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MECHANISMS OF ANTI-CD4-MEDIATED DEPLETION AND IMMUNOTHERAPY

A Study Using a Set of Chimeric Anti-CD4 Antibodies[1]

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A family of rat-mouse chimeric anti-murine CD4 antibodies was used to study the mechanisms of anti-CD4-mediated depletion and immunotherapy. The chimeric antibodies retain identical affinity and specificity as the therapeutically effective prototype antibody, rat GK1.5, but are of different mouse isotypes. GK1.5γ1, GK1.5γ2a, and GK1.5γ2b are significantly more effective at $CD4^+$ cell depletion than rat GK1.5 when low doses of antibody are administered. In contrast, no depletion is seen with GK1.5γ3 at any dose. Depletion of $CD4^+$ cells in vivo is not correlated with either the ability of the antibody to mediate C-dependent cytotoxicity or antibody-dependent cell-mediated cytotoxicity in vitro, implying that additional antibody-mediated cytotoxic mechanisms occur in vivo. The chimeric antibodies were used to investigate the mechanism of GK1.5-mediated immunotherapy in a prototypic model of T cell-mediated autoimmunity, experimental allergic encephalomyelitis. Mice treated with a single dose of 100 μg of either GK1.5, GK1.5γ1, or GK1.5γ2a showed significant recovery within 72 h. In contrast, mice treated with 100 μg of GK1.5γ3 showed only marginal improvement within the first 72 h and regressed within 5 days of treatment initiation. These data suggest that anti-CD4-mediated immunotherapy of murine experimental allergic encephalomyelitis is correlated with depletion of $CD4^+$ cells.

EAE[3] is an inducible autoimmune inflammatory disease of the central nervous system that serves as a murine model for the human disease multiple sclerosis (1). Previous studies have implicated $CD4^+$ Th cells with the development and progression of disease in both EAE (2) and multiple sclerosis (3). These studies were corroborated by experiments that showed that all clinical signs of murine EAE were prevented when a rat anti-murine CD4 mAb GK1.5 (4), was administered before the onset of disease, and were reversed when GK1.5 was administered shortly after the first symptoms of EAE appeared (5). Since this initial demonstration, GK1.5 has been shown to be effective in the treatment of several other autoimmune syndromes including SLE (6), collagen-induced arthritis (7), experimental autoimmune myasthenia gravis (8), and insulin-dependent diabetes mellitis (9, 10). Furthermore, GK1.5 has been shown to prolong allograft survival (11) and to induce tolerance to coadministered antigens (12). This latter effect has also been demonstrated with other anti-murine CD4 antibodies (13).

GK1.5 treatment results in significant, although not complete, depletion of $CD4^+$ T cells in vivo (5, 12, 14–16); however, it is not clear that depletion of $CD4^+$ cells influences the efficacy of the antibody (17). Previous studies addressing the role of depletion of $CD4^+$ cells with non-depleting $F(ab')_2$ fragments of GK1.5 have shown that depletion is not necessary to induce tolerance (18) or suppress autoimmunity in the murine lupus model (17). In these studies repeated administration of $F(ab')_2$ fragments of GK1.5 led to an effective blockade of the CD4 Ag. In our study we address the effects of depletion of $CD4^+$ cells under a therapeutic protocol in which one low dose of antibody is administered. Such a treatment regimen, if effective, may be better suited for human immunotherapy.

We have previously described a family of chimeric rat-mouse GK1.5 antibodies (19) that was designed to provide insight into the possible mechanisms mediated by GK1.5 in cell depletion, the reversal of autoimmune disease, and tolerance induction. The GK1.5 chimeric antibodies, GK1.5γ1, GK1.5γ2a, GK1.5γ2b, and GK1.5γ3, retain identical specificity and affinity for $CD4^+$ T cells as rat GK1.5, but are of different mouse isotypes. With respect to CDC, we have shown that GK1.5γ2a, GK1.5γ2b, and GK1.5γ3 are more effective at lysing $CD4^+$ T cells than rat GK1.5 due to the greater ability of the chimeric antibodies to lyse cells with low cell surface Ag density. GK1.5γ1 does not mediate CDC (19). In this study, we investigate the mechanisms responsible for GK1.5-mediated depletion of $CD4^+$ cells and compare the therapeutic efficacy of GK1.5 and the GK1.5 chimeric antibodies in treating EAE, a prototypic model of T cell-mediated autoimmunity.

