ANTI I-A ANTIBODY SUPPRESSES ACTIVE ENCEPHALOMYELITIS:

Treatment Model for Diseases Linked to IR Genes*

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The in vivo administration of monoclonal antibody to products of the I-A subregion of the immune response gene complex (I region), within the major histocompatibility complex (H-2), has been successful in the prevention of experimental allergic encephalomyelitis (EAE) and experimental autoimmune myasthenia gravis (EAMG) (1, 2). To establish the clinical relevance of this novel therapy for autoimmune disease, we have investigated the effects of such treatment in acute and chronic relapsing EAE by instituting treatment at the onset of paralytic signs and then following the clinical course.

EAE serves as a model for demyelinating disease of the central nervous system, and is frequently used to test potential therapies for demyelinating disease. The disease follows T cell sensitization to myelin basic protein (3), and susceptibility to EAE is in part a dominant trait strongly linked to the genes of the major histocompatibility complex (MHC) (4). Recently, a chronic form of EAE has been established in mice (5, 6). Such a disease is chronic, with relapsing and remitting features resembling multiple sclerosis (MS) clinically and histologically.

Materials and Methods

Animals

SJL/J mice 8–10-wk old, were purchased from The Jackson Laboratory (Bar Harbor, ME).

Monoclonal Antibodies

Monoclonal antibody from hybridoma 10.3.6 recognizes a public specificity (Ia.17) found on I-A^s cells and is an IgG2A (1). BP 107 recognizes I-A^{b,d,q,u,j} and is an IgG1 (2). The binding titers of the hybridomas were $>10^4$ on the appropriate spleen cells. Ascites fluid preparations of the antibody were used in all experiments.

Immunizations

For Induction of Relapsing EAE. Mice were immunized at three sites on the back on days 0, 7, and 21 with 1 mg of syngeneic mouse spinal cord homogenate (MSCH) in 300 µl of 50% phosphate-buffered saline (PBS) and 50% of incomplete Freund's adjuvant to which was added 30 µg of H37RA (Difco Laboratories Inc., Detroit, MI). Mice were examined daily and weighed on alternate days throughout the course of the disease. Therapy rendered was randomized according to the designated cage numbers. For

example, in Experiment 1 all animals in odd numbered cages received monoclonal anti–I-A^s 200 μl (containing 0.6 mg IgG; N. Adelman and H. O. McDevitt, personal communication, reference 7) i.p. weekly (Group I) throughout the period of observation, beginning from the first clinical sign of disease. Control animals (Group II) received no treatment. In Experiments 2 and 3 control animals (Group II) received an irrelevant monoclonal, BP107 anti-I-A^d, 200 μl i.p. weekly. A clinical relapse was defined as an unequivocal new paralysis of a limb or tail, recurrent paralysis of a previously paralyzed limb or tail, or the development of ataxia (inability to right in the absence of appendicular weakness). Weight loss and incontinence without any of the above findings was not recorded as a relapse. At the onset of the first clinical paralytic sign, and weekly thereafter, animals were bled by tail vein for serial antibody measurements. Animals were sacrificed on day 130 and the brain and spinal cord were obtained for histopathology.

Induction of Acute EAE. 5 mg of mouse spinal cord homogenate in 0.1 ml complete Freund's adjuvant containing 400 μg of H37RA mycobacteria was injected into the hind foot pads. Immediately after each immunization and 48 h later, Bordetella pertussis (30 $\times$ 10^9 organism in 0.5 ml PBS) was injected via tail vein. Animals were weighed daily and observed every 8 h from day 10. At the first sign of clinical disease (which was on day 12) animals were divided into two groups. All animals in cages 1–3, received 0.5 ml of an ascitic fluid (containing 1.5 mg IgG) preparation of monoclonal antibody 10.3.6 (anti–I-A^s) intraperitoneally daily. This was continued until day 20. Animals in cages 4–6, received intraperitoneal injections of a similar volume of PBS for the same duration. In a follow-up, control animals were treated with an irrelevant anti-I-A^d monoclonal antibody (BP107), with results similar to those of PBS treatment. Animals were weighed daily and changes in clinical status were observed and recorded. Gradation of disease was scored according to the clinical status as follows: 0, no evidence of clinical disease; 1, paralysis of tail with no paralysis of lower extremities; 2, paralysis of tail with weakness of one or more lower extremities; 3, complete paralysis of lower extremities; 4, moribund; *, mice died during acute attack. Animals were sacrificed on day 27 and histology of brain and cervical spinal cord was evaluated as described previously (1).

