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Crosstalk between α/β T cells and γ/δ T cells *in vivo*: Activation of α/β T-cell responses after γ/δ T-cell modulation with the monoclonal antibody GL3

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ABSTRACT **Although γ/δ T cells express numerous *in vitro* functions similar to α/β T cells, little is known about their biological functioning *in vivo*. Furthermore, it is unclear whether α/β T cells and γ/δ T cells act independently or in a coordinated way. In the present study, γ/δ T cells were modulated *in vivo* by i.p. injection of the anti-γ/δ T-cell receptor (TCR) monoclonal antibody GL3. GL3 administration caused disappearance of the γ/δ TCR in spleen and lymph node cells and the γ/δ TCR was reexpressed after *in vitro* cultivation for a few days. When cultured *in vitro* for 4 days, in the absence of foreign antigens, spleen and lymph node α/β T cells from GL3-modulated mice showed vigorous proliferative responses. CD4 T lymphocytes from GL3-modulated mice produced interleukin 2, and CD8 T cells developed into cytolytic T lymphocytes *in vitro* capable of lysing syngeneic and allogeneic targets. Treatment with heat-inactivated GL3 or with normal hamster immunoglobulin did not cause any of these effects. These findings suggest that the anti-γ/δ TCR monoclonal antibody GL3 modulates γ/δ T cells *in vivo* and that this modulation has profound effects on α/β T-cell reactivity. Hence, the data suggest a role for γ/δ T cells in the regulation of α/β T-cell activation *in vivo*.**

In the periphery, two T-cell populations exist that can be distinguished on the basis of different T-cell receptors (TCRs; refs. 1–4). Although the antigen recognition, genetic restriction, and biological functions of α/β T cells are well understood, γ/δ T cells remain enigmatic. γ/δ T cells produce various interleukins (ILs) and express cytolytic activities *in vitro* and hence they resemble α/β T cells in many functional aspects (2, 3). Yet, it is unclear whether α/β and γ/δ T cells perform similar effector functions *in vivo* and whether α/β T-cell and γ/δ T-cell responses proceed independent of each other or are coordinated by regulatory interactions. The small percentage of γ/δ T cells in lymphoid organs of mice and the difficulties in maintaining antigen-specific γ/δ T-cell lines *in vitro* have markedly hampered analysis of the biological role of γ/δ T cells. We have approached this question by treating mice with the monoclonal antibody (mAb) GL3 specific for γ/δ T cells (5). Our study shows that the *in vivo* modulation of γ/δ T cells with GL3 results in unrestricted α/β T-cell responses *in vitro* and hence suggests a central role for γ/δ T cells in the immunoregulation of α/β T-cell activity.

MATERIALS AND METHODS

Mice and Reagents. C57BL/6 mice of either sex, 10–14 weeks of age and raised under specific pathogen-free conditions were used. Normal hamster immunoglobulin was purchased from Dianova (Hamburg, F.R.G.). Hybridoma supernatants were purified over protein A or G (6). The following hybridoma cells were used: anti-CD3 (145-2C11, ref. 7), anti-γ/δ TCR (GL3, ref. 5; and 3A10, ref. 8), anti-α/β TCR (H57-597, ref. 9). For GL3 inactivation, antibodies were heated at 70°C for 30 min.

Flow Cytometry and Cell Sorting. Ten-thousand spleen cells or lymph node cells were analyzed by flow cytometry using a FACScan (Becton Dickinson). The following mAbs were used: phycoerythrin (PE)-labeled anti-CD4 (GK1.5, Becton Dickinson); fluorescein isothiocyanate (FITC)-labeled anti-CD8 (53-6.7, Becton Dickinson); biotinylated anti-CD8 (53-6.7, Becton Dickinson); FITC-labeled anti-CD3; biotinylated anti-γ/δ TCR (GL3); FITC-labeled or biotinylated α/β TCR (H57-597); FITC-labeled anti-Ly5 (B220, ref. 10; Medac, Hamburg, F.R.G.). When required, PE-conjugated streptavidin was used as a second step reagent. Cells were sorted either with the fluorescence-activated cell sorter [FACStar Plus (Becton Dickinson)] or by the magnetic cell separation system (Miltenyi Biotec GmbH, Bergisch-Gladbach, F.R.G.). For cytofluorimetry, cells were passed over nylon wool columns, stained with biotinylated anti-α/β TCR, FITC-labeled anti-CD8, or PE-labeled anti-CD4 mAb, respectively, and then sorted into positively and negatively stained cells. For sorting by the magnetic cell separation system, cells were first labeled with biotinylated anti-CD8 or biotinylated anti-CD4 mAb and then stained with biotinylated goat anti-mouse IgG. Subsequently, the cells were labeled with FITC-conjugated avidin and then with biotinylated magnetic beads. CD4 T-cell- plus B-cell-depleted and CD8 T-cell- plus B-cell-depleted populations were used for further studies.