MATERIALS AND METHODS

Cells and culture conditions. Cell lines were generated as previously described (19) and maintained in a 7% CO_2 incubator at 37°C.

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[3] Abbreviations used in this paper: EAE, experimental allergic encephalomyelitis, ADCC, antibody dependent cell mediated cytotoxicity, CDC, complement dependent cytotoxicity.

Normal medium contained RPMI 1640 (M.A. Bioproducts, Walkersville, MD) supplemented with 2.0 mM L-glutamine, 1.0 mM sodium pyruvate, and 10% FCS.

Purification of chimeric antibodies. Chimeric antibody was purified from culture supernatant using several chromatography procedures including hydrophobic chromatography, protein A affinity chromatography, anti-bovine Ig affinity chromatography, and molecular size chromatography.

Culture supernatant was harvested and the following added to yield the final concentrations: 0.9 M Na_2SO_4, 0.1% NaN_3, 1 μg/ml aprotinin, 0.2 mM PMSF, and 1.5 M NaCl. The pH was adjusted to 8.5 with 50% Tris base before applying supernatant to the column. The hydrophobic interaction (Sterogene, Arcadia, CA) and protein A (Pharmacia Fine Chemicals, Piscataway, NJ) columns, connected in series, were equilibrated differently for the purification of each antibody isotype. GK1.5γ1 supernatant was equilibrated with 1.5 M glycine, 3M NaCl, 0.9 M Na_2SO_4, pH 9 and GK1.5γ2a, GK1.5γ2b, and GK1.5γ3 supernatants were equilibrated with 0.025 M NaH_2PO_4, 0.1 M NaCl, 0.9 M Na_2SO_4, pH 7. Supernatants were loaded at a flow rate of 0.5 to 2.5 ml/min after which the columns were separated. The protein A column was washed with 1.5 M glycine, 3 M NaCl, pH 9, for GK1.5γ1 supernatant or PBS for GK1.5γ2a, GK1.5γ2b, and GK1.5γ3 supernatants. GK1.5γ1 was eluted with 0.01 M citrate, pH 6, GK1.5γ2a with 0.01 M citrate pH 4.5, and GK1.5γ2b and GK1.5γ3 with 0.01 M citrate pH 5, followed by 0.1 M citrate pH 3. To remove any remaining bovine Ig, each eluate was passed through an Affi-Gel 10 (Bio-Rad, Richmond, CA) column coupled with the IgG fraction of rabbit antiserum against bovine IgG (Sigma Chemical Co., St. Louis, MO). Aggregated antibodies were removed by Superose 6 or 12 chromatography. Antibody was concentrated and sterile filtered through a 0.22 μ low protein binding filter (Millipore, Bedford, MA). Antibody was quantitated by titration on C6VL.1 cells (20) as previously described (19).

Mice. BALB/c or SJL/J mice used in these experiments were females between the ages of 8 to 12 wk purchased from IMR laboratories (Santa Clara, CA) or The Jackson Laboratory (Bar Harbor, ME) respectively.

In vivo depletion studies. BALB/c mice received i.p. injections with 100, 25, 10, or 2 μg of each antibody. After the indicated number of days cell suspensions were made by grinding spleens between two frosted microscope slides in mouse tonicity PBS (0.02 M phosphate, 0.13 M NaCl) supplemented with 5% FCS on ice. Spleen cells were resuspended in 1 ml cold mouse tonicity-PBS-5% FCS and red blood cells lysed by adding 5 ml Gey's solution (21) and incubating on ice for 4 min. The cell suspension was underlayed with FCS and centrifuged at 300 × *g* for 10 min. Cells were washed once in mouse tonicity-PBS-5% FCS, resuspended in staining buffer (PBS, 2% FCS, 0.1% NaN_3) and counted. For staining, 1×10^6 splenocytes were incubated with saturating amounts of the specified fluorochrome coupled antibody for 30 min on ice. The antibodies used vere: anti-mouse CD5 (clone 53.7.3), anti-mouse CD8 (clone 53.6.7), anti-mouse CD4 (GK1.5), anti-rat kappa (MAR 18.5) (Becton Dickinson Immunocytometry Systems, Mountain View, CA), and anti-mouse IgM (D. Buck, Becton Dickinson Monoclonal Center, Mountain View, CA). Cells were washed and analyzed by flow cytometry using a FACScan (Becton Dickinson Immunocytometry Systems) with logarithmic amplifiers. Absolute cell numbers were calculated by multiplying the percentage of cells staining positively by the cell count for individual spleens.

