VARIABLE CAPACITY OF L3T4$^+$ T CELLS TO CAUSE LETHAL GRAFT-VERSUS-HOST DISEASE ACROSS MINOR HISTOCOMPATIBILITY BARRIERS IN MICE

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The transfer of unprimed T cells to lethally irradiated allogeneic mice expressing multiple minor histocompatibility (H)[1] antigen differences can result in a high incidence of lethal graft-versus-host disease (GVHD) (1–4). Which particular T cell subsets are responsible for causing GVHD to minor H differences is the subject of controversy. Earlier studies using in vivo negative selection techniques indicated that H-2K/D-restricted T cells caused GVHD to minor H differences; H-2I-restricted T cells failed to elicit GVHD, and the presence of these cells did not seem to be essential for the function of H-2K/D-restricted T cells (5, 6). Phenotyping studies with antibody and complement (C) supported the notion that only Lyt-2$^+$ and not Lyt-1$^+$,2$^-$ T cells caused minor H GVHD (6). In addition, Lyt-2$^+$ cytotoxic T lymphocytes (CTL) with antirecipient minor H specificity could be isolated from mice during the development of GVHD (7). Recent reports, however, have indicated that Lyt-1$^+$,2$^-$ or L3T4$^+$ T cells can cause GVHD across a full MHC barrier (8–10), especially when class II differences are involved (10). Furthermore, in studies using highly purified T cell subsets, GVHD directed to allo–class II differences was controlled solely by L3T4$^+$ cells, whereas GVHD to class I differences involved only Lyt-2$^+$ and not L3T4$^+$ cells (11).

In view of our prior finding that GVHD to minor H antigen differences appeared to be caused solely by Lyt-2$^+$ and not L3T4$^+$ cells, the possibility arises that GVHD following HLA-matched bone marrow transplantation in man could be abolished by selectively depleting the marrow of CD8$^+$ cells. Because the data on the identity of T cells causing GVHD to non-H-2 differences in mice is based largely on a single strain combination (CBA → B10.BR) and with T cells that were only semipurified, we considered it important to reinvestigate this question with a number of minor H–different strain combinations and to use highly purified T cell subsets for GVHD induction.

In this study we have surveyed six different H-2-compatible, multiple minor H antigen–different strain combinations for the ability of purified L3T4$^+$ or

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[1] *Abbreviations used in this paper:* ATBM, anti-Thy-1 + C–treated bone marrow; ATX, thymectomized; BSS, buffered saline solution; H, histocompatibility; LN, lymph node; MST, median survival time.

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Lyt-2$^+$ T cells to cause lethal GVHD. In four of six of these combinations, lethal GVHD appeared to be caused almost entirely by Lyt-2$^+$ cells, death in recipients of L3T4$^+$ cells being quite low even when presensitized cells were injected. In the remaining two combinations, however, either L3T4$^+$ or Lyt-2$^+$ T cells were able to cause a high incidence of GVHD. These data suggest that the inability of L3T4$^+$ cells to cause GVHD to minor H differences is not an invariable finding.

Materials and Methods

Mice. C3H.SW/SnJ, C57BL/6J (B6), B10.D2/oSnJ, DBA/2J, B10.BR/SgSnJ, CBA/J, BALB/cJ, and SJL/J mice were purchased from The Jackson Laboratory, Bar Harbor, ME. B10.S mice were originally acquired from the Scripps Clinic and Research Foundation, La Jolla, CA, and bred in our own colony. All mice were males, except SJL, and used experimentally as cell donors and irradiated recipients between 8 and 16 wk of age.

Media. Buffered saline solution (BSS) supplemented with 0.1% BSA (Hyclone, Logan, UT) was used for all in vitro manipulations of donor bone marrow cells and lymphocytes. Final suspensions of cells for injection were prepared in BSS only.

Injections. All cell suspensions were given intravenously via the tail vein in a volume of 0.5 ml.

Irradiation. A Shepherd Mark I ^{137}Cs irradiator (75 rad/min) was used for irradiating mice.

Monoclonal Antibodies. Ascites fluid for anti-L3T4 [GK1.5, rat IgG2a, and RL172, rat IgM (12)], anti-Lyt-2 (3.168, rat IgM), anti-Thy-1.2 (J1j, rat IgM), and anti-B (J11d, rat IgM), mAb and guinea pig serum as a source of C were prepared and used as described previously (13). Anti-Lgp100 [30-C7 rat IgG2a (14), culture supernatant] mAb was used for chimera phenotyping. Biotin-conjugated anti-L3T4 (GK1.5), anti-Lyt-2, and anti-Thy-1.2 were prepared with biotin succinimide ester (Vector Laboratories, Burlingame, CA).

