The gray aspects of white matter disease in multiple sclerosis

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The major inflammatory disease of the central nervous system is multiple sclerosis (MS). It is often considered a 2-stage disease with an initial inflammatory attack on myelin involving the 2 hallmarks of adaptive immunity: antigen-specific T cells and antibodies directed to protein and lipid components of the myelin sheath (1). This concerted attack involving the adaptive immune system is followed by a "degenerative" course involving the myelin itself, the oligodendrocytes that produce it, and the underlying axons and their neuronal cell bodies themselves in the gray matter. All of this culminates in significant cerebral atrophy as MS progresses (2). Whether or not there is really such a sequential and bifurcated temporal course between "inflammation" in the white matter, followed by neurodegeneration in the gray matter, is actually an open question. In this issue of PNAS, Derfuss et al. (3) identify, via a multifaceted approach including proteomics, one of the first examples of a target recognized by both T cells and antibodies that is located in gray matter. The target is contactin-2, a homologue of transiently-expressed axonal glycoprotein-1 (TAG-1) expressed on axons and in the juxtaparanodal region of oligodendrocytes producing myelin to insulate these axons (4), and by neurons in gray matter (5, 6).

Gray matter involvement may be a much earlier aspect of the pathology in MS than has been appreciated. Similarly, inflammation may be a much greater component of the so-called secondary progressive phase of MS (7). What has been missing in understanding these issues has been identification of targets of adaptive immunity in gray matter. Derfuss et al. (3) provide a stellar example of a molecule that when attacked produces both white and gray matter pathology.

Many adaptive immune responses to components of white matter have been identified in MS. These include antibody and T cell responses to constitutive myelin proteins, including myelin basic protein (MBP), proteolipid protein (PPL), and myelin-associated glycoprotein (MAG). Large-scale, custom-made arrays of myelin-related proteins and peptides have been designed to identify peptides and recombinant proteins that bind antibodies found in the cerebrospinal fluid of patients with relapsing remitting MS (RRMS) (8, 9). RRMS patients demonstrated significantly increased autoantibodies against various myelin epitopes, including α B crystallin (CRYAB) protein and peptides; J37, a MBP isoform of Golli-MBP; heat shock protein (HSP); and amyloid β (Abeta).

Immunity to myelin proteins is not the only story in MS. Lipid components of the myelin sheath including sulfatides, cerebrosides, and phospholipids are targets of adaptive immunity in brain and spinal fluid in MS (10). The intricate interplay of proteins and their lipid and carbohydrate modifications, and the myelin lipids and carbohydrates in isolation, is a burgeoning field that has not received emphasis. Derfuss et al. (3) decided to focus on the glycoproteins in myelin.

MS patients had increased T cell responses to contactin-2.

Derfuss et al. (3) first identified contactin-2 isolating human myelin on a lentil lectin preparation that preferentially binds glycoproteins, enabling a detailed analysis of this component of myelin sheath. There are many known glycoproteins in myelin including myelin oligodendrocyte glycoprotein (MOG), oligodendrocyte-myelin glycoprotein, and MAG. In this isolation the major proteins of myelin, MBP and PLP, often the subjects of the greatest amount of attention, were excluded. It is a good idea to examine those proteins that are not the usual suspects, as any disease detective ought to know.

After isolation of the less prevalent glycoproteins, the next step was serendipitous. Ig from MS patients receiving an experimental procedure called immunoadsorption (11) was applied to an electrophoresis gel, and a particular spot was identified as contactin-2 with mass spectrometry.

Derfuss et al. (3) then showed that MS patients had increased T cell responses to contactin-2, with production of flagship cytokines of the T helper 1 (TH1) and TH17 pathways. Antibody responses to contactin-2 were seen in the serum in the majority of MS patients. There were also more antibodies to contactin-2 in the cerebrospinal fluid of MS compared with appropriate controls.

Next, Derfuss et al. (3) chose a well-tested strategy to see whether an immune response to contactin-2 might elicit an inflammatory immune disease of the central nervous system. They tried to see whether immunity to contactin-2 would induce experimental autoimmune encephalomyelitis (EAE). EAE is a set of animal models, collectively reflecting many aspects of the inflammatory portion of MS.