ADCC. Thymuses were obtained from BALB/c mice and cell suspensions were made in normal medium by grinding the thymus between two frosted microscope slides. Thymocytes (1×10^6 cells) in normal medium were labeled with 100 μCi of Na[^{51}Cr] (Amersham Corp., Arlington Heights, IL) in 100 μl sterile PBS. Cells were incubated for 2.5 to 3 h at 37°C. Labeled thymocytes were washed three times in normal medium and were used as target cells for ADCC. Effector cells were either BALB/c splenocytes from which red blood cells had been removed by hypotonic shock (21) or peritoneal exudate cells harvested 3 to 4 days after i.p. injection of 2 ml paraffin oil. Effector cells were washed and resuspended to the appropriate concentration in normal medium.

ADCC assays were performed in 96-well U-bottom plates (Costar, Cambridge, MA). Labeled target cells were resuspended at 2×10^5/ml and 50 μl was added to each well containing antibody. Target cells and antibody were incubated at room temperature for 30 min after which 100 μl of effector cells were added. Effector cells were used at a concentration of 5×10^6/ml yielding an E:T ratio of 50:1. Plates were centrifuged at 25 × *g* for 5 minutes and incubated at 37°C in 7% CO_2 for 4 h. Supernatants were harvested using a harvesting system (Skatron, Lier, Norway) and [^{51}Cr] release measured in a gamma counter. Controls consisted of target cells incubated with 100 μl 10% Triton-X (maximum release), 100 μl of medium (medium control), 50 μl of antibody without effector cells (antibody control), and effector cells without antibody (effector control). The mean value from five cultures was used for calculation of the percentage specific [^{51}Cr] release as follows: (mean experimental cpm) − (mean effector control cpm)/(mean maximum release cpm) − (mean effector control cpm) × 100.

Treatment of EAE. EAE was induced in SJL/J mice by immunization in the tail base on day 0 with 5 mg of mouse spinal cord homogenate in 0.1 ml of an emulsion of equal volumes of CFA and PBS containing *Mycobacterium tuberculosis* (H37Ra) at a concentration of 4 mg/ml. Pertussis toxin (400 ng in 0.5 ml PBS) (List Biologics, Campbell, CA) was injected into the tail vein after immunization with mouse spinal cord homogenate on day 0 and day 2.

GK1.5 and the GK1.5 chimeric antibodies (100 and 25 μg) were injected i.p. as purified protein into mice when the initial symptoms of EAE became apparent. Clinical grading of the disease was done blindly with respect to treatment regimen. Severity of disease was graded as follows: 0, no abnormality; 1, decreased tail and body tone; 2, clumsy but otherwise normal gait; 3, definite weakness of one or more limbs; 4, monoplegia or paraplegia; and 5, death.

RESULTS

Depletion of CD4⁺ cells using GK1.5. Depletion of splenic CD4⁺ T cells was measured at various time points after injection of BALB/c mice with 100 μg of GK1.5. Inasmuch as GK1.5 binds to the CD4 Ag on the surface of CD4⁺ cells, the number of CD4⁺ cells was enumerated by counting CD5⁺ (Lyt-1⁺) CD8⁻ (Lyt-2⁻) cells. In addition, fluoresceinated mouse anti-rat κ was used to detect rat GK1.5 on the cell surface and anti-mouse γ1, γ2, or γ3 was used to detect the GK1.5γ1, GK1.5γ2a, and GK1.5γ2b and the GK1.5γ3 antibodies, respectively.

GK1.5 treatment reduced the absolute number of CD4⁺ T cells by approximately 45% within 24 h of injection (Table I). Depletion was specific for CD4⁺ T cells as the number of CD4⁻ T cells, determined by the number of CD5⁺CD8⁺ cells, was not changed. In addition, the number of B cells was found to be equal to control values as detected by determining the number of IgM⁺ cells. By 48 h postinjection the absolute number of CD5⁺CD8⁻ cells in the spleen was reduced by approximately 65% compared to control values. Additional cells were depleted 72 h after injection (Table I), and a plateau of approximately 85 to 90% depletion was reached by day 4. This lasted until days 7 to 14 at which time the CD5⁺CD8⁻ cells began to repopulate the spleen (data not shown).

Inasmuch as EAE is induced in SJL mice depletion of SJL spleen cells after GK1.5 treatment was determined. These results paralleled those seen with the BALB/c mice. Maximum depletion reached a plateau of approximately 90% 4 days after treatment with 100 μg of GK1.5 (data not shown).

