Therapy of Autoimmune Diseases with Antibody to Immune Response Gene Products or to T-Cell Surface Markers[a]

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Over the past five years, the efficacy of monoclonal antibodies for the treatment of autoimmune disease has been demonstrated. Monoclonal antibodies directed against products of the immune response (IR) genes or against T-cell structures involved with recognition of these IR gene products are extraordinarily successful therapeutic agents against autoimmune diseases in experimental animals. In five models of autoimmune disease, monoclonal antibodies against products of the I-A subregion of the major histocompatibility complex or against the L3T4a molecule on helper/inducer T cells either prevented development of clinical signs (when given prior to autoimmunization) or reversed ongoing disease (when administered after clinical signs were apparent).

TREATMENT WITH MONOCLONAL ANTI-I-A ANTIBODIES

Experimental allergic encephalitis (EAE) is an inflammatory disease of the central nervous system resulting in clinical paralysis. One of the primary pathologic events is the development of autoreactive T cells to myelin basic protein (MBP). Susceptibility to EAE and immune responsiveness to MBP is under control of IR genes in a variety of species. In the mouse, we have demonstrated that T-cell clones, which recognize MBP in the context of Ia molecules, can induce EAE.[1] In TABLE 1, we demonstrate that the encephalitogenic T-cell clone, designated 1_1, derived from immunization of SJL/J mice with bovine MBP, responds to MBP only when MBP is presented on spleen cells that express I-A^s.

The linkage of EAE to IR genes can be more precisely analyzed with the strains, PL/J(H-2^u) and (PL/J × SJL/J)F_1 [(PLSJ)F_1]. Following sensitization to guinea pig MBP, inbred mouse strains of SJL/J(H-2^s), PL/J(H-2^u), and (PLSJ)F_1 mice are all found to be susceptible to EAE.[2] Whereas sensitization to the N-terminal 1–37 amino-acid peptide of MBP induces EAE in PL/J mice, immunization to the C-terminal 89–169 peptide of MBP leads to EAE in SJL/J mice.[2] The immune response to MBP in the (PLSJ)F_1 is not codominant, with sensitization to the

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N-terminal (but not the C-terminal) peptide inducing EAE.[2,3] We asked whether T-cell clones raised in $(PLSJ)F_1$ following sensitization to rat MBP would be reactive to N-terminal or C-terminal peptides on MBP and whether they would be restricted to Ia^s, Ia^u, or $Ia^{s/u}$ molecules. We showed that reactivity to self(mouse)-MBP occurs in all clones restricted to $I\text{-}A^u$ or $I\text{-}E^{s/u}$. These mouse MBP reactive clones respond to the N-terminal peptide 1–37. An $I\text{-}A^u$ restricted T-cell clone recognizing peptide 1–37 of MBP induces both classic clinical and histological EAE in the $(PLSJ)F_1$ mouse.[4] The clone-induced disease has three forms, namely, acute and fatal, chronic and stable, and relapsing and remitting. Demyelination is evident histologically, in addition to perivascular cuffing.

Because susceptibility to EAE is controlled in part by IR genes mapping to the I-A subregion, we first asked whether anti-I-A antibodies might prevent acute EAE. Acute EAE is a monophasic disease with high mortality. A small change in immunization protocol produces a chronic relapsing form of EAE (CR-EAE) that resembles multiple sclerosis (MS) in clinical presentation and histology. Like susceptibility to EAE, susceptibility to MS is associated with class II major histocompatibility gene products.

TABLE 1. MHC-Restricted Proliferation of Bovine BP SJL/J Clone 1_1

Strain of APC	I-A Haplotype	Antigen	
		None	BOV BP 100 μg/ml
SJL/J	(s)	1081 ± 778[a]	101,268 ± 15,695
B10.S(9R)	(s)	256 ± 177	29,550 ± 5984
A.TH	(s)	310 ± 320	104,509 ± 10,791
B10.HTT	(s)	91 ± 28	29,811 ± 5012
BALB/c	(d)	3576 ± 1266	6329 ± 1932
C57BL/6	(b)	130 ± 43	524 ± 148
C3H	(k)	349 ± 80	405 ± 129
PL/J	(u)	903 ± 440	1988 ± 1020

[a]Values represent mean ± SD of ^{3}H-thymidine cpm incorporated in quadruplicate cultures.