Treatment of Mice and Determination of Lymphocyte Activities. Mice were treated i.p. with 0.5 mg of purified GL3 or control antibodies (heat-inactivated GL3 or normal hamster immunoglobulin, Dianova). Unless otherwise stated, spleen or lymph nodes were collected on day 6 and single cell suspensions were prepared. Spleen or lymph node cells were cultured in Click's RPMI medium supplemented with 10% fetal calf serum, 0.02 mM 2-mercaptoethanol, 1 mM glutamine, 1 μg of indomethacin per ml, and antibiotics. Cultures were performed in 96-well round-bottom microtiter plates (Nunc) at 37°C, 5% CO_2 in air. Unless otherwise stated, cultures were labeled with 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine ([^{3}H]dThd) after 5 days and proliferative responses were measured 6 hr later. In some experiments, T cells were enriched by passage over nylon wool columns and cells were cultured in the presence of irradiated (3000 rads) syngeneic spleen cells as accessory cells (ACs) as described (11, 12). Supernatants were collected from cultures after 24

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Abbreviations: TCR, T-cell receptor; mAb, monoclonal antibody; IL, interleukin; PE, phycoerythrin; FITC, fluorescein isothiocyanate; AC, accessory cell; IFN, interferon; CTL, cytotoxic T lymphocyte; MHC, major histocompatibility complex; FACS, fluorescence-activated cell sorting.

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hr. Supernatants were tested for IL-2 activities on an IL-2-dependent, IL-4-independent cytotoxic T lymphocyte (CTL) line (6). Interferon γ (IFN-γ), IL-4, and IL-10 activities were determined by ELISA as described (6). Proliferative and IL-2 responses represent means of triplicates with SD <20%. For CTL assays, cells were cultured for 5 days and washed, and their CTL activities were determined using the anti-CD3-producing hybridoma 145-2C11, P815, EL-4, or YAC cells. Target cells were labeled with 375 μCi of ^{51}Cr. Effector T cells and 1×10^3 ^{51}Cr labeled target cells per well were cocultured for 4 hr. Afterward, ^{51}Cr release was determined and specific CTL activity was calculated as described (12). Data in killer assays represent means of triplicates with SD <15%. Each experiment was performed at least twice with comparable results.