Depletion using GK1.5 chimeric antibodies. The kinetics of depletion using GK1.5γ2a were compared to

TABLE I
Depletion of CD4⁺ cells after 100 μg GK1.5

Stain	No. of Cells Remaining ×10⁶					
	Control[a]	Day 1[b]	Day 2	Day 3	Day 4	Day 5
IgM⁺	56.1	62.3	63.3	54.3	46.9	57.7
CD8⁺CD4⁻	8.7	10.0	9.0	7.8	7.9	8.4
CD4⁺CD8⁻	22.6	<0.5	<0.5	<0.5	<0.5	1.0
CD5⁺CD8⁺	8.3	10.2	8.9	7.8	7.3	8.0
CD5⁺CD8⁻	24.1	13.0	7.8	4.9	3.4	4.4
Rat Cκ⁺	<0.5	2.0	1.0	1.0	<0.5	<0.5
Depletion (%)[c]		45	67	80	86	82

[a] One control mouse (injected with an irrelevant antibody) was looked at each day; these are averages of all control values.

[b] Seven test mice were examined per day; SD are < 20%.

[c] Percent depletion was calculated as follows: 100 − (average number of CD5⁺CD8⁻ cells remaining (antibody treated mouse)/average number of CD5⁺CD8⁻ cells remaining (control mouse)) × 100.

that achieved with rat GK1.5. By 24 h after injection of 100 μg of GK1.5γ2a there was 55% depletion of $CD4^+$ cells (Table II). This increased to 75% on day 2 and to 90% by day 4. Inasmuch as depletion plateaued on day 4 using 100 μg of both rat GK1.5 and GK1.5γ2a, depletion was examined on day 4 in further studies.

The depletion seen after injection of 100 μg of either GK1.5γ1 or GK1.5γ2b was similar to that observed with GK1.5γ2a; maximum depletion reached approximately 90%. However, after injection of 100 μg of GK1.5γ3 the absolute number of $CD5^+CD8^-$ cells was not significantly different from control values (Table III). Because it is possible that the cells were depleted, but had repopulated the spleen by day 4, we examined mice receiving injections with 100 μg GK1.5γ3 on day 1. As was seen on day 4, the number of $CD5^+CD8^-$ cells remaining after GK1.5γ3 treatment on day 1 was equal to control values. That GK1.5γ3 was binding to the cell surface was documented by flow microfluorimetry analysis using a fluoresceinated anti-γ3 reagent. For example, on day 4, 49.0 × 10^6 cells stained with anti-γ3, approximately equal to the number of $CD5^+CD8^-$ cells remaining after GK1.5γ3 treatment (Table III). Therefore, although 100 μg of GK1.5, GK1.5γ1, GK1.5γ2a, and GK1.5γ2b depleted 85 to 90% of splenic $CD4^+$ cells, 100 μg of GK1.5γ3 did not deplete cells.

Effects of antibody dose. The effects of antibody dose on depletion of splenic $CD4^+$ T cells were examined. Antibody concentrations were confirmed by determining the titer of each antibody on $CD4^+$ C6VL.1 cells (20), and doses of 2, 10, and 25 μg were administered to BALB/c mice. Lower doses of GK1.5γ1, GK1.5γ2a, and GK1.5γ2b gave significantly greater depletion of $CD5^+CD8-$ cells ($p < 0.0005$; Student's *t*-test) than rat GK1.5 (Table III). Only 25 μg of either the GK1.5γ1, GK1.5γ2a, or GK1.5γ2b antibodies was necessary to deplete the same number of cells as 100 μg of rat GK1.5. In contrast, 25 μg of GK1.5 resulted in significantly less depletion. Furthermore, 2 μg of GK1.5 resulted in no significant depletion of $CD4^+$ cells, although 2 μg of either GK1.5γ1, GK1.5γ2a, or GK1.5γ2b resulted in approximately 40% depletion of $CD5^+CD8^-$ cells. GK1.5γ3 did not deplete $CD4^+$ cells at any dose on days 1 or 4 postinjection.

TABLE II
Depletion of $CD4^+$ cells after 100 μg GK1.5γ2a

Stain	No. of Cells Remaining × 10^6				
	Control	Day 1	Day 2	Day 3	Day 4
$CD5^+CD8^-$	36.1	16.6	10.2	5.6	3.9
Depletion (%)		55	73	85	89

See footnotes for Table I.