Prevention of acute EAE was demonstrated in SJL/J ($H\text{-}2^s$) mice by administering antibodies reactive with $I\text{-}A^s$ prior to immunization with spinal-cord antigen.[5] Clinical disease was evident in 3/28 mice that received anti-$I\text{-}A^s$ antibodies, compared to 19/28 mice that received a noncross-reactive anti-$I\text{-}A^k$ antibody ($p < 0.0001$). Surprisingly, histologic disease was apparent in most of the mice that received anti-$I\text{-}A^s$, though the lesions were not as extensive. This observation indicated that although disease was prevented, autoimmunization to myelin antigens with subsequent entry of cells into the central nervous systems had occurred. Proliferation of T cells to MBP can be demonstrated in MBP-immunized, anti-$I\text{-}A^s$ treated mice, and this corroborates our view that following immunization with MBP in complete Freund's adjuvant (CFA), autoimmunization is not prevented. In spite of the generation of autoreactive T lymphocytes, there was decreased migration of lymphocytes to the central nervous system in mice treated with $I\text{-}A^s$.[6] This was demonstrated by injecting ^{51}Cr-labeled lymphocytes intravenously into mice. The mice had been injected with anti-$I\text{-}A^s$ prior to immunization with spinal cord in CFA. The homing of ^{51}Cr lymphocytes to brain and spinal cord was clearly diminished in mice given anti-$I\text{-}A^s$ (TABLE 2).

In order to be clinically relevant, any putative therapy must be successful when given after disease onset. Thus, we investigated whether treatment with anti-I-A

TABLE 2. Accumulation of ^{51}Cr-Lymphocytes in Spinal Cords of Mice Treated with Anti-I-A^s

Treatment	Clinical EAE			Mean Weight Loss
	Mild	Severe	Total	
Anti-I-A^s	1/20	3/20	4/20[a]	−0.3 ± 2.2 g[b]
Anti-I-A^d	5/40	15/40	20/40[a]	−1.1 ± 2.0 g[b]

Treatment	^{51}Cr Accumulation (lymph-node cells)
Anti-I-A^s (clinically well)	6160 ± 1308[c]
Anti-I-A^d (clinically well)	9357 ± 4239
Anti-I-A^d (severe disease)	24,751 ± 4331[c]

[a] $\chi^2 = 3.84$ with continuity correction; $p < 0.05$.
[b] $p < 0.07$, Student's t test.
[c] $p < 0.00001$, Student's t test.

antibodies might prove effective in mitigating ongoing acute EAE and CR-EAE.[7] Anti-I-A antibodies were administered at the onset of paralytic signs and the subsequent clinical course was followed (see TABLE 3). In CR-EAE, mice first displayed the initial attack of paralysis around day 32. These SJL/L mice were then given either anti-I-A^s (0.6 mg weekly, intraperitoneally) or a control monoclonal anti-I-A that does not cross-react with I-A^s. During a 4½-month observation period, 18 clinical relapses were seen in a control group containing 18 mice, while 7 relapses were seen in a group of 18 mice given anti-I-A^s ($p < 0.001$). There was no mortality (0/18) in the anti-I-A^s treated group, while control mice had 7/23 deaths ($p < 0.04$), with 5 occurring in the first attack. Weekly measurement of antibody to MBP revealed a decrease in anti-MBP levels in mice given anti-I-A^s. The reduction occurred five weeks after treatment started and continued throughout the full period of observations. In acute EAE, mice were treated with anti-I-A antibody at the first signs of paralysis.

