ABSTRACT

Experimental autoimmune encephalomyelitis (EAE) is the primary animal model used for studying multiple sclerosis (MS). Here we discuss 3 examples of molecular targets identified through studies of EAE and comment on their application in the treatment of MS. The anti-very late antigen 4 antibody, natalizumab, has shown marked therapeutic effects through the blockade of T-cell interactions with vascular endothelium of the blood-brain barrier. Insulin-like growth factor 1 stimulation of myelin production, in contrast, has had variable effects. Finally, inhibition of Nogo-A, neurite outgrowth inhibitor, shows promise as therapeutic means to combat axonal transection. Thus studies of EAE have provided insights into potential therapies targeting MS at many different levels.

THE EAE MODEL

The animal model for multiple sclerosis (MS) is experimental autoimmune encephalomyelitis (EAE). The clinical course of the disease mimics certain types of MS whereas histopathology shows marked resemblance to central nervous system (CNS) lesions seen in MS. Although the model has shortcomings, several key molecular observations have translated to MS. This article will review some of the molecular targets in the EAE model that have found or are finding their way to human clinical trials.

PROCEEDINGS

PROMOTING NEUROPROTECTION: LESSONS LEARNED FROM EAE*

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in humans is unclear. These T cells then begin secreting matrix metalloproteinases, which digest the basement membrane allowing access to the CNS. As part of the inflammatory process, microglia secrete cytokines, such as interleukin (IL)-12 and IL-23, which stimulate the infiltrating T cells to produce interferon γ (IFNγ) or IL-17. These cytokines contribute to an inflammatory environment resulting in the activation of immune cells, including macrophages, T cells, and B cells, to attack oligodendrocytes and neurons. The best characterized effector mechanisms at present involve immunoglobulins and glutamate; however, other mechanisms are believed to be involved as well. A major target is, of course, the myelin sheath; however, axonal transection is also common and may be responsible for the permanent accumulation of disability associated with MS. Identification of critical proteins at each phase of the disease process provides a potential target for therapeutic intervention.

**DISRUPTION OF VLA-4/VCAM-1 INTERACTIONS**

The identity of the adhesion proteins responsible for lymphocyte interaction with the blood-brain barrier was determined by an in vitro cell adhesion (Stamper-Woodruff) assay using tissue slices from the brains of Lewis rats with EAE. Using monocytes or lymphocytes, the researchers found that these cells interact specifically with vascular tissue. This interaction could be disrupted with antibodies specific for very late antigen 4 (VLA-4, α4β1 integrin) but not for other members of the integrin family. Additionally, antibodies against a ligand for VLA-4, vascular cell adhesion molecule-1 (VCAM-1), also disrupted cellular interaction with the vascular endothelium. These results were somewhat surprising as other integrins play an important role in lymphocyte attachment to endothelium in other tissues. The authors then examined the effect of the anti-VLA-4 antibody in vivo. They again employed the Lewis rat EAE model, administering anti-VLA-4 or control antibody 2 days after administering pathogenic T-cell clones. Paralysis was blocked in approximately 75% of rats treated with the anti-VLA-4 antibody and in the remaining 25%, disease intensity was significantly diminished.

This exciting result engendered a series of additional studies, first in the EAE model and subsequently in patients with MS. For human therapy, a humanized anti-VLA-4 antibody (natalizumab) was constructed and confirmed to have anti-VLA-4 activity. It successfully passed through phase I trials and was then examined as a therapeutic agent for the treatment of MS in several larger trials. The Natalizumab Safety and Efficacy in RRMS (AFFIRM) trial examined the effect of natalizumab on patients with RRMS over a 2-year period and reported a 42% reduced risk (hazard ratio, 0.58; confidence interval, 0.43–0.77; P < .001) of sustained progression of disability. Additionally, the study reported a 92% decrease in gadolinium-enhancing lesions in the natalizumab arm in years 1 and 2 (P < .001).

Although the adverse effects seen in the AFFIRM study seemed manageable, an extremely serious complication, progressive multifocal leukoencephalopathy (PML), was observed in 2 patients from the Safety and Efficacy of Natalizumab in Combination with IFNβ-1a in Patients with RRMS trial, in which natalizumab and IFNβ-1a were combined. An additional PML case was seen in a natalizumab-treated patient with Crohn’s disease. JC virus was observed in CNS lesions in 2 of these patients. This led others to consider the possibility that natalizumab may be inhibiting general immune surveillance within the CNS.

To test this hypothesis, Stüve et al examined cerebrospinal fluid (CSF) from 23 patients with MS taking natalizumab and compared these samples to those from 35 control patients with MS and 16 control patients with other neurologic diseases. As shown in Figure 1, there was a marked decrease in the number of white blood cells (WBC) in CSF from natalizumab-treated patients compared with untreated controls. Interestingly, 6 months after cessation of natalizumab treatment, 13 patients were rescreened and CSF WBC remained low. This is somewhat surprising as the pharmacologic half-life of natalizumab is approximately 11 days, suggesting that the bound antibody has a significantly longer half-life. Should a patient develop PML, cessation of natalizumab treatment would likely not reverse the drug’s effect on immune surveillance. Natalizumab was voluntarily withdrawn from the market until further safety studies could be performed and in July 2006, the US Food and Drug Administration approved restricted distribution of the drug to patients with MS under strict guidelines. In considering the usefulness of the EAE model as a means to identify targets and test therapeutic strategies, it is worth noting...
that PML does not occur in rodents. Thus, it would have been impossible to predict that this adverse effect would occur on the basis of testing in this model.

