P2-Reactive T Cells in Inflammatory Demyelination of the Peripheral Nerve

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Lewis rats immunized with P2 protein, a 14.5-kDa protein of the peripheral nerve myelin, develop experimental allergic neuritis, a paralytic disorder with clinical, histologic, and electrophysiologic features similar to those of human Guillain-Barré syndrome (GBS). T cells reactive to P2 protein or a peptide corresponding to 53-78 residues of the protein can transfer the disease to naive animals. The mechanisms by which these T cells induce demyelination are not well understood; however, they may induce inflammation and demyelination in the nerves by production of Th1 cytokines. Th2 cytokines may lead to suppression of the inflammation and eventual recovery. There is no conclusive evidence that P2 protein plays a role in the pathogenesis of GBS, with or without association with Campylobacter jejuni; however, studies of the immunopathogenesis of P2 protein–induced experimentally allergic neuritis are important for understanding the pathogenesis of inflammatory demyelination in the peripheral nerves, the hallmark of GBS.

The search for the cause of Guillain-Barré syndrome (GBS) has been a long and arduous process. Various viruses and bacteria have been implicated in the etiology of the disease, either directly or through presumed antigenic mimicry and autoimmunity; however, a cause-and-effect relationship has not been established.

The autoimmune theory of causation of GBS was first proposed in 1995, mainly by analogy to experimental allergic neuritis (EAN; an animal model of GBS) induced in rodents by immunization with peripheral nerve tissues in complete Freund’s adjuvant [1, 2]. Over the ensuing years, the search for the antigen in the peripheral nerve led to the discovery of P2 protein of the peripheral nerve myelin as the main antigen responsible for the induction of EAN [3, 4]. P2 protein is a component of the peripheral nervous system (PNS) myelin and has a molecular mass of 14.5 kDa. It is comprised of 131 amino acids [3, 5].

P2-Reactive T Cells in EAN

P2 protein, when mixed with complete Freund’s adjuvant and injected subcutaneously into Lewis rats, produces a paralytic disorder with histologic and electrophysiologic features similar to those of GBS [4]. A synthetic peptide corresponding to residues 53-78 of bovine P2 protein (SP26) has also been shown to produce EAN in Lewis rats [6]. Lymph node cells from P2- or SP26-immunized rats respond to these antigens in vitro and can transfer EAN to naive syngeneic rats [7, 8] (figure 1). The recipient rats do not show antibodies to these antigens. Systemic injection of the sera from these animals does not induce demyelination in the PNS; however, intraneural injection (thereby bypassing the blood-nerve barrier) of high-titer anti-P2 serum can induce demyelination in the sciatic nerve of rats [9]. Systemic injection of antibody to galactocerebroside, a glycolipid component of the myelin sheath, in addition to reactive T cells into Lewis rats produces severe demyelination in the PNS. The demyelination is greater than that produced by SP26-reactive T cells alone, suggesting a synergistic role of T cell autoimmunity and humoral response in the inflammatory demyelination of Lewis rat EAN [10]. Activated T cells nonreactive to neural antigens have also been shown to open the blood-nerve barrier to circulating demyelinating antibodies [11].

The clinical course of EAN induced by active immunization with P2 or SP26 is different from that of EAN induced by passive transfer of P2- or SP26-reactive T cells. Typically, in active EAN in the Lewis rat, the disease starts ~10 days after immunization, peaks around day 16, and resolves by day 30. In passive EAN in the Lewis rat, the disease starts earlier, usually on day 4, peaks around day 8, and resolves by day 20 after the transfer of P2- or SP26-reactive T cells [8] (figure 2). These T cells can undergo apoptosis in situ and terminate the autoimmune attack on the nerve [12]. P2- or SP26-reactive T cells capable of transferring EAN are CD4+ T cells, and preliminary observation suggests that they are of the Th1 subtype [8].

The exact mechanism by which P2-reactive T cells cause demyelination in the PNS is not clear. They may do their damage through production of various cytokines by Th1 cells, and recovery from the disease could result from action of inhibitory cytokines produced by Th2 cells. In fact, administration of interferon (IFN)-γ, a product of Th1 cells, can exacerbate EAN, and monoclonal antibody against IFN-γ can suppress EAN [13]; moreover, serum levels of IFN-γ increased in paral-
the axons when the nerve milieu becomes less inflammatory, but what factors suppress the inflammation in the nerve? T cells can undergo apoptosis in situ and gradually terminate the autoimmune attack on the nerve [12]. Alternatively or concomitantly, the shift of balance of the inflammatory cells in the nerves may favor a Th2 response that can counteract the effect of Th1 cytokines, such as INF-\(\gamma\), and suppress the inflammation.

My colleagues and I did a longitudinal study on two major Th2 cytokines, interleukin (IL)-10 and IL-4, in active EAN [15]. Using quantitative RT-PCR, we measured the amount of message for these cytokines at different time points, from immunization to recovery, during EAN. There was a slight decrease in IL-10 expression at day 10 after immunization, which corresponded with days 1–2 before clinical onset, when there was an increase in IFN-\(\gamma\) message. IL-10 production, like IFN-\(\gamma\) production, reached maximum at day 16 after immunization. No IL-4 message could be detected in the nerves during the course of EAN or in control rat nerves. IL-10 is a potent suppressor of macrophages, T cells, and NK cells. It suppresses the transformation and growth of Th1 cells and production of IFN-\(\gamma\) by those cells. It also suppresses the production of tumor necrosis factor-\(\alpha\), IL-6, granulocyte-macrophage colony-stimulating factor, and IL-10 itself [18, 19].

