesterol in astrocytes during disease in MS models and perhaps in MS clinical trials (39–43).

Consistent with our region-specific transcriptomics data, clinical studies in MS have suggested that altered levels of cholesterol precursors and oxysterols in blood and cerebrospinal fluid may be biomarkers for neurodegeneration (44, 45). Treatment with statins in MS clinical trials has focused on antiinflammatory effects, but whether statins can enter the CNS during disease to affect cholesterol homeostasis in astrocytes in a deleterious or beneficial manner warrants investigation (46–48). Together, our data in spinal cord and optic nerve suggest that targeting cholesterol synthesis in astrocytes in MS may affect walking and vision, respectively.

Regarding other MS disabilities, there are implications for cholesterol metabolism in the hippocampus, given the association of apolipoprotein E (apoE) allele inheritance in cognitive disability in MS (49–51). Cognition is frequently affected in MS, including hippocampal-dependent verbal and spatial memory (52). Hippocampal atrophy and neuropathology have been shown in EAE (18, 21, 53–55) and MS (56–58). However, we found relatively few cholesterol gene expression changes in astrocytes in hippocampus in EAE. That said, an effect of disease on cholesterol pathways in another cell type (neurons, oligodendrocytes) in hippocampus during EAE remains possible.

Findings herein also have implications for the role of astrocytes in the immunopathogenesis of MS and EAE. It is known that there are regional differences in local inflammatory infiltrates in MS and EAE. There is diffuse and localized microglial and astrocyte activation with high levels of T lymphocyte and macrophage infiltration in white matter while there is diffuse microglial and astrocyte activation with a relative paucity of localized T lymphocyte and macrophage infiltration in gray matter, each accompanied by axonal and/or synaptic loss (11, 21, 59, 60). Inflammatory infiltrates in EAE white matter can also vary qualitatively from one CNS region to another (61–63). A central question is whether differences in local inflammatory infiltrates are responsible for regional differences in astrocyte gene expression, or whether regional differences in astrocyte gene expression are responsible for differences in local inflammatory infiltrates. Either is possible as a causal driver of the other. Since differences in gene expression in astrocytes exist in healthy states in the absence of inflammation (1), regional differences in astrocytes could drive regional differences in immune infiltration during EAE. For example, lipocalin2 (LCN2), an autocrine mediator of astrocyte activation (64), drives expression of CCL2 and CXCL10 to promote microglia activation and peripheral immune infiltration in optic nerve and spinal cord during EAE (65–67). Here, the astrocyte transcriptome during EAE showed that Lcn2 gene expression was increased in astrocytes from white, but not gray, matter (Dataset S1). Conversely, differential
production of IFNγ and interleukin-17 (IL-17) in peripheral immune cells has led to differential inflammation in white matter regions during autoimmune inflammation (62, 63), and LCN2 expression in astrocytes could be induced by IFNγ, IL-17, and tumor necrosis factor α (TNFα) (68, 69). Thus, these mechanisms are not mutually exclusive and may coexist in a self-perpetuating feedback loop. Regardless of causality, regional differences in astrocyte gene expression and immune infiltration may provide a rationale for a therapeutic strategy optimally targeting each region and its associated disability.

In conclusion, discovery of cell-specific and region-specific molecular signatures of disease in preclinical models can provide novel targets tailored for each neurological pathway in MS as a strategy to optimize neuroprotective treatment development. Accordingly, a clinical trial designed to reverse the molecular signature within a specific neurological pathway should have a primary outcome measure sensitive to change in a specific disability, rather than a composite that pools different disabilities. This disability-specific approach that begins with discovery and ends with clinical trial design represents a strategy for finding neuroprotective treatments, not only for MS but also for other multifocal neurodegenerative diseases.

Materials and Methods

Mice. RiboTag mice (9) were purchased from The Jackson Laboratory. Mice expressing HA-tagged ribosomal protein RPL22 in astrocytes were generated by crossing RiboTag mice with GFAP-Cre mice (13). All animal experiments were approved by the University of California, Los Angeles Animal Research Committee. Informed consent was attained for research involving human autopsy tissues.