TABLE III
Effect of dose on depletion of $CD4^+$ cells

Dose (μg)	No. of $CD5^+CD8^-$ Cells × 10^6[a] (% Depletion)[b]				
	GK1.5	GK1.5γ1	GK1.5γ2a	GK1.5γ2b	GK1.5γ3
100	5.1 (86%)	3.5 (90%)	3.9 (89%)	5.0 (86%)	41.7 (NS)
25	13.3 (63%)	4.8 (87%)	5.8 (84%)	4.6 (87%)	36.0 (NS)
10	16.9 (53%)	8.6 (76%)	8.8 (76%)	7.9 (78%)	ND
2	33.9 (NS)	20.2 (45%)	23.2 (35%)	19.5 (45%)	ND

[a] There were seven test mice (receiving injections with the indicated antibody) and one control mouse (injected with PBS) in each group; SD are <20%.

[b] The average number of $CD5^+CD8^-$ cells from all control mice in this series of experiments was used to calculate percent depletion as stated in the footnotes to Table I.

ADCC using lymphocytes as effector cells. The ability of rat GK1.5 and the GK1.5 chimeric antibodies to mediate ADCC was investigated using murine thymocytes as target cells. Approximately 95% of thymocytes express CD4 on their surface. Murine splenocytes, from which red blood cells had been removed, were used as a source of effector cells. GK1.5γ3 was the most effective isotype at mediating ADCC, lysing approximately 50% of the target cells (Fig. 1). Rat GK1.5, GK1.5γ1, GK1.5γ2a, and GK1.5γ2b were less effective under the same conditions ($p < 0.0005$). Maximal lysis was always less than 20% with each of these antibodies and there were no significant differences between them.

ADCC using peritoneal macrophages as effector cells. The ability of rat GK1.5 and the GK1.5 chimeric antibodies to mediate ADCC with paraffin oil-induced peritoneal cells as effector cells was determined. Previous studies using oil-induced peritoneal cells have shown that the population consisted of approximately 80% macrophages (22). Our results show that GK1.5γ1 and GK1.5γ2a were more effective than GK1.5γ2b, GK1.5γ3, and rat GK1.5 (Fig. 2) at macrophage-mediated ADCC. GK1.5γ1 and GK1.5γ2a lysed 17 to 18% of the target cells compared to 7% for the other antibodies ($p < 0.005$).

Immunotherapeutic effects of GK1.5 in treating EAE. In the initial studies that demonstrated that GK1.5 can 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 after the initial treatment (5). Inasmuch as approximately 90% depletion of splenic $CD4^+$ cells occurred with as little as 100 μg of antibody, we first determined whether lower doses of GK1.5 could reverse EAE. After administration of 100 μg of GK1.5, five of eight diseased mice fully recovered within 72 h of treatment (Table IV). By comparison, none of the control mice, those receiving injections with an irrelevant antibody, recovered over this time period. These results indicated that one dose of 100 μg of GK1.5 was sufficient to induce complete reversal of EAE (χ^2 vs control group, $p < 0.05$). When 25 μg of GK1.5 were

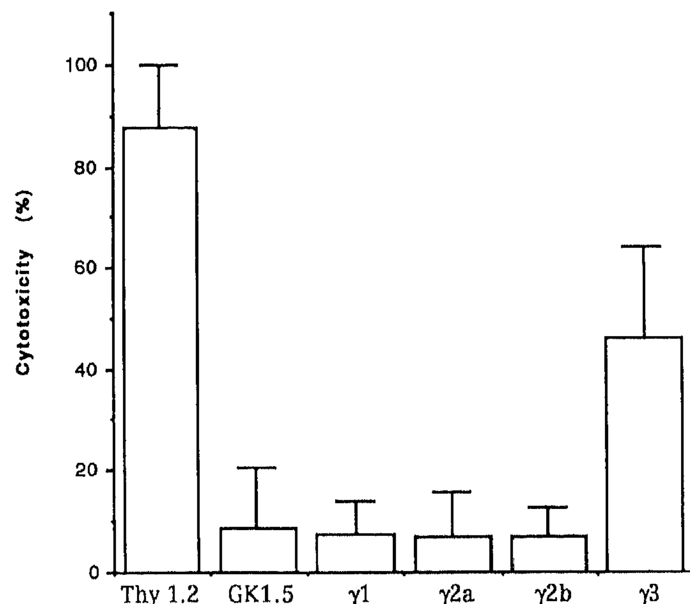

Figure 1. ADCC using lymphocytes as effector cells. ADCC was determined for each of the antibodies at a concentration of 10 μg/ml using thymocytes as targets and lymphocytes as effectors.