TABLE 3. Clinical Features of Anti-I-A Antibody Treatment in Chronic Relapsing EAE

	Number of Mice	Date of Onset (d) (mean ± S.D.)	Mortality
Initial Attack			
Group I	18	32 ± 9	0/18
Group II	23	32 ± 12	5/23
First Relapse			
Group I	5/18[a]	78 ± 14[b]	0/5
Group II	12/18[a]	61 ± 20[b]	2/12
Second Relapse			
Group I	2/18	105 ± 12	0/2
Group II	5/16[c]	99 ± 16	0/5
Cumulative Totals	Number of Relapses		Mortality at Day 130
Group I	7[d]		0/18[a]
Group II	18[d]		7/23[a]

[a] $p < 0.04$.
[b] $p < 0.08$.
[c] One mouse had a third relapse.
[d] $p < 0.001$.

These mice showed a dramatic reversal of paralytic signs and a rapid recovery, sometimes over a period of as little as a few hours.[3]

These experiments with acute EAE and CR-EAE extend our view that anti-I-A therapy acts after the generation of autoreactive effector T lymphocytes. Though we can demonstrate that anti-I-A antibody blocks macrophage presentation of myelin basic protein to T-cell clones in an *in vitro* presentation assay, it is unlikely that this is the only mechanism of action for anti-I-A.[2,4] *In vivo* administration of anti-I-A antibody can induce suppressor T cells that abrogate a delayed type hypersensitivity response to tumor antigens.[8] Anti-I-A treatment may also lead to the induction of suppressor T cells that attenuate the autoimmune response to MBP.[9] However, the extreme rapidity of action of anti-I-A in acute EAE indicates that it probably plays a role in suppressing the immune response at the sites of inflammation. Thus, although lymphocytes and monocytes are observed in the central nervous system in mice given anti-I-A^s antibody,[5,7] it is possible that their function is impaired or altered. Cerebral capillary endothelial cells may play an important role in the pathogenesis of EAE. These endothelial cells express I-A antigens in guinea pigs with EAE.[10] Following the binding of anti-I-A antibody to I-A positive endothelial cells, there may be an alteration in endothelial function with attenuation of local immune reactivity.

We have studied anti-I-A therapy in experimental autoimmune myasthenia gravis (EAMG), which is a model for human myasthenia gravis where antibodies to

TABLE 4. Anti-I-A Induces Suppressor T Cells

Cell Transferred	Antibody Titer (Anti-AChR Standard)	% Inhibition
Anti-I-A^sT	3.7 ± 1.7	66
Anti-I-A^sT + Anti-Thy + C	9.0 ± 1.3	17
Anti-I-A^dT	11.4 ± 1.2	−6
Anti-I-A^dT + Anti-Thy + C	8.5 ± 1.1	21
No Monoclonal	10.8 ± 1.7	0

acetylcholine receptor (AChR) impair neuromuscular transmission by mediating the loss of AChR in the postsynaptic membrane. Increased susceptibility to myasthenia gravis is associated with HLA-DR3 in man, while in the mouse, immune responses to AChR map to the I-A region.

In two different strains of mice susceptible to EAMG, treatment with the appropriate strain-specific monoclonal anti-I-A abolished antibody responses to AChR, while the irrelevant anti-I-A had no effect.[11] Clinical manifestations of EAMG appeared to be suppressed. In addition, while antibody and proliferation responses to AChR were reduced in anti-I-A treated mice, reactivity to the purified protein derivative of tuberculin remained intact. Thus, there seems to be some specificity in the immune suppression that is induced by anti-I-A treatment.

It can be demonstrated, with an adoptive transfer system, that *in vivo* injection of anti-I-A induces a suppressor T cell capable of suppressing the antibody response to AChR. Thus, lethally irradiated SJL/J mice were reconstituted with AChR-primed spleen cells plus T cells from other groups of SJL mice that were given a primary and secondary immunization with AChR and given either (1) *in vivo* anti-I-A^s, (2) an irrelevant monoclonal anti-I-A^d, or (3) no monoclonal. In TABLE 4, it can be seen that T cells from anti-I-A^s treated mice could inhibit anti-AChR titers by 66%, while T cells from anti-I-A^d mice did not inhibit at all. The T suppressor cell was Thy positive.

