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From Treatment of Experimental Allergic Encephalomyelitis to Clinical Trials in Multiple Sclerosis

Lawrence Steinman, John W. Lindsey, Susan Alters, and Suzanne Hodgkinson
Stanford University School of Medicine, Stanford, California

The CD4 co-receptor on T lymphocytes binds to a nonpolymorphic region of the MHC class II molecule and contributes to the interaction of TCR with the MHC class II molecule. Experiments with animal models of autoimmune disease have indicated that anti-CD4 antibodies are effective in reversing various spontaneous and induced diseases, even in advanced clinical stages in rodents and in nonhuman primates (1–8). Despite the potential risk of opportunistic infection that might ensue following the inactivation or depletion of $CD4^+$ lymphoid cells, a few clinicians have now instituted trials of anti-CD4 in the treatment of several autoimmune conditions including rheumatoid arthritis, multiple sclerosis, psoriasis vulgaris, and systemic lupus erythematosus (9–15).

Brostoff and Mason (1) and our group (2) first demonstrated that administration of a monoclonal antibody against CD4 prevents development of experimental autoimmune encephalomyelitis (EAE). Furthermore, treatment with anti-CD4 reverses EAE when the antibody is given to paralyzed animals. In vivo injection of anti-CD4 selectively depleted CD4-bearing T cells from lymph node and spleen in the mouse EAE model (2) but did not deplete CD4 T cells to an appreciable extent in the rat model (1).

Injection of anti-CD4 prevented the clinical and histologic manifestations of EAE when the antibody was administered after autoimmune T cells capable of transferring EAE had already been generated (see Table 1). Nine days after immunization with mouse spinal cord homogenate

Table 1 Prevention of EAE with Anti-CD4 Antibody

mAb treatment of MSCH-immunized mice	Day injected	Clinical EAE	Histological EAE
anti-CD4	9,10,11,12,14, 16,18,20,22	0/10 ($p < 0.001$)	1/6
anti-CD4	9,10,11,12	8/18 ($p < 0.02$)	1/8
anti-CD4	−2,−1,1	4/15 ($p < 0.002$)	0/9
anti-CD 8	−2,−1,1	8/9	5/5
anti-CD8	9,10,11,12	17/19	11/12
PBS	−2,−1,1	26/30	13/13

Treatment with anti-CD4 was effective even when mice were injected with the antibody after the first signs of EAE were apparent (on days 12–14). In this protocol, mice were observed daily and were randomly selected to receive anti-CD4 or saline injection once the first signs of EAE (tail weakness, paraparesis, and weight loss) appeared. Unlike the control mice, the anti-CD4 treated mice did not progress to hind limb paralysis, quadriplegia, or death, and by 72 hours after the initiation of anti-CD4 treatment, 90% of the treated mice showed clinical improvement with no residual neurologic deficit (Table 2). Treatment of quadriplegic or moribund mice with anti-CD4 did not ameliorate paralysis or prevent death.

Table 2 Reversal of EAE with Anti-CD4 Monoclonal Antibody

	Paralyzed mice				
		72 h after RX			
Treatment	Prior to RX, mild	Normal	Mild	Severe	Dead
Anti-CD4	16	14	1	1	1
PBS	16	1	2	13	6

(MSCH), mice have already developed a T-cell population that can transfer EAE to naive recipients. Such MSCH-immunized mice fail to develop EAE when injected repeatedly with anti-CD4 beginning on day 9. When anti-CD4 was injected on the two days preceding and the day following immunization for induction of EAE, no mice exhibited disease 2 weeks later—a time when nearly 90% of saline-injected controls were paralyzed (see Table 1). Similar treatments with a monoclonal anti-CD8 antibody, which does not bind to CD4 peripheral T cells, did not significantly influence the incidence of EAE (see Table 2).