INSULINLIKE GROWTH FACTOR 1 TREATMENT IN MS

Insulinlike growth factor 1 (IGF-1) was identified as a differentiation factor for oligodendrocytes through in vitro experiments examining survival in cerebellar cultures. Consistent with this observation, IGF-1 transgenic mice showed increased myelination and expression of myelin-associated proteins. Conversely, disruption of the IGF-1 gene resulted in hypomyelination. These data prompted others to examine IGF-1 in the Lewis rat EAE model. Recombinant IGF-1, or placebo, was administered 12 days after EAE induction for a period of 8 days, after which the animals were killed and brains examined for lesions. IGF-1 markedly improved CNS myelination post-EAE induction, both in terms of inhibition of demyelination and promotion of remyelination.

An obstacle to this therapy being used in humans is that high-dose IGF-1 is likely to promote hypoglycemia through binding to insulin receptors. As others have shown that a complex of IGF-1 with its major serum binding protein, IGFBP3, has pharmacokinetic properties that avoid insulin receptor activation, another EAE study was performed comparing IGF-1 to the IGF-1/IGFBP3 complex. Surprisingly, the effect of this treatment was quite complex. At early times EAE was inhibited and disease onset was delayed. However, at later times the disease caught up with the control group and then surpassed it. Histology showed clearly that at early times IGF-1/IGFBP3 inhibited the inflammatory process whereas at later times inflammation was much worse. IGF-1 therapy also was studied in an SJL mouse EAE model. No significant clinical effect was noted. Nevertheless, a small clinical trial was initiated looking at IGF-1 treatment in patients with MS. The results were variable. As shown in Figure 2, some patients responded quite well whereas others responded poorly. Several potential mechanisms for this heterogeneity can be invoked. IGF-1 serves as an inducer of myelination and as a growth factor that has some effect on immune function.

It is clear from these studies that IGF-1 has very different effects on different models of EAE. In the Lewis rat model, the disease is monophasic, whereas in the SJL mouse, the disease is relapsing-remitting. This underscores the need to pay close attention to the clinical course within the disease model.

Figure 1. Natalizumab Decreases the Number of Lymphocytes in CSF

Figure 2. IGF-1 Effect on Number of Gd-Enhancing Lesions
INHIBITION OF NOGO EXPRESSION

Induction of neurite sprouting could potentially reverse the injury caused by axonal transection. Nogo-A is a neurite outgrowth inhibitory protein expressed in oligodendrocytes. Inhibition of Nogo signaling has been demonstrated to result in a marked increase in long distance axonal regeneration and neurite sprouting.\textsuperscript{22-24} Preliminary data involving an RNA interference-based strategy to inhibit Nogo-A demonstrate a decrease in the clinical score in a murine EAE model, perhaps due to enhanced neurite sprouting (Lovett-Racke A and Racke M, Unpublished data). Clinical trials involving the inhibition of Nogo-A are in the planning stage.

CONCLUSIONS

Here we have given 3 examples of therapeutic strategies for MS in various stages of development, which are derived from studies of the EAE model. T-cell entry has been blocked with an anti–VLA-4 antibody in several clinical trials, resulting in a marked positive effect that has, for the moment, been marred by a critical adverse effect in a small number of patients. IGF-1 has been shown to have beneficial effects in a monophasic model of EAE but equivocal effects in a relapsing-remitting EAE model. An initial clinical trial in patients with MS had variable effects. Finally, preliminary data indicate that inhibition of Nogo-A has a positive effect in a rodent EAE model. These examples underscore the usefulness of this animal model for the study of MS.

DISCUSSION

Q: I have a question about the IGF-1. How long were people on IGF-1? There was an article in Science showing that, at least in the C elegans model, IGF-1 seemed to be one of the triggers for Alzheimer’s disease by shutting down heat shock factor 1. Have you run into any problems with people on IGF-1 having any other deficits?

Dr Racke: I actually only did the mouse studies. Dr Richert, do you want to comment on that?

Dr Richert: There are several caveats to the IGF-1 study that we did at the National Institutes of Health. First of all, it was done in patients with secondary progressive MS (SPMS), thus even though there were inflammatory lesions, these were all classified as patients with SPMS. The study was only for 6 months, therefore, we really cannot comment on Alzheimer’s disease.

Dr Racke: The reason I think it was able to inhibit the gadolinium-enhancing lesions and inflammation in some patients is that it had some effects on the endothelium’s expression of VCAM-1. That may have explained why some of the animals had a delayed onset of disease.

Also, I should mention that not only do you get this whopping expansion of all these cell populations in the immune system, the mice grow significantly more than mice that did not receive IGF-1. The bottom line is that these molecules have really pleiotropic effects and thus are going to be difficult to use as therapeutic agents unless a significant number of these pleiotropic effects have a positive benefit on the disease process.

Dr Bar-Or: You alluded to the possibility from previous reports that suggest that intervening with Nogo-A may modulate the immune response. Sam David just published a paper in Neuron looking at intervening with Nogo-A in the context of peripheral nerve disease and showed that Nogo-A molecules are expressed on macrophages, that those cells are modulated, and that there is a very major change in the biology of peripheral injury in the context of Nogo-A intervention. So, have you looked at macrophage invasion, mobility, and any other read-outs?

Dr Racke: I think we have been trying to address that from a different point. We are putting our small interfering RNA (siRNA) into in vitro cultures and asking what effects does that have on gene expression of a number of molecules. Also, we are labeling a number of different siRNAs to look where they go and what things seem to be induced. In terms of the CNS, one of the things I think is important to realize is that we really have only been able to see labeled siRNA go into EAE lesions and not into other parts of the brain.

REFERENCES


