

## MULTIPLE SCLEROSIS

## T cells take aim at a ubiquitous autoantigen in multiple sclerosis

Joseph J. Sabatino Jr. and Scott S. Zamvil\*

CD4<sup>+</sup> T cells from multiple sclerosis lesions target a ubiquitous self-antigen that is shared by gut commensal bacteria (Planas *et al.*, this issue).

Multiple sclerosis (MS) is an inflammatory demyelinating condition of the central nervous system (CNS) that causes relapsing and progressive neurologic deficits. CD4<sup>+</sup> T cells have long been implicated in MS pathogenesis, given the strong association of MS with the major histocompatibility complex (MHC) II allele HLA-DR2 (*DRB1\*15:01, DQB\*06:02*), the presence of CD4<sup>+</sup> T cells in MS lesions, and the central role of CD4<sup>+</sup> T cells in the MS animal model experimental autoimmune encephalomyelitis (EAE) (1). Although myelin is considered a putative CNS autoantigen in MS, evidence that myelin is the primary target of the adaptive immune system is limited (2). Thus, identification of the specific targets of the cellular immune response in MS remains an active area of research. In this issue of *Science Translational Medicine*, Planas *et al.* (3) used an exploratory approach to determine the antigen specificity of a CD4<sup>+</sup> T cell clone (termed TCC21.1) isolated from the cerebrospinal fluid (CSF) of a patient with MS.

The T cell receptor (TCR) sequence of TCC21.1 was previously found to be enriched in two brain lesions of the same patient with MS (4). To assess the antigen reactivity of TCC21.1, the authors used a decapeptide positional scanning library where each single amino acid was alternately fixed at a given position with random distribution of 20 amino acids at all other positions. The authors identified two stimulatory partially overlapping peptides derived from a protein not previously associated with MS called guanosine diphosphate (GDP)-L-fucose synthase. GDP-L-fucose synthase (also known as protein FX) is an NADP(H)-binding protein that converts GDP-4-keto-6-D-deoxymannose to GDP-L-fucose and is widely expressed in all human cell types (5). Expression of GDP-L-fucose synthase protein was confirmed by proteomic

analysis of white and gray matter postmortem brain tissue samples from patients with and without MS, thus supporting the possibility that it is a CNS autoantigen. TCC21.1 responded most robustly to GDP-L-fucose synthase when stimulated by antigen-presenting cells (APCs) expressing HLA-*DRB1\*15:01*, an MHC II allele that confers an increased risk of MS (6). Phenotypically, TCC21.1 produced predominantly not only anti-inflammatory T helper 2 (T<sub>H</sub>2) cytokines [e.g., interleukin-4 (IL-4), IL-5, and IL-13] but also proinflammatory interferon- $\gamma$  (IFN- $\gamma$ ) and granulocyte-macrophage colony-stimulating factor in response to GDP-L-fucose synthase peptide restimulation.

The authors expanded their search for additional CD4<sup>+</sup> T cell epitopes of GDP-L-fucose synthase by generating an overlapping peptide library spanning the entire length of the GDP-L-fucose synthase protein. Using a mitogen to expand CSF CD4<sup>+</sup> T cells from the same patient from which TCC21.1 was derived, an additional CD4<sup>+</sup> T cell line was demonstrated to have reactivity against a separate GDP-L-fucose synthase epitope. Interestingly, this T cell line was composed of three TCR clonotypes, two of which were enriched in a brain lesion of the same patient, suggesting that there may be clonal expansion of other CNS-infiltrating CD4<sup>+</sup> T cells specific for GDP-L-fucose synthase in this patient.

Having demonstrated that there was expansion of GDP-L-fucose synthase-specific T cells in the CNS of one patient with MS, they addressed whether there was expansion of GDP-L-fucose synthase-specific T cells in other MS patients. To test this possibility, the authors generated CD4<sup>+</sup> T cell libraries from the CSF of 31 patients with MS or clinically isolated syndrome (CIS), that is, patients who had experienced only a first clinical attack.

Nearly 40% of the patients demonstrated moderate to high reactivity to 14 different GDP-L-fucose synthase peptides, most of which elicited responses in CD4<sup>+</sup> T cells from multiple patients. In contrast to the TCC21.1 clone, most of the GDP-L-fucose synthase-reactive CD4<sup>+</sup> T cells were of an IFN- $\gamma$ -producing (i.e., T<sub>H</sub>1) phenotype. The authors observed a correlation between patients with the highest GDP-L-fucose synthase reactivity and the presence of myelin-specific CD4<sup>+</sup> T cells, particularly those T cells that were specific for the immunodominant myelin basic protein epitope 83–99. Importantly, the authors found co-responsiveness of GDP-L-fucose synthase-reactive and myelin-reactive CD4<sup>+</sup> T cells but did not demonstrate cross-reactivity to both antigens by an individual T cell clone, a requirement of molecular mimicry (i.e., TCR cross-reactivity between two distinct antigens that share either sequence or structural similarity). Interestingly, the patients with the highest GDP-L-fucose synthase reactivity were all HLA-*DRB3\*02:02* positive, an MHC II allele that is not strongly associated with MS. Whether GDP-L-fucose synthase reactivity is associated with other MS-linked HLA alleles will require further study.