Unlike EAE and EAMG, the autoimmune syndrome that develops in NZB/W F_1

mice is spontaneous. This disease bears a strong resemblance to the human disease of systemic lupus erythematosus (SLE) and is characterized by antibodies to nuclear antigens and an immune-complex-mediated glomerulonephritis. Susceptibility to SLE in humans is associated with HLA-DR2 and HLA-DR3. In NZB/W F_1 ($H\text{-}2^{d/z}$) mice, a gene closely linked to $H\text{-}2^z$ haplotype is associated with the development of renal disease. Hugh McDevitt and his colleagues, Nancy Adelman and David Watling, studied suppression of NZB/W F_1 disease with anti-I-A antibody. When monoclonal anti-$I\text{-}A^z$ was administered to NZB/W F_1 mice that already exhibited signs of renal disease, there was a 90% increase in survival as compared to control mice receiving no monoclonal antibody.[12] Anti-$I\text{-}A^d$ treatment also significantly increased the survival of treated mice.

Thus, therapy with antibody to IR gene products is clearly helpful in several experimental autoimmune conditions where susceptibility is linked to specific IR genes. These results with anti-I-A have been extended to include prevention of collagen-induced arthritis[13] and experimental autoimmune thyroiditis.[14]

Anti-I-A antibodies have been successful in disease prevention, as well as in ameliorating clinical deficits in acute and chronic situations. Therapy was successful in induced model systems like EAE, EAMG, collagen arthritis,[13] and experimental

TABLE 5. Anti-I-A Treatment Depletes IgM^+, IgD^+ B Cells from Spleen and Lymph Node

Days After Anti-I-A Treatment	IgM^+, IgD^+ Cells (percents)[a]			
	SJL/J Mice		CKB Mice	
	Spleen	Lymph Node	Spleen	Lymph Node
untreated	31 ± 5	10 ± 3	42 ± 6	15 ± 3
2	11 (9,12,12)	3 (2,3,3)	24 (17,30)	10 (10,10)
5	5 ± 1	1 (1,1)	14 ± 3	6 (5,6)
14	18 ± 3	4 ± 2	36 (35,36)	8 (7.8)
49	34 (33,34)	10 (10,10)	36 (35,36)	11 (10,11)

[a]Means and individual mouse values are indicated except if four or more mice were tested, in which case standard deviations are given.

autoimmune thyroiditis,[14] where the disease provoking self-antigens is intentionally administered, and in spontaneous diseases like NZB/W F_1 nephritis, where the etiology of the autoimmune process remains unsolved.

One of the intriguing aspects of anti-I-A therapy is the haplotype specificity of its action. When an anti-$I\text{-}A^k$ monoclonal antibody is administered to a (C3H-CWB)F_1 ($H\text{-}2^{k/b}$), antibody production to the synthetic polypeptide of (H,G)-A--L, which is regulated by $I\text{-}A^k$, is suppressed, while antibody production to (T,G)-A--L, which is controlled by $I\text{-}A^b$, is unaffected.[15] Because most humans are heterozygous at the critical HLA-D loci that play a role in conferring disease susceptibility, haplotype-specific therapy offers the possibility of suppressing autoimmune responses linked to particular alleles at HLA-D region loci without global immune suppression.

A note of caution should be raised regarding human therapy protocols based on injection of anti-Ia antibodies. Fluorescent-activated cell sorter (FACS) multiparameter analysis of the B-cell populations shows that anti-I-A treatment causes severe and prolonged depletions of splenic and lymph-node B cells (TABLE 5). Maximum depletion occurs around five days after treatment and recovery of some B-cell subpopulations is still incomplete one month later.[16] SJL mice are more sensitive to this

TABLE 6. Anti-I-A Treatment Impairs IgG_1 Anti-DNP Responses More Severely than IgG_{2a} and IgG_{2b} Anti-DNP Responses

Treatment[a]	DNP Immunization[b]	Splenic B Cells on Day of Immunization	IgG Anti-DNP Responses[c]		
			IgG1	IgG2b	IgG2a
	Day	Estimated percent	No. impaired/no. tested		
Anti-I-A (Day 0,2,7,9)	1	(24)	8/10	5/10	7/10
	8	(8)	6/10	0/5	2/10
	70	(39)	9/10	0/10	0/5
None	2	(39)	1/10	0/5	0/10
	8	(39)	0/10	0/5	0/10
	70	(39)	0/10	0/5	1/10

[a]Treated animals received 4 mg of anti-I-A antibody (0.5 ml 10-3.6 ascites) on days 0, 2, 7, and 9.