RESULTS

Phenotype Analysis in Normal and γ/δ T-Cell-Modulated Mice. Mice were treated with GL3 or left untreated. Six days later, relative proportions of T-lymphocyte populations among lymph node and spleen cells were determined. Marginal to no detectable differences were observed in the proportion of total T cells, CD4 T cells, CD8 T cells, γ/δ T cells, and α/β T cells in lymph nodes and spleens between untreated and GL3-treated mice. Consistent with published data, γ/δ T cells did not express a demonstrable density of CD4 and/or CD8 markers (ref. 13; data not shown). CD4$^-$8$^-$ γ/δTCR$^+$ or CD4$^-$8$^-$ α/βTCR$^+$ cells made up 0.6% or 1.6%, respectively, of all lymphoid cells in control C57BL/6 mice and 0.03% or 1.4%, respectively, in GL3-treated mice. We conclude that GL3 treatment did not affect CD4$^-$8$^-$ α/β TCR$^+$ lymphocytes. To improve detection of γ/δ T cells, spleen cells were stained with FITC-labeled B220 mAb, FITC-labeled α/βTCR mAb, and PE-biotin-labeled γ/δTCR mAb, and the B220$^-$ and α/βTCR$^-$ lymphoid cell population was gated. In this population lymphoid cells brightly staining for γ/δ TCR expression could be identified with GL3 among cells from control mice (Fig. 1). In contrast, cells from GL3-treated mice were devoid of γ/δ TCR^{bright} cells and a population of γ/δ TCR^{dull} cells became detectable (Fig. 1). These findings suggest γ/δ TCR modulation *in vivo* by GL3 treatment. In additional control experiments, B220$^-$ spleen cells from normal or GL3-treated mice were labeled (*i*) with GL3 or (*ii*) with another anti-γ/δ TCR mAb, 3A10, or (*iii*) remained untreated. Subsequently, these cells were stained with FITC-labeled anti-hamster immunoglobulin antibodies. In control mice, the anti-hamster immunoglobulin antibodies alone did not stain cells by themselves, whereas the GL3 or 3A10 mAb plus anti-hamster immunoglobulin antibodies stained an equal proportion of cells (1% of lymphoid cells). In contrast, none of these stainings caused a detectable signal in B220$^-$ cells from GL3-treated mice (data not shown). We, therefore, consider blocking of the γ/δ TCR by continued *in vivo* presence of the GL3 mAb unlikely. Rather, we assume that GL3 treatment caused γ/δ TCR down-regulation *in vivo*. Concomitant with the γ/δ TCR the CD3 molecule was also down-regulated (data not shown). After *in vitro* cultivation of spleen cells in the absence of exogenous stimuli for up to 6 days, some γ/δ TCR^{bright} cells reemerged (data not shown). Already on day 1 of *in vitro* cultivation, some γ/δ TCR^{bright} cells were demonstrable. These findings suggest that GL3 down-modulates γ/δ TCR expression *in vivo* but does not physically eliminate the γ/δ T-cell population.

Unrestricted α/β T-Cell Proliferation after γ/δ T-Cell Modulation. Graded numbers of spleen cells, lymph node cells, or nylon wool-enriched T cells from GL3-treated or untreated mice were cultured in the presence of syngeneic ACs for 4 days. Cells from GL3-treated mice strongly proliferated in the absence of exogenous stimuli, whereas those from normal mice showed only marginal to no responses (Table 1). These findings indicate that GL3 modulation