Gut microbiota have been linked to MS. GDP-L-fucose synthase is an evolutionarily conserved enzyme also used by gut microbiota. Thus, the authors examined the reactivity of CSF-derived CD4<sup>+</sup> T cells to GDP-L-fucose synthase homologs from gut commensal bacterial species that are known to be associated with MS and for which the protein sequence is known. CD4<sup>+</sup> T cells expanded from the CSF of four patients with MS demonstrated reactivity to at least three bacterial peptides with greater than 40% sequence similarity to the human GDP-L-fucose synthase epitope. Notably, peptides from *Akkermansia* and *Prevotella*, two bacterial genera previously reported to be disproportionately overabundant in the gut of patients with MS (7), elicited the highest CD4<sup>+</sup> T cell responses.

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Determining the antigen specificity of T cells in the brain lesions and CSF of patients with MS is considered to be a prerequisite to determining their role in pathogenesis. Thus, the findings of Planas *et al.* represent a big advance in our understanding of potential self-antigen targets in MS. As with any technically challenging study, there are limitations. The authors used their antigen discovery strategy with a single CD4<sup>+</sup> T cell clone; thus, the reactivity of many other CNS-infiltrating T cell clonotypes remains to be explored. The authors performed robust *in vitro* enrichment and expansion of CSF CD4<sup>+</sup> T cells to detect GDP-L-fucose synthase reactivity, suggesting that the frequency of these cells is quite low. Although the TCC21.1 clonotype was enriched in an MS CNS lesion, there were about 50 additional clonotypes that were present at similar or higher frequencies in the same lesion (4). Many of the clonally expanded TCR sequences were from CD8<sup>+</sup> T cells, which vastly outnumber CD4<sup>+</sup> T cells in most MS lesions (1), underscoring the importance of investigating the specificity of both clonally enriched CD4<sup>+</sup> and CD8<sup>+</sup> T cells.

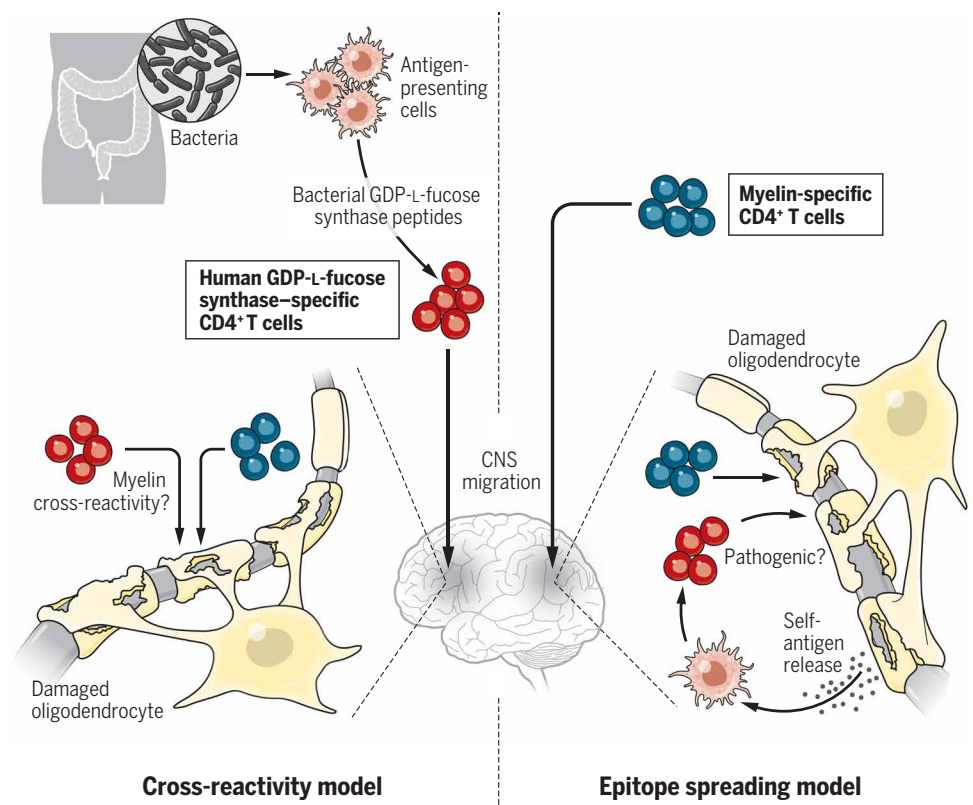
A key question raised by this study is how CNS T cell reactivity to GDP-L-fucose synthase, a ubiquitous self-antigen, is elicited in MS. There are at least two possibilities. As Planas *et al.* suggest, one potential mechanism involves two separate steps of CD4<sup>+</sup> T cell cross-reactivity—one between bacterial and human GDP-L-fucose synthase homologs, and another between GDP-L-fucose synthase and myelin autoantigen (Fig. 1, left). T cells activated in response to gastrointestinal bacterial GDP-L-fucose synthase, perhaps expressed by gut bacterial species over-represented in MS, can traffic to the CNS, where they could encounter human GDP-L-fucose synthase and myelin. In this model, GDP-L-fucose synthase may have a direct role in triggering CNS inflammation. The authors demonstrated reactivity to homologous peptides of human and bacterial GDP-L-fucose synthase by CSF CD4<sup>+</sup> T cells isolated from patients with MS. However, they did not test whether individual T cell clones reacted to both the human and bacterial homolog of GDP-L-fucose synthase peptides, a requirement for demonstrating antigen cross-reactivity through TCR engagement. Second, T cell cross-reactivity between GDP-L-fucose synthase and myelin autoantigen was not tested at the clonal level.