IL-10 has been shown to suppress clinical EAN in rats [20]. Therefore, the rise in IL-10 at the height of clinical EAN can be viewed as an attempt by Th2 cells to down-regulate the proinflammatory cytokines and lead to recovery from EAN. Other antiinflammatory cytokines, such as transforming growth factor-\(\beta\), may also play a role in recovery from EAN [21–23].

**Figure 1.** A, Longitudinal section of rat sciatic nerve 11 days after intracardiac injection of P2-reactive T cells. Segmental demyelination (arrows) is associated with dense mononuclear cell accumulations. Bar = 20 \(\mu\)m. B, Longitudinal section of rat sciatic nerve 12 days after intracardiac injection of purified protein derivative–stimulated T cells. Morphology is normal. Bar = 20 \(\mu\)m. Adapted with permission from [7].

el with the clinical course of EAN, and positive IFN-\(\gamma\) immunoreactivity in the cauda equina was shown at the peak of clinical disease [14].

Recently, using quantitative reverse transcriptase–polymerase chain reaction (RT-PCR), my colleagues and I demonstrated that the expression of IFN-\(\gamma\) message in the cauda equina of rats with EAN starts to increase prior to the onset of clinical disease [15]. This increase parallels the rise in the severity of clinical paralysis. The main source of IFN-\(\gamma\) at this stage of the disease is most likely Th1 cells infiltrating the nerves, although nonimmune cells within the peripheral nerves, such as Schwann cells, produce cytokines [16, 17]. It is possible that the macrophages that have infiltrated the nerves become activated by IFN-\(\gamma\) and damage the myelin sheath.

Most animals actively immunized and passively transferred with P2-reactive T cells recover from EAN. The mechanism of this recovery is not clear. Schwann cells can remyelinate the P2-Reacte T Cells in GBS

Based on the observation that P2 proteins can induce EAN and P2-reactive T cells can transfer the disease, several laboratories have studied the potential of P2 protein as an antigen in GBS or chronic inflammatory demyelinating polyneuropathy. A mild degree of cell-mediated immunity to P2 protein in GBS has been reported in some studies [24–26] but not in others [27, 28].

P2-reactive T cells can be isolated from GBS and normal subjects by in vitro sensitization, using P2 protein in the culture medium [29]. These observations suggest that some autoreactive T cells are not entirely deleted from the repertoire during immunologic maturation.

Is there a role for T cells reactive to P2 protein or other PNS antigens in C. jejuni–associated GBS? There are no studies to specifically address this question. However, one can assume that C. jejuni infection, perhaps through antigenic mimicry and induction of an autoimmune response, can damage the PNS myelin. This process can release P2 and other proteins and nonprotein antigens from the damaged myelin sheath. Immune response against these antigens, which are normally hidden
behind the relatively immunologically privileged blood-nerve barrier, can generate another autoimmune attack against the PNS myelin and produce further inflammation and demyelination. T cells reactive to the myelin antigens can either directly (most likely through cytokines) damage the myelin or increase the permeability of the blood-nerve barrier, thereby allowing humoral factors to gain access to the nerve and produce demyelination.

Patients with GBS and C. jejuni infection often have higher antibody to the bacteria. These antibodies may cross-react with GM₁ ganglioside, a component of PNS myelin [30, 31]. My colleagues and I tested the effect of serum from a GBS patient with high-titer anti-C. jejuni antibody on a group of Lewis rats to see if they produced demyelination in vivo and consequent paralysis, as seen in EAN and GBS. Before receiving intraperitoneal injections of the sera, the rats were injected with a small number of P₂-reactive T cells. The number of cells was slightly lower than the amount that can produce paralysis but large enough to open the blood-nerve barrier and allow the antibodies to access the nerve [10, 11]. High-titer anti-C. jejuni serum was injected into these rats for several days after T cell transfer. Another group of similarly treated rats received control sera from a healthy donor.

In preliminary studies, my colleagues and I observed no paralysis or histologic signs of EAN. These studies need to be repeated, and if the results are confirmed, they suggest that antibodies to C. jejuni infection may not be the only cause of demyelination in C. jejuni-associated GBS; other factors may be needed to enhance the demyelinating effect of the antibodies.

T cell–mediated immunity to various components of C. jejuni bacteria has not been fully studied. Such components may induce demyelination in the same way as P₂-reactive T cells do in EAN. Activation of the complement cascade may play an important role in the process of demyelination. Deposition of complement on Schwann cells in the demyelinating form of GBS [32] and on axolemma in acute motor axonal neuropathy associated with C. jejuni infection [33] has been reported. Association of C. jejuni infection with GBS is an intriguing observation. Further studies on the role of this bacterium in GBS and establishment of a cause-and-effect relationship can lead to a better understanding of the pathogenesis of GBS, even in the cases not associated with this infection.

References