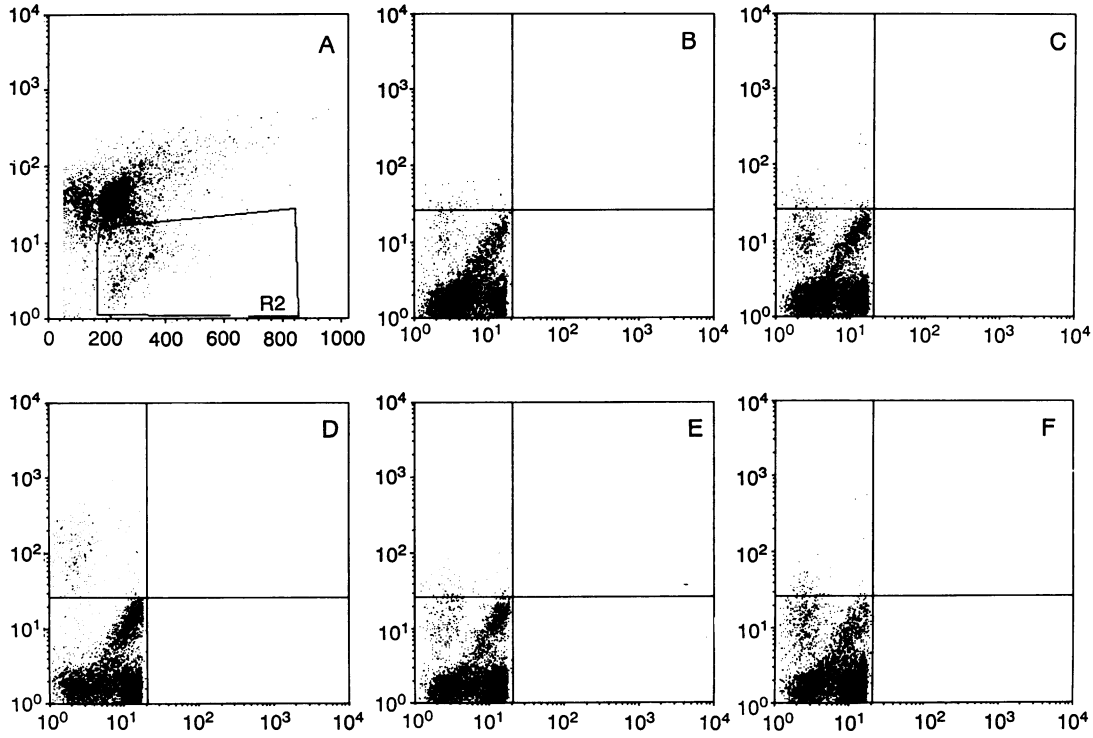

FIG. 1. *In vivo* longevity of γ/δ TCR down-regulation. Mice were treated with GL3 or left untreated. Spleen cells were stained with FITC-labeled B220 mAb, FITC-labeled α/β TCR mAb, and PE-biotin-labeled γ/δ TCR mAb. The B220$^-$, α/β TCR$^-$ lymphoid population was gated and the proportion of γ/δ TCR$^+$ cells in this population was analyzed. (*A*) Forward light scatter (*x* axis) vs. B220 plus α/β TCR staining ($\log_{10}$ fluorescence, *y* axis). The gated population is indicated. (*B–F*) B220 plus α/βTCR staining ($\log_{10}$ fluorescence, *x* axis) vs. γ/δTCR staining ($\log_{10}$ fluorescence, *y* axis) on day 1 (*B*), day 3 (*E*), day 6 (*C*), and day 8 (*F*) after GL3 treatment or without GL3 treatment (*D*).

Table 1. GL3, but not heat-inactivated GL3 nor normal hamster immunoglobulin, causes proliferation

Cells, no. × 10^{-4}	Proliferative response ([^{3}H]dThd, cpm)		
	Control	GL3	Control Ab
Experiment I: Heat-inactivated GL3 as control Ab*			
10	5,500	27,000	9,100
5	1,500	13,900	1,500
2.5	500	2,900	300
1.2	170	1,000	200
0.6	60	80	80
0.3	60	60	70
0	60	60	60
Experiment II: Normal hamster immunoglobulin as control Ab			
20	5,800	30,200	5,900
10	2,900	16,900	2,800
3	690	3,300	500
1	270	600	300
0.3	130	100	100

Mice were treated with GL3, with control Ab, or remained untreated; 6 days later lymph node cells were cultured together with 2.5×10^5 syngeneic ACs for 4 days, the last 6 hr in the presence of 1 μCi of [^{3}H]dThd.
*GL3 was inactivated by heating at 70°C for 30 min.