Thus, the possibility of molecular mimicry as a driver of GDP-L-fucose synthase reactivity remains to be confirmed.

Alternatively, CD4<sup>+</sup> T cell reactivity toward GDP-L-fucose synthase may represent a secondary immune response to CNS inflammation (Fig. 1, right). As in EAE, and possibly in MS, CNS autoantigen-specific T cells direct the initial wave of CNS inflammation. Localized CNS cellular injury causes release of cryptic self-antigens, promoting recruitment and expansion of additional self-reactive CD4<sup>+</sup> T cell clonotypes. In this model of epitope spreading, GDP-L-fucose synthase may be a secondary (8) rather than a primary pathogenic target in the CNS. This is reminiscent of a recent report that demonstrated that oligoclonal bands, antibodies restricted to the CSF in most patients with MS, may also

target ubiquitous intracellular self-antigens (8, 9). The finding that CD4<sup>+</sup> T cell reactivity against GDP-L-fucose synthase in patients with MS is also associated with detectable myelin-specific CD4<sup>+</sup> T cells is consistent with this possibility.

Discovery of T cell reactivity to GDP-L-fucose synthase in MS will stimulate further research to determine how it may serve as a potential autoantigen. In future studies, GDP-L-fucose synthase-specific CD4<sup>+</sup> T cells could be tested for their capability to induce EAE directly or, alternatively, modulate EAE induced by myelin antigen. Amplification of GDP-L-fucose synthase-specific CD4<sup>+</sup> T cells after myelin peptide-induced EAE would lend support to the notion of secondary (bystander) T cell activation. In the new work by Planas *et al.*, reactivity to GDP-L-fucose



**Fig. 1. Possible mechanisms for expansion of GDP-L-fucose synthase-reactive CD4<sup>+</sup> T cells in MS.** More than one model can explain how GDP-L-fucose synthase-reactive CD4<sup>+</sup> T cells participate in CNS inflammation. In the cross-reactivity model (left), GDP-L-fucose synthase protein is expressed by commensal bacteria, perhaps by species over-represented in the gastrointestinal tract of patients with MS. These peptides are presented by local APCs, thereby activating CD4<sup>+</sup> T cells that cross-react with human GDP-L-fucose synthase. Activated GDP-L-fucose-reactive T cells can migrate to the CNS, where they may contribute to MS pathology, either by (i) direct cross-reactivity to pathogenic determinants of myelin antigens or (ii) acting synergistically with CD4<sup>+</sup> T cells specific for myelin. In the model of epitope spreading (right), myelin-reactive CD4<sup>+</sup> T cells traffic from the periphery to the CNS and initiate inflammation and demyelination. Cellular debris, including ubiquitous self-antigens, is released and processed by local APCs, leading to a secondary activation and expansion of GDP-L-fucose synthase-reactive CD4<sup>+</sup> T cells. Currently, whether GDP-L-fucose synthase-specific T cells are inert (i.e., bystander), suppress or propagate CNS inflammation is unknown.

synthase relied entirely on stimulation with decapeptides, which do not require processing by APCs. In general, native antigens require internalization and processing by APCs for presentation of the epitopes recognized by CD4<sup>+</sup> T cells. In this respect, it is known that de novo antigen processing and presentation of native CNS myelin protein are required for the induction of disease by myelin-reactive CD4<sup>+</sup> T cells (10). Thus, it will be important to determine whether the identified epitopes of GDP-L-fucose synthase correspond to naturally processed peptides. Finally, although the authors demonstrated clear CD4<sup>+</sup> T cell reactivity against GDP-L-fucose synthase in the CSF of a subset of patients with MS or CIS, there were no control populations for comparison. Thus, it is not known whether similar reactivity may be found in the CSF of patients with other neurologic conditions or even healthy controls. Further studies will be necessary to determine whether GDP-L-fucose synthase is a unique target of the immune response in MS.

The identification of the antigen specificity of an MS lesion-infiltrating CD4<sup>+</sup> T cell clone-type in one patient with MS, and confirmation of similar reactivity in the CSF of other patients with MS, is a big step forward in elucidating MS immunopathology. Further study is still needed to determine the biological significance of the GDP-L-fucose synthase-specific CD4<sup>+</sup> T cell response in MS. Uncovering the antigen specificities of all arms of the adaptive immune response in MS will be vital to understanding MS immunopathogenesis and the development of potential strategies to induce tolerance to these autoantigens and alleviate symptoms of this devastating disease.

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