To determine whether immunoglobulin isotype plays a role in the therapy of EAE with anti-CD4 antibody, an isotype switch variant family

of the mouse IgG1 antirat CD4 antibody, known as W3/25, was isolated with the fluorescence-activated cell sorter. The IgG1, IgG2b, and IgG2a W3/25 isotype variants all had identical binding capacities for rat $CD4^+$ T cells (16). Although all three W3/25 isotypes showed some beneficial effects in the amelioration of EAE, the IgG1 and IgG2a W3/25 antibodies were superior to the IgG2b W3/25 in the treatment of EAE. Multiparameter fluorescence-activated cell sorter analysis of T-cell subpopulations from treated rats showed that none of the antibodies of the W3/25 isotype switch variant family substantially depleted $CD4^+$ target cells in vivo. These experiments demonstrate that immunoglobulin isotype is important in the monoclonal antibody therapy of autoimmune disease. They indicate that therapy of EAE may be successful without a major depletion of $CD4^+$ lymphocytes. Immunotherapy may be optimized by selecting an appropriate isotype of a monoclonal antibody (16).

Optimization of the therapy of EAE with anti-CD4 antibodies was studied in finer detail using a family of molecules derived from anti-CD4 V regions, using the rat antimouse CD4 antibody GK1.5, and mouse IgG1, IgG2a and b, and IgG3 constant regions. We investigated further the role of isotype in anti-CD4 therapy. The rat-mouse chimeric antibodies are specific for murine CD4 and with identical binding curves as native GK 1.5 on $CD4^+$ T cells. The chimeric GK1.5 IgG2a, GK1.5 IgG2b, and GK1.5 and IgG3 antibodies are more efficient than rat GK1.5 at complement-mediated cytotoxicity. This is attributed to the enhanced capacity of the chimeric antibodies, compared to rat GK1.5, to lyse $CD4^+$ cells with a low cell surface antigen density. The GK1.5 IgG3 antibody does not deplete CD4 antibodies when administered in vivo, while the chimeric IgG1, IgG2a and IgG2b constructs were more efficient in vivo (depleted better at lower doses) than the native GK 1.5 antibody (17,18).

In our initial studies (2) demonstrating that GK1.5 could reverse EAE, a total of 500 μg of antibody was injected into diseased mice, 300 μg after the mice exhibited mild EAE, and 100 μg on each of the 2 days following the initial treatment. Ninety percent depletion of splenic $CD4^+$ cells occurred with as little as 100 μg of antibody, therefore, the ability of lower doses of GK1.5 to reverse EAE was examined. Adjusting the dose downward we saw that one dose of 100 μg of GK1.5 was sufficient to induce complete reversal of EAE. However, mice who received a single dose of 25 μg of GK1.5 showed only marginal improvement relative to the control group.

One dose of 100 μg of GK1.5 appeared to be effective at curing EAE, therefore, this dose was chosen for additional EAE experiments using the GK1.5 chimeric antibodies. The results of these experiments are shown in Table 3.

Table 3 Effect of Anti-CD4 on Treatment of EAE

A.	Grade	Clinical Symptoms
	0	No abnormality
	1	Decreased tail and body tone
	2	Clumsy but otherwise normal gait
	3	Weakness of one or more limbs
	4	Monoplegia or paraplegia
	5	Death

		Clinical Disease			
B.	Antibody (dose)	Grade 2	Recovered	Improved	Worse
	GK1.5 (100 μg)	2.9	5/8	2/8	1/8
	GK1.5 (25 μg)	2.8	1/8	2/8	5/8
	Control (100 μg)	2.8	0/8	1/8	7/8

As can be seen, 100 μg of GK1.5 was sufficient to reverse EAE. Mice treated with 100 μg of GK1.5 IgG1 or GK1.5 IgG2a also recovered from EAE. It appeared that GK1.5 IgG2a was slightly more effective than GK1.5; this might have been due to the faster depletion of $CD4^+$ cells seen with GK1.5 IgG2a, relative to rat GK1.5. In contrast to GK1.5, GK1.5 IgG1, and GK1.5 IgG2a, GK1.5 IgG3 was not effective. The number of mice who recovered after treatment with GK1.5 IgG3 was not significantly different from mice treated with an irrelevant control antibody. Although mice treated with GK1.5 IgG3 did not show significant recovery, they did show marginal improvement 72 hours posttreatment. These experiments emphasize the critical role of optimal isotype in therapeutic trials of anti-CD4.