Measurement of Antibody to Myelin Basic Protein

A microtiter ELISA assay was adopted to measure the antibody titer to MBP. Briefly, to titrate anti-MBP antibody, 2.5 μg of bovine basic protein (an amount in antigen excess) diluted in 50 μl phosphate buffer (pH 6.0) was coated on to Falcon polystyrene plates (Oxnard, CA) and incubated for 2 h. The plates were rinsed with wash solution containing 0.9% NaCl and 0.05% Tween. The wells were then serially incubated for 1 h with 0.2% bovine serum albumin (BSA), mouse anti-MBP serum (dilution 1:250, 1:500, or 1:2,000 in PO$_4$-Tween solution) and 1% horse serum in PBS, with rinsing in between incubation. Similar results were obtained at all three dilutions. Control wells were incubated with normal mouse serum (diluted either 1:250, 1:500, or 1:2,000). Beta galactosidase conjugated sheep anti-mouse antibody (Fab)$_2$ (Bethesda Research Laboratories, Gaithersburg, MD) diluted 1:200 in PO$_4$-Tween buffer was then added and incubated again for 1 h. The plates were rinsed and the substrate, p-nitrophenyl B-D-galactopyranoside was added. The degree of substrate catalysis was determined from absorbance at 405 nM using an automated spectrophotometer.

Results

To determine whether anti I-A^s antibody would alter the progression of chronic EAE in SJL mice (H-2^s), we followed the clinical course of anti-I-A^s antibody treated and untreated animals for 4½ mo. Table I describes features of the clinical course of the disease in two groups of animals. Treatment was begun at the earliest onset of clinical signs: group I (the treatment group) received monoclonal anti–I-A^s antibody weekly while group II (the control) received either a non–cross-reactive monoclonal antibody (BP 107, anti I-A^d) (9 animals) or no treatment at all (14 animals). Five animals (21%) died during the acute attack in group II, while there was no mortality in the anti–I-A^s treated group.

TABLE I

Clinical Features of Anti–I-A Antibody Treatment in Chronic Relapsing EAE

Initial attack	Number of mice	Date of onset (d) (mean ± SD)	Mortality
Group I	18	32 ± 9	0/18
Group II	23	32 ± 12	5/23
First relapse			
Group I	5/18*	78 ± 14‡	0/5
Group II	12/18*	61 ± 20‡	2/12
Second relapse			
Group I	2/18	105 ± 12	0/2
Group II	5/16§	99 ± 16	0/5
Cumulative totals	*Number of relapses*	*Mortality at day 130*	
Group I	7$^\parallel$	0/18*	
Group II	18$^\parallel$	7/23*	

* $P < 0.04$.
$\ddagger$ $P < 0.08$.
$\S$ One mouse had a third relapse.
$\parallel$ $P < 0.001$.

Five initial relapses were observed in the treated group (Group I), while of the surviving animals in the control (Group II) 12 mice had an initial relapse, $\chi^2 = 4.007$ ($P < 0.04$). At day 130 there was a cumulative total of 18 relapses in group II, while in group I only 7 relapses were seen, $\chi^2 = 13.1$ ($P < 0.001$). There was no mortality (0/18) in the I-A^s treated group, while mice that received anti-I-A^d antibody or no treatment had a 30% (7/23) mortality, $\chi^2 = 4.34$ ($P < 0.04$). Animals that received such long-term therapy showed no obvious deleterious effects.

Weekly measurement of antibody to myelin basic protein (MBP) showed a decrease in antibody levels in animals that received anti-I-A^s antibody (Fig. 1). This reduction in levels occurred 5 wk after the start of treatment and continued throughout the period of observation. In individual animals the level of antibody did not correlate clearly with changes in the clinical state. When the animal was bled in temporal proximity to a clinical relapse, there was no consistent change in antibody levels compared with the previous weeks.