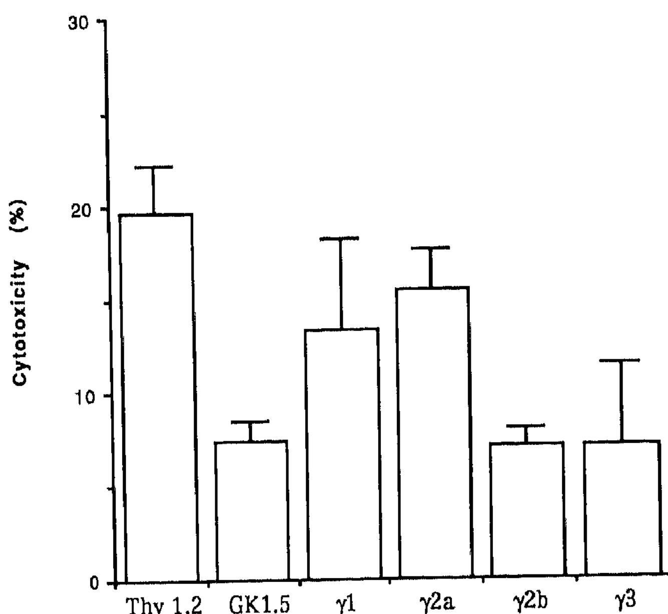

Figure 2. ADCC using peritoneal macrophages as effector cells. ADCC was determined for each of the antibodies at a concentration of 10 μg/ml using thymocytes as targets and oil-induced peritoneal macrophages as effectors.

TABLE IV
Total of 100 μg GK1.5 is effective in treatment of EAE

Antibody (Dose)	Clinical Disease			
	Grade[a]	Recovered[b]	Improved[c]	Worse[d]
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

[a] Mean grade of mice at onset of treatment.
[b] Mean grade post treatment = 0.
[c] Mean grade post treatment is less than at onset of treatment.
[d] Mean grade post treatment is more than at onset of treatment.

TABLE V
Results of EAE experiments 72 h after administration of antibody

Antibody	Clinical Disease			
	Grade	Recovered (p)[a]	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 (NS)	5/9	2/9
Control	2.7	1/18	4/18	13/18

See footnotes for Table IV.
[a] p values were computed by comparing the treated groups to the control group using χ^2 with Yates' correction.

administered to mice with EAE only one of eight recovered, although two mice showed some improvement in symptoms.

Immunotherapeutic effects of chimeric GK1.5 antibodies in treating EAE. One dose of 100 μg of GK1.5 was effective at reversing 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 V. As in the previous experiment, 100 μg of GK1.5 was sufficient to reverse EAE. Mice treated with 100 μg of GK1.5γ1 or GK1.5γ2a also recovered from EAE. In contrast to GK1.5, GK1.5γ1, and GK1.5γ2a, mice treated with GK1.5γ3 showed only marginal improvement and the number of mice that recovered 72 h after treatment with GK1.5γ3 was not significantly different from mice treated with an irrelevent control antibody (Table V). Furthermore, by day 5 after treatment with GK1.5γ3, the two mice that had appeared to recover within 72 h had regressed, and five of nine mice appeared worse.

Effect of antibody dose on treatment of EAE. The ability of 25 μg of the chimeric antibodies to treat EAE was examined. In contrast to the results with rat GK1.5, mice treated with 25 μg of either GK1.5γ2a or GK1.5γ2b showed significant improvement relative to the control group (Table VI). Interestingly, treatment with 25 μg of GK1.5γ1 was not effective.

DISCUSSION

We compared the efficacy of a family of chimeric mouse anti-murine CD4 antibodies to deplete $CD4^+$ cells to determine the mechanism by which cell depletion is mediated in vivo. Furthermore, we examined the role of depletion of $CD4^+$ cells in GK1.5-mediated immunotherapy of EAE, a prototypic model of T cell-mediated autoimmunity. We reported previously that the GK1.5 chimeric antibodies retained the same specificity and affinity for $CD4^+$ cells as rat GK1.5 (19), therefore our depletion, ADCC, CDC, and EAE treatment results (summarized in Table VII) reflect differences in isotype function.