activated T cells *in vivo* causing marked T-cell proliferation *in vitro*. We were concerned that the activities seen *in vitro* actually reflected antigen-specific T-cell responses against epitopes shared by the GL3 hamster mAb and immunoglobulin present in the fetal calf serum used for *in vitro* assays. To exclude this possibility, mice were injected with heat-inactivated GL3 or with normal hamster immunoglobulin, respectively. Spleen cells from these mice behaved like those of untreated mice (Table 1). We, therefore, consider the possibility unlikely that the *in vitro* responses reflected specific immunity against immunoglobulin. Administration of GL3 had rapid and long-lasting effects: already after 1 day, elevated proliferation was detected, which was further increased by day 8 (data not shown). Concomitantly, γ/δ TCR expression was down-modulated *in vivo* as early as day 1 and remained so until day 8 after mAb administration (Fig. 1). GL3-induced proliferation of T cells was significantly diminished in the absence of indomethacin (data not shown), suggesting abundant prostaglandin secretion by spleen cells from GL3-modulated mice that inhibited proliferative responses. All experiments reported here were performed in the presence of indomethacin. Nylon wool-enriched T cells from GL3-treated or control mice were sorted by cytofluorimetry into α/β TCR$^+$ and α/β TCR$^-$ populations that were than cultured as described. Table 2 shows that proliferative responses resided in the selected α/β TCR$^+$ population, whereas the α/β TCR$^-$ population gave no responses. We conclude that α/β T cells proliferate *in vitro* after *in vivo* modulation with GL3. *In vitro* labeling of spleen cells from control mice with the anti-α/β TCR mAb did not cause proliferation excluding nonspecific α/β T-cell stimulation by this mAb (Table 2). Similarly, the selected CD4$^+$ plus CD8$^+$ cell population but not the selected CD4$^-$ plus CD8$^-$ cell population from GL3-treated, but not untreated, mice showed strong proliferative responses (data not shown). These findings suggest α/β T-cell activation by *in vivo* modulation with GL3. Nylon wool-enriched T cells from GL3-treated or control mice were incubated with PE-labeled anti-CD4 mAb or with FITC-labeled anti-CD8 mAb and, afterward, CD4$^+$ T cells and CD8$^+$ T cells were sorted by cytofluorimetry. The CD4$^+$ and the CD8$^+$ T-cell populations were then cultured in the presence of ACs and proliferative responses were determined as described. As shown in Table 3, proliferation induced by *in vivo* treatment with GL3 exclusively resided in the CD4$^+$ T-cell population, and selection of CD4$^+$ T cells resulted in a >5-fold increase in proliferation. In the absence of exogeneous IL-2, selected CD8$^+$ T cells from GL3-treated mice did not proliferate and neither CD4$^+$ T cells nor CD8$^+$ T cells from control mice showed any proliferative response (Table 3). Proliferation of selected CD4$^-$ CD8$^+$ T cells from GL3-treated mice, however, could be reconstituted by addition of exogenous IL-2 {proliferative responses ([^{3}H]dThd uptake in cpm) of 10^5 CD8$^+$ T cells in the absence or presence of IL-2: 420 cpm or 12,220 cpm, respectively}. These findings suggest that CD4 and CD8 T cells are activated by *in vivo* modulation of γ/δ T cells, with CD8 T cells being dependent on CD4 T cells.

Table 2. Evidence for α/β T-cell activation in γ/δ T-cell-modulated mice

Cells, no. × 10^{-4}	Proliferative response ([^{3}H]dThd, cpm) of cells from GL3-modulated mice			
	Untreated cells*	Unsorted cells†	α/β TCR$^+$ cells‡	α/β TCR$^-$ cells§
α/β TCR sorted cells from GL3-treated mice				
10	20,900	12,500	19,600	800
5	11,900	7,700	7,200	80
α/β TCR sorted cells from control mice				
10	5,500	2,300	3,000	500
5	1,100	860	2,000	110

Mice were treated with GL3 or remained untreated; after 6 days, spleen cells were sorted into α/β TCR$^+$ and α/β TCR$^-$ populations by fluorescence-activated cell sorting (FACS). Cells were then cultured in the presence of ACs for 5 days, the last 16 hr in the presence of 1 μCi of [^{3}H]dThd.
*Culture initiated after nylon wool treatment.
†Culture performed with stained, unsorted cells.
‡Positively sorted population.
§Negatively sorted population.

IL-2 Secretion by α/β CD4 T Cells from GL3-Modulated Mice. Nylon wool-purified spleen cells from mice treated with GL3 or with heat-inactivated GL3 or from untreated mice were cultured for 24 hr, and, afterward, IL-2, IL-4, and IFN-γ activities were determined. Neither IFN-γ, IL-4, nor IL-10 was detectable by ELISA techniques with a detection threshold of 0.1 unit for IL-10 or IL-4 and 0.05 unit for IFN-γ per 50-μl supernatant. On the other hand, cell cultures from GL3-treated mice produced IL-2, whereas cells from untreated mice or mice treated with heat-inactivated GL3 failed to do so (data not shown). Next, spleen cells were sorted into α/β TCR$^+$ and α/β TCR$^-$ cells by FACS. Selected α/β TCR$^+$ cells from GL3-treated mice produced IL-2, whereas selected α/β TCR$^-$ cells failed to do so (Table 4). In contrast, IL-2 was not produced by selected α/β TCR$^+$ cells from control mice excluding activation by the anti-α/β TCR mAb