[b]DNP-KLH (100 μg) on alum on day 1 and day 8, and 50 μg of DNP-CGG on alum on day 70.

[c]Mice were bled one week after the first immunization and two weeks after the second and third immunizations. Anti-DNP responses, measured with a solid-phase radioimmune binding assay,[17] were scored as impaired if they were less than two standard deviations below the mean of the appropriate control (non-anti-I-A injected) anti-DNP responses.

B-cell depletion and recover more slowly than CKB mice. Despite the overall depletion of B cells, IgG responses to DNP and to KLH are not as severely impaired for the IgG2a and IgG2b isotypes as they are for the IgG1 isotype (TABLES 6 and 7).

TREATMENT WITH MONOCLONAL ANTIBODIES TO T-CELL SUBSET ANTIGENS

The L3T4 antigen expressed on helper T cells is near the site of the aspect of the T-cell receptor that recognizes I-A. Thus, anti-L3T4 antibody can block antigen-driven activation of antigen-specific T-cell clones.[1,17] We have demonstrated that administration of a mAb (GK1.5) directed against the L3T4 marker present on helper T cells prevents development of EAE. Furthermore, treatment with mAb GK1.5 reverses EAE when the antibody is given to paralyzed animals. *In vivo* injection of mAb GK1.5 also selectively depleted L3T4-bearing helper T cells from lymph node and spleen.[17]

Injection of mAb GK1.5 prevented the clinical and histologic manifestations of

TABLE 7. Impairment of Secondary IgG Anticarrier Responses by Anti-I-A Treatment

Treatment[a]	Immunization[a]	IgG Anti-KLH Responses[a]		
		IgG1	IgG2b	IgG2a
		No. impaired/no. tested		
Anti-I-A	DNP-KLH twice	10/10	4/5	5/10
None	DNP-KLH twice	0/10	0/5	1/10

[a]Treatment, immunization, and bleed schedules, as well as responses and scoring, are described in the footnote for TABLE 6.

EAE when the antibody was administered after autoimmune T cells capable of transferring EAE had already been generated (TABLE 8). Nine days after immunization with mouse spinal-cord homogenate (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 mAb GK1.5 beginning on day 9. When mAb GK1.5 was injected on the two days preceding and the day following immunization for induction of EAE, no mice exhibited disease two weeks later—a time when nearly 90% of saline-injected controls were paralyzed (TABLE 8). Similar treatments with a monoclonal anti-Lyt2 antibody, which does not bind to L3T4$^+$

TABLE 8. Prevention of Experimental Allergic Encephalomyelitis with mAb GK1.5[a]

Monoclonal Antibody Treatment of MSCH-Immunized Mice (day 0)		Clinical Disease			Perivascular Cuffs
Monoclonal Antibody[b]	Injection days %	Cumulative Incidence[c]	Significance[d] (p value)	Mean Onset[e] (day)	Frequency[f]
GK1.5 (anti-L3T4)	9, 10, 11, 12 12, 14, 16, 18, 20, 22	0/10	0.001	—	1/6
(anti-L3T4)	9, 10, 11, 12	8/18	0.02	19	1/8
(anti-L3T4)	−2, −1, 1	4/15	0.002	27	0/9
53-6.7 (anti-Lyt2)	−2, −1, 1	8/9	n.s.	12	5/5
(anti-Lyt2)	9, 10, 11, 12	17/19	n.s.	14	11/12
none (PBS)	−2, −1, 1	26/30	—	14	13/13

[a]On day 0, all mice were immunized with 5 mg of mouse spinal-cord homogenate (MSCH) in 0.1 ml of a 1:1 emulsion of complete Freund's adjuvant and phosphate-buffered solution (PBS) containing 4 mg/ml H37Ra mycobacteria in the hind footpads. *Bordetella pertussis* (30×10^9) organisms in 0.5 ml PBS were injected into the tail vein before immunization with MSCH on day 0 and again on day 2.