Purification of T Cell Subsets. In a modification of a technique described in detail elsewhere (13), purified T cell subsets were obtained by treating pooled lymph node (LN) cells with mAb plus C, followed by positive panning on antibody-coated petri plates. To prepare Lyt-2$^+$ cells, LN cells were treated with a mixture of anti-B (J11d) and anti-L3T4 (RL172) mAb plus C, washed, and then incubated on ice with anti-Lyt-2 (3.168) mAb for 30 min. Cells were washed again and allowed to adhere to a plate coated with goat anti–rat IgM Ab (Cooper Biomedical, Malvern, PA) for 1 h at 4°C. After gentle but extensive washing of nonadherent cells from the plates, the adherent cells were eluted by vigorous pipetting. The eluted cells were routinely tested for phenotype and contained no detectable L3T4$^+$ cells. An analogous procedure was used to prepare pure L3T4$^+$ cells, i.e., pretreatment of LN cells with J11d and anti-Lyt-2 mAb plus C, followed by positive panning on anti-L3T4 (GK1.5)-coated plates.

Phenotyping. Antibody-mediated lysis in the presence of C was measured in a one-step trypan blue exclusion assay. For fluorescent analysis with an Ortho 50H Cytofluorograf, 0.5×10^6 cells were labeled with appropriate biotin-conjugated mAb for 30 min at 4°C, washed, and incubated for 10 min with FITC-conjugated avidin DCS (Vector Laboratories), and washed three more times before use. The level of FITC excitation and forward and right-angle scatter were determined relative to unlabeled control cells. A similar procedure was used for analysis of chimerism with anti-Lgp100 mAb and FITC–goat anti–rat IgG, Fc portion–specific (Cooper Biomedical) secondary labeling.

Mortality Assay for GVHD. As described previously (1), 2- to 4-mo-old recipient mice were exposed to a midlethal dose of irradiation (750–820 rad), and then ~6 h later were injected intravenously with a mixture of T cell–depleted donor bone marrow (anti-Thy-1 + C–treated bone marrow [ATBM]; 4×10^6 viable cells) together with the purified T cell subsets. Recipients of ATBM cells alone served as controls. Mice were given food and water ad libitum and were checked routinely for morbidity and mortality. Experiments were terminated after 80 d. Median survival times (MST) were calculated for pooled experiments as previously described (10), and statistical comparisons between groups made by the Mann-Whitney two-sample rank test.

TABLE I

Surface Markers of Lyt-2$^+$ and L3T4$^+$ T Cell Subsets Purified From C3H.SW LN Cells

Group	Pretreatment of C3H.SW LN*	Assay for typing cells*	Percentage of cells positive for:	
			L3T4	Lyt-2
Whole T	J11d (anti-B cell) + C′	Complement-mediated cytotoxicity	64.2	33.0
		FACS- fluorescein-avidin	63.7	36.4
L3T4$^+$	J11d + anti-Lyt-2 + C′; pan on anti-L3T4 plate	Complement-mediated cytotoxicity	96.8	0.0
		FACS-fluorescein-avidin	95.7	0.0
Lyt-2$^+$	J11d + anti-L3T4 + C′; anti-Lyt-2 alone; pan on goat anti-rat IgM plate		0.0	96.4
			0.0	92.8

* For purification and phenotyping procedures, see Materials and Methods.

Presensitization of Donor Cells. 6-wk-old C3H.SW mice were thymectomized (ATX) and injected intraperitoneally 2 wk later with 2×10^6 B6 spleen cells. They were used as LN donors for the GVHD assay 3 wk after immunization, at which time they were also tested for the generation of secondary anti-minor H CTL responses. Cells were cultured (4×10^6 cells/well of a Costar 24-well plate) with an equal number of irradiated (1,500 rad) B6 spleen stimulator cells for 7 d at 37°C, 8% CO_2. Cells were then tested for cytotoxic activity in microtiter plates against Cr^{51} labeled 2-d-old Con A blast cells in a standard 4 h release assay. The percent specific release was determined relative to spontaneous and maximum release controls.

In Vitro Proliferation. Aliquots of 2×10^5 responder LN L3T4$^+$ cells, purified by the procedures described above, were cultured in flat-bottom microtiter plates with 8×10^5 irradiated (2000 rad) spleen cells as stimulators in a volume of 200 μl, and then pulsed with 0.25 μCi [^{3}H]TdR 18 h before harvest (13).