Table 3. Evidence for CD4$^+$ T-cell proliferation

Cells, no. × 10^{-4}	Proliferative response ([^{3}H]dThd, cpm)			
	Untreated cells*	Unsorted cells†	CD4$^+$ T cells	CD8$^+$ T cells
GL3-treated mice				
10	28,800	32,600	176,400	600
5	14,000	17,000	106,800	130
Control mice				
10	5,200	5,600	1,100	180
5	1,000	1,500	500	250

Mice were treated with GL3 or remained untreated; after 6 days, spleen cells were incubated with mAb, and the CD4$^+$ and CD8$^+$ T-cell populations were sorted by FACS. Cells were then cultured in the presence of ACs for 5 days, the last 16 hr in the presence of 1 μCi of [^{3}H]dThd.
*Culture initiated after nylon wool treatment.
†Culture performed with stained, unsorted cells.

Table 4. IL-2 production by CD4 α/β T cells

Cells, no. $\times 10^{-4}$	IL-2 production ([^{3}H]dThd uptake by CTLs, cpm)					
	Untreated cells*	Unsorted cells†	α/β TCR$^+$ cells‡	α/β TCR$^-$ cells§	CD4$^+$ cells	CD8$^+$ cells
	α/β TCR sorted cells from GL3-treated mice					
10	13,300	29,000	22,500	196	ND	ND
5	10,000	15,700	17,900	100	ND	ND
	CD4 and CD8 sorted cells from GL3-treated mice					
10	33,600	50,100	ND	ND	63,600	230
5	12,800	35,600	ND	ND	41,400	170
	α/β TCR sorted cells from control mice					
10	140	530	560	227	ND	ND
5	130	360	300	70	ND	ND
	CD4 and CD8 sorted cells from control mice					
10	340	200	ND	ND	370	140
5	200	220	ND	ND	270	170

Mice were treated with GL3 or remained untreated; after 6 days, spleen cells were sorted by FACS. Cells were cultured in the presence of ACs and after 24 hr, supernatants were collected and tested for IL-2 activity on CTLs. CTL proliferation induced by recombinant IL-2: 1.2 units per well = 101,900 cpm; 0.6 unit per well = 81,500 cpm; 0.3 unit per well = 65,600 cpm; 0.015 unit per well = 54,500 cpm; 0.08 unit per well = 31,800 cpm; 0.04 unit per well = 17,600 cpm; 0.02 unit per well = 7100 cpm; 0.01 unit per well = 2500 cpm. ND, not done.
*Culture initiated after nylon wool purification.
†Culture performed with stained, unsorted cells.
‡Positively sorted population.
§Negatively sorted population.

(Table 4). Similarly, the selected CD4$^+$ plus CD8$^+$ cell population, but not the CD4$^-$ plus CD8$^-$ cell population, produced IL-2 (data not shown). Finally, CD4$^+$ cells and CD8$^+$ cells were sorted by cytofluorimetry and cultured as described above. Table 4 shows that IL-2 was exclusively produced by CD4 T cells but not by CD8 T cells. Thus, *in vivo* treatment with GL3 activated CD4 T cells to produce IL-2 *in vitro*.

GL3 Modulation *in Vivo* Causes *in Vitro* Activation of CD8 α/β CTLs. We wondered whether T cells from GL3-treated mice differentiate into specific CTLs. T cells were cultured for 5 days in the presence of syngeneic ACs and then cytolytic activities were assessed. Potent CTLs developed from spleen cells of GL3-treated mice, but not from untreated mice, as shown by antibody redirected killing of anti-CD3 hybridoma cells (Fig. 2). In addition, allogeneic (P815) and syngeneic (EL4) targets were lysed, whereas natural killer activities (assayed on the mouse lymphoma cells, YAC-1) were low to absent (Fig. 2). For control purposes, spleen cells from mice treated with hamster immunoglobulin were employed. Only negligible CTL activities were observed (data not shown). We conclude that *in vivo* modulation of γ/δ T cells committed CTL precursors for differentiation into effector cells recognizing allogeneic as well as syngeneic targets. This finding indicates that control of autoreactive CTLs in the periphery involves γ/δ T lymphocytes. To characterize the T-cell subset(s) responsible for CTL activities, spleen cells from mice that had been treated with GL3 were cultured *in vitro* for 5 days. In one experiment, CD4 and CD8 T cells were depleted by magnetic cell sorting and subsequently CTL activities were assessed on CD3-producing hybridoma cells. CD8 T-cell depletion markedly reduced killer activities, whereas depletion of CD4 T cells had no such effect (Fig. 3*A*). In a second experiment, CTL assays were performed in the presence of anti-CD4 mAb or anti-CD8 mAb. As shown in Fig. 3*B*, blocking with anti-CD8 antibodies caused a significant reduction of CTL activities, whereas blocking with anti-CD4 mAb had only marginal effects. We conclude that CTL activities resided in the major histocompatibility complex (MHC) class-I-restricted CD8 T-cell subset.