A low dose of rat GK1.5 (25 μg) was not effective in treatment of EAE. However, since the initial results indicated that depletion of $CD4^+$ cells correlated with therapeutic efficacy, and since 25 μg of GK1.5 IgG1, GK1.5 IgG2a, and GK1.5 IgG2b depleted a significantly greater percentage of cells than rat GK1.5, the ability of a low dose of the chimeric antibodies to treat EAE was examined. In contrast to the results with low-dose GK1.5, mice treated with 25 μg of either GK1.5 IgG2a or GK1.5 IgG2b showed significant improvement relative to the control group (Table 4). This was consistent with the notion that depletion of $CD4^+$ cells correlated with therapeutic efficacy. As expected, treatment with the nondepleting GK1.5 IgG3 antibody was not effective. However, treatment with 25 μg of GK1.5

Table 4 Effect of Anti-CD4 Isotype on Treatment of EAE

	Clinical disease			
Antibody	Grade 2	Recovered (p)	Improved	Worse
GK1.5	2.6	8/18 (<0.05)	8/18	2/18
GK1.5γ1	2.8	5/9 (<0.05)	3/9	1/9
GK1.5γ2a	2.7	6/10 (<0.01)	3/10	1/10
GK1.5γ3	2.4	2/9 (N.S.)	5/9	2/9
Control	2.7	1/18	4/18	13/18

IgG1, which at this dose depleted approximately 85% of $CD4^+$ cells, was also not effective.

Recently the first trials with anti-CD4 in human autoimmune diseases have been reported (9–15). Patients with multiple sclerosis tolerated infusions of murine anti-CD4 antibody without side effects (10). In rheumatoid arthritis patients, treatment with the anti-CD4 murine monoclonal antibody M-T151 was well tolerated and some measures of clinical function indicated improvement (9). However, typical of murine monoclonal antibodies, a short free circulating half-life (8 h) and a human antimouse antibody (HAMA) response were observed (9,10). Similar HAMA responses were seen by Hafler and colleagues when murine anti-CD4 was tried for treatment of chronic progressive MS.

In Hafler's study chronic progressive MS patients received five daily infusions (0.2 mg/kg/d) of either anti-CD2 or anti-CD4 murine monoclonal antibodies. It was found that the in vivo anti–T-cell monoclonal antibody infusion suppressed in vitro measurements of the immune response. The anti-CD2 monoclonal antibody blocked T-cell activation by way of the CD2 sheep red blood cell binding protein. The anti-CD4 antibody infusions reduced the in vitro pokeweed mitogen induction of immunoglobulin synthesis. Eighteen hours after the first anti-CD4 infusion, there was an approximately 50% decrease in circulating $CD4^+$ lymphocytes accompanied by a twofold increase in the percentage of circulating $CD8^+$ cells (10).

Based on these results with murine anti-CD4 antibodies, a concerted effort to produce chimeric anti-CD4 antibodies was undertaken by Rietmuller and colleagues at Centocor. Repeated therapy with murine monoclonal antibodies in humans has been limited by the development of a humoral antimurine immune response (19–22). This antimurine immune response may block the monoclonal antibody from binding to its target and thus diminish the antibody's therapeutic effect. In addition, devel-

opment of an antimurine immune response increases the likelihood of serious toxicity, due to the formation of immune complexes. In the case of OKT3, antimurine responses have been directed both at the constant region and at the idiotypic portions of the variable region (23). Similar results have been noted with the use of anti-CD5 and with equivalent antibodies (19,20).

To address these concerns, a chimeric murine-human anti-CD4 monoclonal antibody (chimeric M-T412) was developed with high affinity, epitope specificity for CD4 and the capacity to inhibit T-helper functions, similar to MT151 used in the aforementioned rheumatoid arthritis trial (9). The chimeric M-T412 consists of the antigen-binding V regions of the murine anti-CD4 antibody M-T412, and the constant regions of a human IgG1, kappa immunoglobulin. Results in early phase I trials in rheumatoid arthritis have recently been reported (13–15).

Treatment with chimeric anti-CD4 in refractory rheumatoid arthritis patients induced a sustained depression of circulating CD4 T cells, but was well tolerated without opportunistic infections in 58 patients followed for over 6 months. In some patients there was a reduction in joint swelling and tender joint counts. We are currently evaluating a phase I open label study on 29 patients with chronic progressive MS.

Anti-CD4 therapy has great potential for the treatment of autoimmune disease. If toxicity is not a problem, and if repeated doses do not elicit limiting HAMA responses, then the role of CD4 cells in autoimmune diseases in humans can be directly analyzed. Given the success in animal models of autoimmune disease, ablation of CD4 T cells in autoimmune disease in humans might provide benefits.

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