Histopathology of brain showed qualitative differences between control and treated groups. Animals that received anti-I-A^s antibody had fewer parenchymal infiltrates and a lesser degree of demyelination (S. DeArmond, S. Sriram, and L. Steinman, manuscript in preparation).

In order to test the efficacy of anti-I-A antibody in acute EAE, monoclonal anti-I-A^s antibody was administered during the first signs of acute clinical disease. Beginning on day 11, mice were observed every 8 h. While most animals developed tail weakness as the first sign, six animals displayed hind limb paralysis and three animals became moribund after appearing normal 8 h earlier. Treatment with anti-I-A^s antibody produced striking clinical improvement. With the exception of two animals that were already moribund at the start of anti-I-A^s antibody therapy, all other animals that received anti-I-A^s antibody showed no features of clinical progression (Fig. 2). Animals in clinical stages II and III showed a dramatic improvement within 24–48 h, and by 72 h they all appeared clinically well. After 1 wk of such therapy, and then observation for one more week, no mice relapsed or developed disease de novo. Mice that did not receive
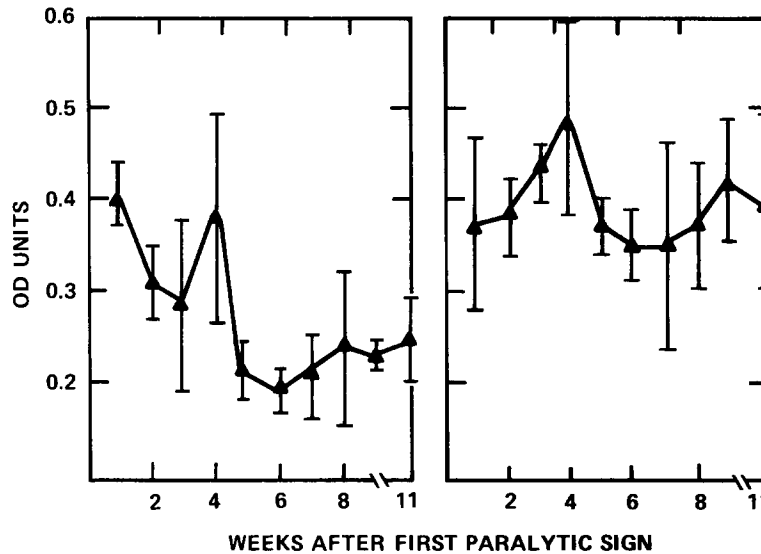
FIGURE 1. Antibody levels to MBP in animals with chronic relapsing EAE after onset of paralytic signs. Left panel shows antibody levels to MBP in animals that received weekly injections of anti–I-A^s antibody. Right panel shows levels in control animals. (Serum dilution 1:250).

the appropriate monoclonal antibody, getting either PBS or the irrelevant monoclonal antibody anti–I-A^d, showed the characteristic features of acute EAE. The degree of severity of the paralysis increased on days 13 and 14 with gradual recovery by the end of 1 wk. At the time of termination of the experiment, all eight animals in the treated group were clinically normal. In contrast, in the control group, of the surviving eight animals three were grade II, two were grade I, and three were clinically normal. Weight changes paralleled closely the clinical status of the animals (data not shown). Routine histopathology of the brain and cervical spinal cord showed the presence of inflammatory cells in both treated and untreated groups. Using standard histological criteria (1), no difference was observed between the two groups.

Discussion

These experiments extend earlier observations on the efficacy of in vivo anti–I-A antibody therapy in autoimmune disease (1, 2, 7). The mechanism of action of anti–I-A antibody to IR gene products in the treatment of acute and chronic EAE remains speculative. Since suppression of disease occurs after an established T cell response, it is unlikely, though possible, that suppression works by blocking macrophage presentation of the encephalitogenic determinant to T cells. Administration of anti–I-A antibody also has been shown to induce suppressor cells in vivo (8). Such a mechanism could be involved in explaining the action of anti–I-A^s in chronic EAE.