DISCUSSION

The present report describes α/β T-cell activation following *in vivo* modulation of γ/δ T cells. The biological activities identified thus far comprise IL-2 production and CTL effector functions against syngeneic and allogeneic target cells. Hence, our findings indicate crosstalk between γ/δ T cells

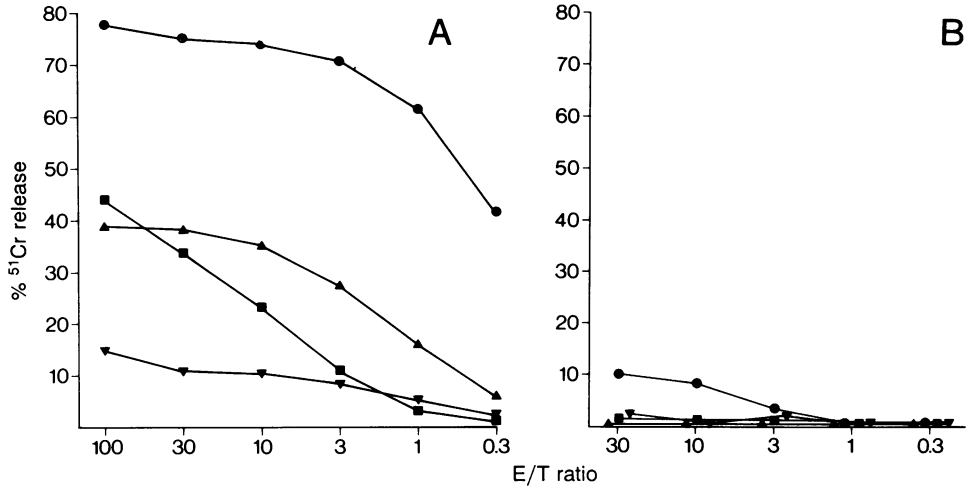

FIG. 2. Activation of alloreactive and autoreactive CTLs from spleen cells of γ/δ T-cell-modulated mice. Mice were treated with GL3 (*A*) or left untreated (*B*). Spleens were removed on day 5 and spleen cells cultured for 6 days. Afterward, CTL activities were assessed on anti-CD3 hybridoma (●), P815 (■), EL-4 (▲), or YAC-1 (▼) cells. E/T, effector/target.