GK1.5γ3 differed dramatically from the other antibodies with respect to in vivo cytotoxic capabilities. One dose of 100 μg of rat GK1.5, GK1.5γ1, GK1.5γ2a, or GK1.5γ2b was sufficient to deplete 90% of splenic $CD4^+$ cells, whereas the same dose of GK1.5γ3 did not deplete cells in either normal or diseased mice. Furthermore, GK1.5γ1, GK1.5γ2a, and GK1.5γ2b were more effective at depleting cells than rat GK1.5 when lower doses of antibody were administered.

The ability of the antibodies to mediate ADCC in vitro did not correlate with in vivo cell depletion. The nondepleting GK1.5γ3 antibody was more efficient at ADCC than rat GK1.5 and any of the other GK1.5 chimeric antibodies when lymphocytes were used as effector cells

TABLE VI
A total of 25 μg of GK1.5γ2a and GK1.5γ2b improves clinical condition

Antibody	Clinical Disease		
	Grade	Recovered or Improved (p)	Worse
GK1.5	2.7	7/17 (NS)	10/17
GK1.5γ1	2.6	3/8 (NS)	5/8
GK1.5γ2a	2.9	7/9 (<0.05)	2/9
GK1.5γ2b	2.7	6/9 (<0.05)	3/9
GK1.5γ3	2.9	3/10 (NS)	7/10
Control	2.4	2/16	14/16

See footnotes for Table V.

TABLE VII
Summary of results

Antibody	CDC[a]		ADCC[b]		Depletion[c]		EAE[d]	
	$CD4^+$	$CD4^{dull}$	Lym	Mφ	100μg	25μg	100μg	25μg
GK1.5	75%	45%	10%	7%	86%	63%	+++	–
GK1.5γ1	0%	0%	10%	17%	90%	87%	+++	–
GK1.5γ2a	95%	70%	10%	18%	89%	84%	+++	+
GK1.5γ2b	95%	65%	10%	7%	86%	87%	N.D.	+
GK1.5γ3	100%	60%	50%	7%	0%	0%	–	–

[a] CDC on $CD4^+$ or $CD4^{dull}$ (low density $CD4^+$) cells (19).
[b] ADCC using lymphocytes (Lym) or macrophages (Mø) as effector cells.
[c] Depletion of splenic $CD4^+$ cells.
[d] Therapeutic efficacy in the treatment of EAE.

in vitro (Fig. 1). The difference in efficacy in macrophage-mediated ADCC was not as distinct as when spleen cells were used as effector cells. However, when peritoneal macrophages were used, GK1.5γ2b was significantly less effective at mediating ADCC (Fig. 2) than GK1.5γ1 and GK1.5γ2a although all showed similar abilities to deplete cells in vivo. GK1.5γ3, which did not deplete cells, was as effective at mediating ADCC as rat GK1.5 and GK1.5γ2b. Therefore, although others have shown that the ability to mediate ADCC is correlated with antitumor activity (23, 24), the ability to deplete $CD4^+$ cells with the GK1.5 antibodies did not correlate with their ability to mediate ADCC.

We showed previously (19) that GK1.5γ1 does not mediate CDC, however GK1.5γ1 depletes cells in vivo to a greater extent than rat GK1.5, which is very effective in mediating CDC. Therefore, the ability to mediate CDC is not required for in vivo depletion of $CD4^+$ cells by GK1.5. This is consistent with previous work showing mAb-mediated cell depletion in mice congenitally deficient in C component C5 (25). Inasmuch as GK1.5γ3 mediates CDC well, but does not deplete cells in vivo, we conclude that the ability to kill cells by CDC is also not sufficient for in vivo cell depletion.

If depletion of $CD4^+$ cells by GK1.5 does not correlate with the ability of the antibody to mediate CDC or ADCC, how are the cells depleted? One possibility is that depletion of $CD4^+$ cells results from C3b-receptor-mediated clearance. In this mechanism interaction of the C3b receptor on monocytes or macrophages with target cell-bound C3b results in phagocytosis of antibody-coated target cells. It is interesting to note that GK1.5-mediated depletion of $CD4^+$ cells is significantly more difficult to achieve in NZB/NZW F1 mice, which spontaneously develop murine SLE (6), and that C3b receptor-mediated clearance was found to be defective in some patients with SLE (26). We are investigating whether C3b receptor-mediated clearance is the mechanism of $CD4^+$ T cell depletion. Alternatively, depletion of $CD4^+$ cells may be dependent on the ability of the antibody to sequester the target cells in the vicinity of appropriate effector cells or to modulate surface Ag.

