Toward identification of personalized immunological profiles in multiple sclerosis

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The diversity of four previously unidentified autoantigens found in multiple sclerosis mirrors its notorious clinical variability.

Multiple sclerosis (MS) is the most common inflammatory disease of the central nervous system (CNS), affecting about 2.5 million to 3 million people worldwide. Despite considerable progress in the treatment of MS, the disease often leads to permanent neurological disability. Many investigators consider MS an autoimmune disorder, that is, caused by a misdirected immune response against antigens within the CNS. Identifying these molecules (called autoantigens) is a prerequisite not only for obtaining clues to MS pathogenesis but also for improving the diagnosis and treatment of the condition. The path toward discovery has been difficult for many reasons, but two in particular stand out: Antigen identification is technically demanding, and a unifying autoantigen may not exist in MS.

**ANTIGEN DISCOVERY**

In this issue of *Science Advances*, Bronge *et al.* (1) report on the identification of four novel autoantigens recognized by T cells in MS. Searching for target antigens of T cells requires fundamentally different techniques than searching for antibody targets. Unlike B cells and antibodies that bind with high affinity to native, unprocessed antigens, T cells only recognize peptide fragments generated by the antigen-processing machinery of an antigen-presenting cell (APC). The processed peptides are bound to molecules encoded by the major histocompatibility complex (called HLA in humans), and the peptide-HLA complexes are displayed on the surface of the APC where they can interact with the antigen-specific T cell receptor (TCR) of an antigen-responsive T cell. Because T cells recognize processed proteins, antigen screening is performed by stimulating the T cells in culture and measuring their functional response. Bronge *et al.* (1) applied a method known as FluoroSpot assay to assess the production of three proinflammatory cytokines by T cells in response to antigenic stimulation (Fig. 1).

The authors screened blood cells from patients with MS and matched healthy controls for T cell reactivity against fragments of a panel of 63 different proteins. The selected panel of protein antigens included completely new and previously suspected antigens, all of which known to be expressed in the CNS, the site of MS pathology. The stimulating antigens were narrowed to four source proteins: fatty acid binding protein 7, prokineticin-2, reticulin-3, and synaptoase-associated protein 91 kDa. Testing full-length recombinant versions of the novel candidate autoantigens in a validation cohort confirmed significantly elevated cytokine responses for all four novel antigens, as well as for three myelin autoantigens known to induce CNS autoimmune disease in animal models of MS—myelin oligodendrocyte glycoprotein, myelin basic protein, and proteolipid protein (2).

In the initial screenings, T cells were obtained from patients with MS who were treated with natalizumab, a therapeutic monoclonal antibody that might confound the results of antigen screening. Therefore, the candidate antigens were tested in patients with untreated MS. Again, elevated cytokine responses were noted against all four novel autoantigens, as well as the three myelin autoantigens in the blood of patients with MS.

Importantly, patients with MS differed in the strength and pattern of their individual T cell responses to the seven tested autoantigens. Using receiver operating characteristic analyses, the investigators designed composite tests that allowed them to confirm or rule out an MS diagnosis, based on the individual T cell response profiles measured with the FluoroSpot technique.

**RAISING FUNDAMENTAL QUESTIONS ABOUT T CELL RESPONSES**

Several additional interesting observations were made in this study. First, the elevated autoreactive T cell responses in patients with MS were mostly due to CD4 T cells with some contribution of CD8 T cells, inviting further studies to define more precisely the role of these two major T cell subsets. Second, male patients reacted more strongly than female patients to five of the seven (four new and three previously established) autoantigens. Last, despite the investigation of a large collection of plasma from patients with MS and healthy controls (>500 per group), no increase in autoantibodies against the four new autoantigens was detected, indicating a puzzling divergence between autoreactive T and B cell responses.

Bronge *et al.* (1) add four novel autoantigens to the list of about a dozen previously proposed candidates. If 4 of the 63 selected autoantigens tested turn out to be recognized by T cells from patients with MS, then the full array of autoantigens in MS is probably much wider.

Several fundamental questions regarding these autoreactive T cell responses could be raised: (i) Are they primary or secondary to the disease process? (ii) Are they related to established environmental factors such as Epstein-Barr virus (3)? (iii) Are they directly involved in the destructive inflammatory process within the CNS? In addition, (iv) Can we use them as “biomarkers” for diagnostic, prognostic, or therapeutic purposes?

One experimental approach to the first question is to assess autoreactivity very early on in the disease process, ideally even before

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the clinical onset of MS. This is possible by 
surveying large cohorts of individuals, some of 
whom will later on develop MS (3), or 
small cohorts of identical twins discordant 
for MS, the healthy twin being prone to later 
develop MS (4). As noted by Bronge et al. 
(1), the strongest autoreactivity was detected a 
few years after disease onset, arguing rather 
in favor of a secondary process, possibly 
resulting from CNS tissue damage and subse-
quent immune stimulation. This would be 
consistent with previous observations demon-
strating that some of the antibodies pro-
duced by B cells in the cerebrospinal fluid of 
patients with MS recognize intracellular auto-
antigens released during CNS injury (5).

The autoreactive T cell responses could be 
pathogenic and therefore represent a valid 
therapeutic target. However, demonstrating 
their deleterious potential poses a real 
challenge. One approach to this challenge is 
a detailed molecular characterization of auto-
reactive T cells through functional charac-
teristic assessment [such as cytokine production 
as performed by Bronge et al. (1)], their 
migratory potential, and their differentia-
tion status. Addressing their destructive 
potential in animal models through deliberate 
immunization with individual or combined 
autoantigens is a further step forward. Ulti-
mately, the final answer regarding the role 
of these autoreactive T cells in the MS 
disease process will be based on the clinical 
benefit afforded by their selective therapeu-
tic inhibition (6).

**DEFINING INDIVIDUAL PROFILES**

No consistent autoreactive T or B cell 
responses have so far been identified in MS with 
diagnostic or prognostic value. Current data 
(1, 7) suggest that the spectrum of autoreac-
tivity differs not only between patients but, 
possibly, also in individual patients over 
time. The real value of screening for autore-
active T cell responses may lie in identifying 
individual immunological response profiles. 
Specifically, individual profiling of auto-
reactive T cell responses in the blood and/or 
cerebrospinal fluid may help to define sub-
groups of patients with MS who are at high 
risk for a severe course of MS, thus repres-
enting a step toward personalized risk assess-
tment. This, in turn, will help calibrate the 
immunotherapy armamentarium to the indi-
vidualized aggressiveness of the disease.

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