[b]Monoclonal anti-L3T4 and anti-Lyt2 antibodies were purified from culture supernatants of hybridomas GK1.5[17] and 53-6.7[17] grown in serum-free medium HB101. One hundred μg of antibody were given intraperitoneally on each day.

[c]Number of mice sick/total. Mice were examined at least through day 32. Mice were scored as sick if they exhibited any signs of illness.

[d]p values were computed by comparing the treated groups to the PBS group by utilizing the continuity correction and taking account of multiple comparisons (n.s. = nonsignificant).

[e]The standard deviation for all these values is ± 2 days.

[f]Number of mice with perivascular cuffs/total number of mice examined histologically. Six standard sections of brain and spinal cord were examined for each mouse.[17] Slides were coded and read by an observer who was blind with regard to the treatment protocol.

peripheral T cells, but does bind to suppressor/cytotoxic T cells, did not significantly influence the incidence of EAE (TABLE 8).

Treatment with mAb GK1.5 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 mAb GK1.5 or phosphate-buffered saline (PBS) injection once the first signs of EAE (tail weakness, paraparesis, and weight loss) appeared. Unlike the control mice, the mAb GK1.5 treated mice did not progress to hind-limb paralysis, quadriplegia, or death, and by 72

TABLE 9. Reversal of EAE with mAb GK1.5

Treatment[a]	Number of Mice Exhibiting Clinical Symptoms				
	Before Treatment (mild)[b]	72 Hours After Treatment			
		none	mild	severe	Deaths[c]
mAb GK1.5	16	14	1	1	1
none (PBS)	16	1	2	13	6

[a]Treatment was initiated when mice exhibited mild EAE. At this time, mice received 300 μg of mAb GK1.5 intraperitoneally. Also, 100 μg of mAb GK1.5 were injected on each of the two days following treatment initiation.

[b]The clinical status of the mice was graded according to the following scale: none = no neurologic symptoms or residual tail weakness with weight gain; mild = a flaccid tail and paraparesis with weight loss and poor coat texture; severe = quadriplegia with hind-limb scissoring. The clinical conditions were graded by an observer who was blind with regard to the treatment protocol.

[c]Seven days after treatment initiation.

hours after the initiation of mAb GK1.5 treatment, 90% of the treated mice showed clinical improvement with no residual neurologic deficit (TABLE 9). Treatment of quadriplegic or moribund mice with mAb GK1.5 did not ameliorate paralysis or prevent death.

In TABLE 10, the potency of a monoclonal anti-I-A^s mouse (monoclonal 10-3.6) is compared to the anti-L3T4 antibody (the rat monoclonal GK1.5).

We used multiparameter fluorescent-activated cell sorter (FACS) analyses to investigate the changes in the frequencies of T cells belonging to different T-cell subsets following treatment with mAb GK1.5. We utilized the Ly1 and Lyt2 surface markers. T helper/inducer cells are $Ly1^+Lyt2^-$, while suppressor/cytotoxic cells are $Ly1^+Lyt2^+$. With dual immunofluorescence analyses, these surface markers provide a measure of $L3T4^+$ T-cell frequency since $L3T4^+$ cells are $Ly1^+Lyt2^-$ and $L3T4^-$ cells are $Ly1^+Lyt2^+$.[17]

Anti-L3T4 antibody treatment selectively depletes $L3T4^+$ T cells. Two injections of mAb GK1.5 at 24-hour intervals are sufficient to deplete about half of the splenic $L3T4^+$ cells and nearly all of this T-cell subset from lymph nodes. Similar depletions of the $L3T4^+$ subset occur in mice that have already been immunized for the induction of

TABLE 10. Comparison of Dose Response for Monoclonal Anti-I-A versus Monoclonal Anti-L3T4 in Prevention of EAE

Treatment[a]	Incidence of EAE	Number Dead
0	9/10	1/10
120 μg anti I-A	7/9	5/9
600 μg anti I-A	7/9	2/9
3,000 μg anti I-A	1/7	0/7
15,000 μg anti I-A	0/5	0/5
0	7/10	1/10
5 μg anti L3T4	7/8	1/8
20 μg anti L3T4	4/8	4/8
100 μg anti L3T4	0/8	0/8
500 μg anti L3T4	0/8	0/8

[a]One injection of mAb i.p. on the day prior to immunization with MSCH-CFA. Anti-I-A employed was 10-3.6, anti-I-A^s. Anti-L3T4 was GK1.5.