In most instances, depletion of $CD4^+$ cells correlated with the therapeutic efficacy of each antibody in treating EAE. Mice treated with 100 μg of any of the depleting antibodies, GK1.5, GK1.5γ1, and GK1.5γ2a, recovered from EAE whereas mice treated with the same dose of the nondepleting GK1.5γ3 antibody showed only marginal and short lived improvement in symptoms. Furthermore, low doses (25 μg) of GK1.5γ2a and GK1.5γ2b, but not rat GK1.5, was enough to improve the condition of mice with EAE. With respect to depletion, 25 μg of GK1.5γ2a or GK1.5γ2b depleted a significantly greater number of cells than 25 μg of rat GK1.5 (Table III).

Mice treated with 100 μg of GK1.5γ1 showed 90% depletion of $CD4^+$ cells and recovered from EAE. However, in contrast to GK1.5γ2a and GK1.5γ2b, when low doses of GK1.5γ1 were administered, the correlation between depletion and therapeutic efficacy was not found. The reasons for this are unclear. It is possible that depletion of a specific subset of $CD4^+$ cells is required for successful treatment of disease, and, unlike GK1.5γ2a and GK1.5γ2b, GK1.5γ1 is unable to deplete this subset. In fact, we reported previously a correlation between antibody isotype and the ability to lyse low Ag density $CD4^+$ cells by CDC in vitro (19). If some subset of low density $CD4^+$ cells is in part responsible for EAE, this may explain why 25 μg of GK1.5γ1 was less effective at treating EAE. Alternatively the antibodies may differ in their abilities to deplete a population of $CD4^+$ cells other than splenic lymphocytes.

A recent study using $F(ab')_2$ fragments of GK1.5 showed that suppression of a murine model of SLE occurs without depletion of $CD4^+$ cells (17). The discrepancy between this result with SLE and our results may be due in part to the disease model treated. Although both are autoimmune diseases, EAE is an experimentally induced disease in SJL mice whereas SLE is a spontaneous disease of NZB/NZW F1 mice. EAE can be induced with $CD4^+$ T cell clones specific for myelin basic protein (27), whereas the role of $CD4^+$ cells is not as well established in SLE. Furthermore, the disparate observations in treatment of EAE and SLE may be due to differences in the treatment regimens. In the EAE experiments, a single dose of 100 μg of antibody was administered when the first symptoms of disease appeared. Under this treatment regimen any of the antibodies that were capable of depleting $CD4^+$ cells were able to reverse EAE, whereas the nondepleting GK1.5γ3 antibody was less effective. In contrast, in the SLE study 0.5 mg of GK1.5 $F(ab')_2$ fragments was administered to mice three times per wk for the duration of the experiments. Under this treatment regimen the $CD4^+$ cells were not depleted. However, the CD4 Ag was probably coated with GK1.5 $F(ab')_2$ fragments throughout the duration of the experiment because administration of only 100 μg of GK1.5 was sufficient to allow detection of circulating antibody in serum 3 days after administration (28). Therefore, although depletion of $CD4^+$ cells was not necessary in the SLE model, larger doses of antibody were administered continuously leading to an effective blockade of the CD4 Ag. Our experiments indicate that a single 100 μg dose of a $CD4^+$ cell-depleting antibody resulted in significant recovery from EAE. Under this treatment regimen recovery occurred without the need for continual large doses of antibody. However, under such a treatment regimen a nondepleting anti-CD4 antibody may not be as effective.

We have shown a correlation between the ability of 100 μg of each of the GK1.5 antibodies to deplete $CD4^+$ cells with the ability of the antibody to reverse EAE. Previous work with anti-Thy 1.2, which recognizes an Ag found on all T cells, has shown that anti-Thy 1.2 depletes approximately 60% of splenic $CD4^+$ cells and is ineffective in EAE (29). This is similar to our data with 25μg of rat GK1.5 (which also depletes 60% of $CD4^+$ cells and is not effective in the treatment of EAE) and is consistent with the correlation between depletion and successful immunotherapy. However, these results are in contrast to those found in the rat EAE model. Although isotype was important in anti-rat CD4-mediated immunotherapy of rat EAE (30), depletion of $CD4^+$ cells was not necessary. It is clear from these kinds of studies that successful anti-CD4-mediated immunotherapy may be dependent on a variety of factors pertaining both to the antibody (epitope, affinity, isotype, avidity) and the immune response (humoral, cellular, alloreactive, memory). We are continuing our studies using the GK1.5 chimeric antibodies in these areas.

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