The discovery of EAE in 1933 was marked by its diamond anniversary in 2008 (12, 13). Twenty-eight years ago researchers, including one of the senior authors of ref. 3, demonstrated that T cell lines could induce EAE (14). Specific T cell clones were then produced that could induce relapses and remissions characteristic of early MS (15). Now inbred animals carrying the T cell receptors for such clones have been genetically engineered, making it possible to take a programmed mouse off the rack, which will get EAE spontaneously (16). EAE is often used to probe the biological activity of a molecule in brain inflammation.

When T cells specific for TAG-1 were transferred to naïve animals, they developed hind limb paralysis, with pathological evidence of inflammation with involvement of cortex and spinal cord gray matter. To induce demyelination and axonal injury Derfuss et al. (3) augmented the anti-TAG-1 T cell response with infusion of a monoclonal antibody to another glycoprotein (MOG). After this, there was demyelination in gray and white matter. The lesions in the cerebral cortex had many features of lesions in the same region with early MS. The discovery has now opened a very realistic animal model for gray matter involvement in MS.

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Attempting to induce EAE, with a newly-discovered target discovered in MS is a good idea, but this approach is not without its problems. This strategy is flawed when it fails, but is, of course, invaluable when it succeeds. A notorious example of how a field can be retarded, pursuing this strategy emphasizes why we should be cautious about habitually returning to testing a molecule to see whether it causes EAE.

Perhaps the strongest adaptive immune response seen in the spinal fluid of MS patients is directed to a small heat shock protein CRYAB (Fig 1). CRYAB is expressed in the myelin sheath and in axons in MS lesions (9, 17). CRYAB is produced in copious amounts at the site of pathology in gray-matter diseases like Alzheimer's disease and in white-matter diseases like the leucodystrophy known as Alexander's disease (9, 18). It is also the main constituent of the lens of the eye. CRYAB is an inducible protein that appears in copious amounts in various types of MS lesions, including active and chronic types (19). After CRYAB was first discovered as one of the strongest targets of adaptive immunity elicited in patients from MS myelin, investigators spent much of the next decade trying to induce EAE with it.

Ten years of research lead to the disappointing conclusion that immunity to CRYAB did not induce EAE at all. In fact, EAE was used to establish what this molecule might be doing in MS. In this case paralytic EAE was more severe when CRYAB was deleted in gene knockouts. It was never possible to induce EAE on its own with CRYAB. Instead we learned that CRYAB is a major guardian protein that can protect myelin, neurons, and axons from degeneration (9, 20). CRYAB inhibits cleavage of caspase 3 and blocks activation of major inflammatory pathways like p38 kinase. An immune response to CRYAB does not elicit EAE, instead it neutralizes a potent brake on inflammation and neurodegeneration. CRYAB maintains immune privilege in the brain, as it does in the eye lens.

Contactin-2 and CRYAB are 2 molecules found in gray matter and white matter that are major targets of the immune response in MS. Because contactin-2 triggers EAE in experimental animals, we might conclude that it is a molecule with an important structural role in axons, myelin, and particularly the node of Ranvier, where currents flow in myelinated fibers. CRYAB plays less of a "structural" role in normal physiology, and in fact does not appear normally in brain. Its function is less of a structural bulwark in the nervous system, and instead it has "regulatory" activities. So, when the brain is under immune attack CRYAB is a "protector." In MS, CRYAB itself is then attacked, neutralizing its role. CRYAB does not elicit EAE, in part because it is not present in normal brain and in part because its role in any case is to inhibit inflammation.

There are many other molecules that have yet to be discovered that comprise the targets of adaptive immunity in MS. Some, like contactin-2, will be found in gray matter and white matter and may give us significant insights about why both these components of the nervous system are involved in MS. Other molecules will likely be discovered that are guardians like CRYAB, not even normally present in white or gray matter, yet induced in both places, when the brain is under siege. For some of these guardians an immune attack against the induced protective molecule impairs the ability of the nervous system to fight back and heal itself against gray and white matter damage. Defining the targets of immunity in the gray and the white matter, and then understanding their biological and pathological roles, will allow us to devise more effective therapeutic measures for the treatment of MS.

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