Although T cells are sufficient to transfer EAE to susceptible animals, the role of B cells in the demyelinative process is less certain. B cells are observed in the inflammatory cuff and oligoclonal bands appear in the CSF of animals with EAE (9, 10). Also, prevention of B cell differentiation in suckling rats leads to a
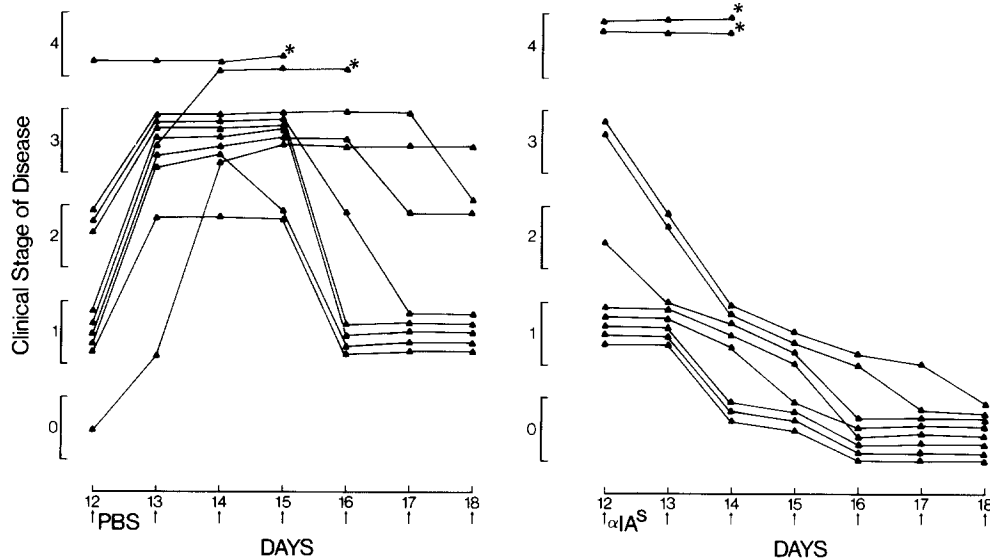
FIGURE 2. Suppression of acute EAE with monoclonal antibody to immune response gene product. Right-hand panel represents the clinical course of animals that received monoclonal anti I-A^s antibody; left-hand panel shows clinical course in control animals. Gradation of clinical disease explained in text.

decrease in the severity of acute EAE (11). Since sensitization to MBP is sufficient to cause relapsing EAE (12), the observed reduction in anti-MBP titers may explain in part the therapeutic effects of anti–I-A treatment.

The cause of the dramatic and rapid reversal of the clinical course in acute EAE merits other considerations. Anti–I-A antibody could block the effector function of I-A-positive cells at the inflammatory site. Macrophages, B and T cells are seen in the perivascular cuff and are shown to be involved in experimental demyelination (9, 13) and multiple sclerosis (14). Thus, although lymphocytes and monocytes are observed in perivascular cuffs in mice that had received anti–I-A^s antibody, it is possible that the effector functions of these cells were impaired. Furthermore, endothelial cells are known to express I-A antigens (15) and may play a role in local cell-mediated immunity.

Several of the known human autoimmune diseases and MS are linked to the IR genes (HLA-D) (16) in man. It is quite likely that the autoantigens that cause EAE are not analogous to those causing human demyelinating disease such as MS. However, both the experimental autoimmune model and multiple sclerosis might share a final common pathway where the putative "antigen(s)" that trigger the pathologic process, are recognized through a common immune response gene product. Suppression of the allelic product associated with disease susceptibility offers a novel means of immunological therapy (7). Such an approach could be tested in man when the appropriate antibodies become available.

Summary

To test the clinical relevance of monoclonal anti–I-A antibody in autoimmune disease, we investigated the effects of such a therapy in acute and chronic relapsing experimental allergic encephalomyelitis (EAE) by instituting treatment after the onset of paralytic signs and following the clinical course. In chronic

relapsing EAE, animals treated with anti–I-A^s antibody had no mortality and fewer relapses when compared with control animals. Antibody levels to myelin basic protein were lower and histopathology showed milder lesions in the treated group. Similarly, in the acute EAE model, animals treated with anti–I-A^s antibody showed a dramatic reversal of paralytic signs and a rapid recovery. The mechanisms of action of antibody to IR gene products in autoimmune disease are discussed.

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