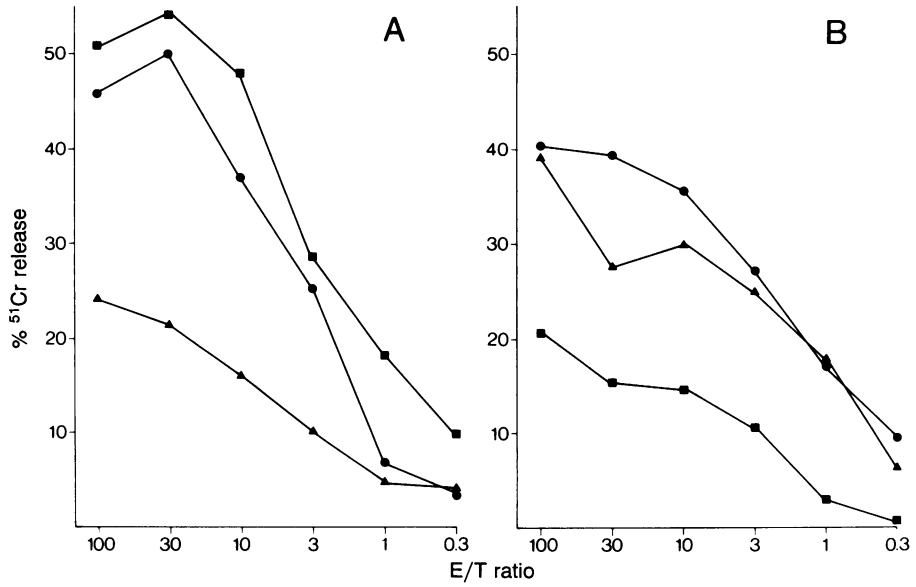

FIG. 3. Phenotype of CTLs. Spleen cells from GL3-treated mice were cultured *in vitro*. (*A*) Cells were depleted of CD4 T cells (■) or CD8 T cells (▲) or left untreated (●) and afterward tested in CTL assays using anti-CD3 hybridoma cells as targets. (*B*) CTL assays were performed in the presence of anti-CD4 mAb (▲) or anti-CD8 mAb (■) or in the absence of mAb (●) using EL-4 cells as targets. E/T, effector/target.

and α/β T cells *in vivo* and suggest control of α/β T-cell activation by γ/δ T cells. In our study, the hamster mAb GL3 was used for *in vivo* modulation of γ/δ T cells. Using another hamster mAb with specificity for the γ/δ TCR, UC7, evidence for *in vivo* depletion of γ/δ T cells from the lymphoid organs has been obtained (13). We consider conventional depletion of γ/δ T cells by GL3 via activation of complement and/or natural killer cells unlikely because we detected a γ/δ TCR^{dull} lymphoid population among gated B220$^-$, α/β TCR$^-$ lymphoid cells from GL3-treated mice different from the γ/δ TCR^{bright} lymphoid cells seen in controls. Furthermore, following *in vitro* culture of spleen cells from GL3-treated mice, γ/δ TCR^{bright} cells reemerged. Finally, the two unrelated anti-γ/δ TCR mAbs, GL3 and 3A10, gave comparable staining results with spleen cells from GL3-treated and control mice, and we failed to detect any residual GL3 mAb on spleen cells from GL3-treated mice by using FITC-labeled anti-hamster immunoglobulin antibodies. We, therefore, conclude that GL3 treatment induced down-modulation of the γ/δ TCR and, in parallel, of the CD3 molecule. In the *in vivo* setting, γ/δ TCR remained down-modulated for up to 20 days. Reexpression of γ/δ TCR was observed, however, during *in vitro* cultivation for a few days. At the moment we cannot decide whether GL3 administration activated or inactivated γ/δ T cells *in vivo*. Yet, our findings emphasize the importance of considering more complex mechanisms than mere target cell depletion in interpreting data from *in vivo* mAb administration experiments (14).

How could the relatively small fraction of γ/δ T cells regulate the much larger population of α/β T cells? Regulation could be achieved via IL secretion. *In vitro*, immobilized GL3 has been found to activate γ/δ T cells for IL secretion and target cell lysis (15, 16). Alternatively, γ/δ T cells and α/β T cells could interact with each other via cell surface ligands in a regulatory network, the disturbance of which causes elevated reactivities in α/β T cells. Though ligands involved in the crosstalk between α/β T cells and γ/δ T cells remain undefined, heat shock proteins and self-MHC products represent possible candidates (17, 18). Evidence has been presented that γ/δ T cells participate in the immune response against intracellular bacteria and contribute to autoimmune pathogenesis as effector cells (13, 19–21). In contrast, our data suggest an immunoregulatory, rather than an effector, role of γ/δ T cells that seems to control the activation of α/β T cells. Consistent with a regulatory role of γ/δ T cells, Doherty and coworkers (22) observed that γ/δ T cells accumulate late in the lung of mice infected with influenza virus at a time when the virus had already been cleared from the organ. Furthermore, recent data from our laboratory indicate that γ/δ T cells control granuloma induction by α/β T cells in murine listeriosis (23). Hence, γ/δ T cells seem essential for maintaining immune homeostasis.

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