EAE and treated with mAb GK1.5 on days 9–12. The T-cell depletion was specific for the $L3T4^+$ subset since the numbers of $Ly1^+Lyt2^+$ ($L3T4^-$ T cells) were not altered by the mAb GK1.5 treatment. Interestingly, although nearly all thymocytes express L3T4,[17] the percentage of $L3T4^+$ thymocytes was not significantly changed in treated mice.

Similar results with anti-L3T4 antibodies in the suppression of NZB/W F_1 disease were reported by Seamens and Wofsey[18] and similar results in suppression of EAE in rats with W3/25 were reported by Brostoff and Mason.[19] These results in EAE and in NZB/W F_1 disease suggest that manipulation of the human equivalent of the $L3T4^+$ T-cell subset (namely, Leu-3 or OkT-4 T cells) with monoclonal antibodies may provide effective therapy for autoimmune diseases mediated by this cell subset.

Before either anti-Ia or anti-L3T4 antibody therapy can be tried in humans, the potential toxicities of these antibodies must be evaluated. Preliminary studies indicate that although treatment with anti-I-A antibodies substantially depletes B cells,[16] anamnestic antibody responses remain intact in treated mice. In addition, for therapy of chronic disease states in humans with these mouse monoclonal antibodies, overcoming the anti-mouse response may be critical. For this purpose, chimeric immunoglobulins, combining a human constant region with a mouse variable region,[20] may be a solution. We are also generating isotype switch variants of monoclonal antibodies for use in therapy.

Therapeutic trials with either anti-Ia or anti-Leu-3 antibodies for diseases like multiple sclerosis are currently being planned. Testing with anti-Ia and anti-Leu-3 reagents in the treatment of EAE in monkeys is under way. If the results seen in animal models prove relevant, then the promise of these therapies would seem great.

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DISCUSSION OF THE PAPER

UNIDENTIFIED DISCUSSANT: Do you think you are simply removing the cells responsible for inducing the disease or might you perhaps be allowing for the development of Ly2–3 positive cells that are involved in suppression?

S. SRIRAM (*Stanford University School of Medicine, Stanford, CA*): If you immunize animals that have been pretreated with anti-L3T4 with tetanus toxoid and abrogate the primary anti-tetanus antibody response, the secondary anti-tetanus is nevertheless intact. Therefore, I do not think that I am immunoregulating them with an Ly2-positive suppressor cell population.

UNIDENTIFIED DISCUSSANT: Have you checked to see if there is a compromise of the immune response against cells infected by virus when you use anti-Ia antibodies?

SRIRAM: I have not yet tested them against the antibody response to a specific virus like flu or measles. However, the animals that lived for four or five months following Ia

treatment did not succumb to any unusual illnesses. The same is true of animals that received anti-L3T4. If you challenge them with tetanus toxoid when they are T4 deleted, antibody titers are lowered, but still present.

D. HAFLER (*Harvard Medical School, Boston, MA*): I have treated a number of MS patients with anti-T11, anti-T4 monoclonal antibodies. The major problem has been that the antibodies attach to the T-cell surface and remain there. Have you observed this in your animals?

SRIRAM: We do not find it.

UNIDENTIFIED DISCUSSANT: Is it true that monkeys given anti-Ia antibody died?

SRIRAM: I think I will ask E. A. Clark to answer this question because he is involved in these experiments.

E. A. CLARK (*University of Washington, Seattle, WA*): Three or four years ago Paul Martin at the Fred Hutchinson Cancer Center had given quite a few monkeys anti-Ia antibodies with no effect. We have given anti-Ia antibodies of all different types to primates with no toxic effect and are in the process of testing their efficacy in EAE in nonhuman primates. Therefore, I think that the report by Billings and Terisaki might have had some technical problems.