Results

Preparation of T Cell Subsets. In all of the experiments presented below, pooled LN cells from appropriate donor mice (five to eight mice depending upon the particular strain) were prepared by a combination of antibody plus C depletion and panning techniques (see Materials and Methods) to obtain purified T cell subsets. Every preparation of cells was phenotyped by cytofluorescent analysis using a biotin-antibody–FITC-avidin system. A typical example of the results that are obtained with the purification procedure is shown in Table I, in which C3H.SW cells are analyzed by both antibody cytotoxicity and FITC staining. Treatment of normal C3H.SW LN cells with J11d mAb plus C alone yielded a B-depleted population containing an approximately 2:1 ratio of L3T4$^+$ to Lyt-2$^+$ cells (Group A); with B6 mice and the B10 H-2 congenic lines, the ratio of L3T4$^+$ to Lyt-2$^+$ cells approached 1:1 (13). LN cells treated with J11d and 3.168 (anti-Lyt-2) mAb plus C followed by positive panning for L3T4$^+$ cells (Group B) were >95% L3T4$^+$ and contained no detectable Lyt-2$^+$ cells by both measures of analysis. Conversely, LN cells treated with J11d and RL172 (anti-L3T4) mAb plus C and panned for Lyt-2$^+$ cells (Group C) were 92–96% Lyt-2$^+$ and appeared to be devoid of L3T4$^+$ cells (the failure to observe 100% purity

by FITC staining might have reflected interference by residual unlabeled antibody from the panning step).

GVHD Mortality Across Minor H Barriers with T Cell Subsets. The general experimental approach was to irradiate recipient mice with 750–820 rad and, 6 h later, transfer a mixture of 4×10^6 donor anti-Thy-1 plus C–treated (T cell–depleted) bone marrow (ATBM) along with either whole T cells (J11d plus C treated LN), purified L3T4$^+$, or Lyt-2$^+$ T cells; the dosage of T cells was kept at 10^6 for all experiments. Six different H-2-compatible, minor H antigen–disparate strain combinations were tested for the capacity of T cell subsets to cause lethal GVHD: C3H.SW → B6 (Fig. 1*a*); DBA/2 → B10.D2 (Fig. 1*b*); B10.BR → CBA (Fig. 1*c*); B10.S → SJL (Fig. 1*d*); B10.D2 → DBA/2 (Fig. 1*e*); and B10.D2 → BALB/c (Fig. 1*f*). The individual minor H and other known loci differences between donor and recipient, and the overall mortality rates are summarized in Table II. In viewing the results as a whole, the following points should be noted.

First, the transfer of 10^6 whole T cells led to (*a*) late-onset GVHD in the C3H.SW → B6 combination, (*b*) minimal disease in the DBA/2 → B10.D2 group, and (*c*) severe GVHD in the remaining four strain combinations, with high incidence and early onset (with a particularly acute GVHD response in the B10.D2 → BALB/c group).

Second, in three of the strain combinations (C3H.SW → B6, DBA/2 → B10.D2, and B10.S → SJL) the transfer of L3T4$^+$ T cells was clearly ineffective at mediating lethal GVHD. Except for a few mice, the recipients remained healthy and did not exhibit any typical clinical symptoms of disease. A few late-onset deaths occurred in the B10.BR → CBA combination, suggesting a mild incidence of GVHD. In two combinations, i.e., B10.D2 → DBA/2 and B10.D2 → BALB/c, the L3T4$^+$ cells were able to induce a high incidence of GVHD with an MST only slightly greater than with transfer of whole T cells (45 vs. 38 d, and 24 vs. 20 d, respectively).

Third, in all six combinations, Lyt-2$^+$ T cells were capable of mediating GVHD. In the C3H.SW → B6 and DBA/2 → B10.D2 groups, Lyt-2$^+$ cells appeared to be more potent than whole T cells, possibly reflecting the two- to threefold enrichment for Lyt-2$^+$ T cells; in terms of MST, however, this difference was not statistically significant. In the B10.BR → CBA and B10.S → SJL strain combinations, the incidence of GVHD and survival patterns were virtually the same for the whole T cell and Lyt-2$^+$ cell groups; the onset of GVHD occurred early in these two groups in comparison to the first two strain combinations. In the B10.D2 → DBA/2 and B10.D2 → BALB/c groups, Lyt-2$^+$ cells seemed to be less potent than either whole T cells or L3T4$^+$ cells, as reflected by a lower incidence of GVHD and a prolonged MST value; this difference was statistically significant only in the B10.D2 → BALB/c combination.

GVHD with Preimmunized T Cell Subsets. Because the precursor frequency of minor H antigen–specific L3T4$^+$ cells is probably quite low in unprimed populations, it was of interest to determine whether primed L3T4$^+$ cells might be more potent mediators of lethal GVHD. Donor C3H.SW mice were first thymectomized (ATX) to prevent the further influx of unprimed T cells into the peripheral lymphoid organs; 2 wk later the mice were immunized intraperito-

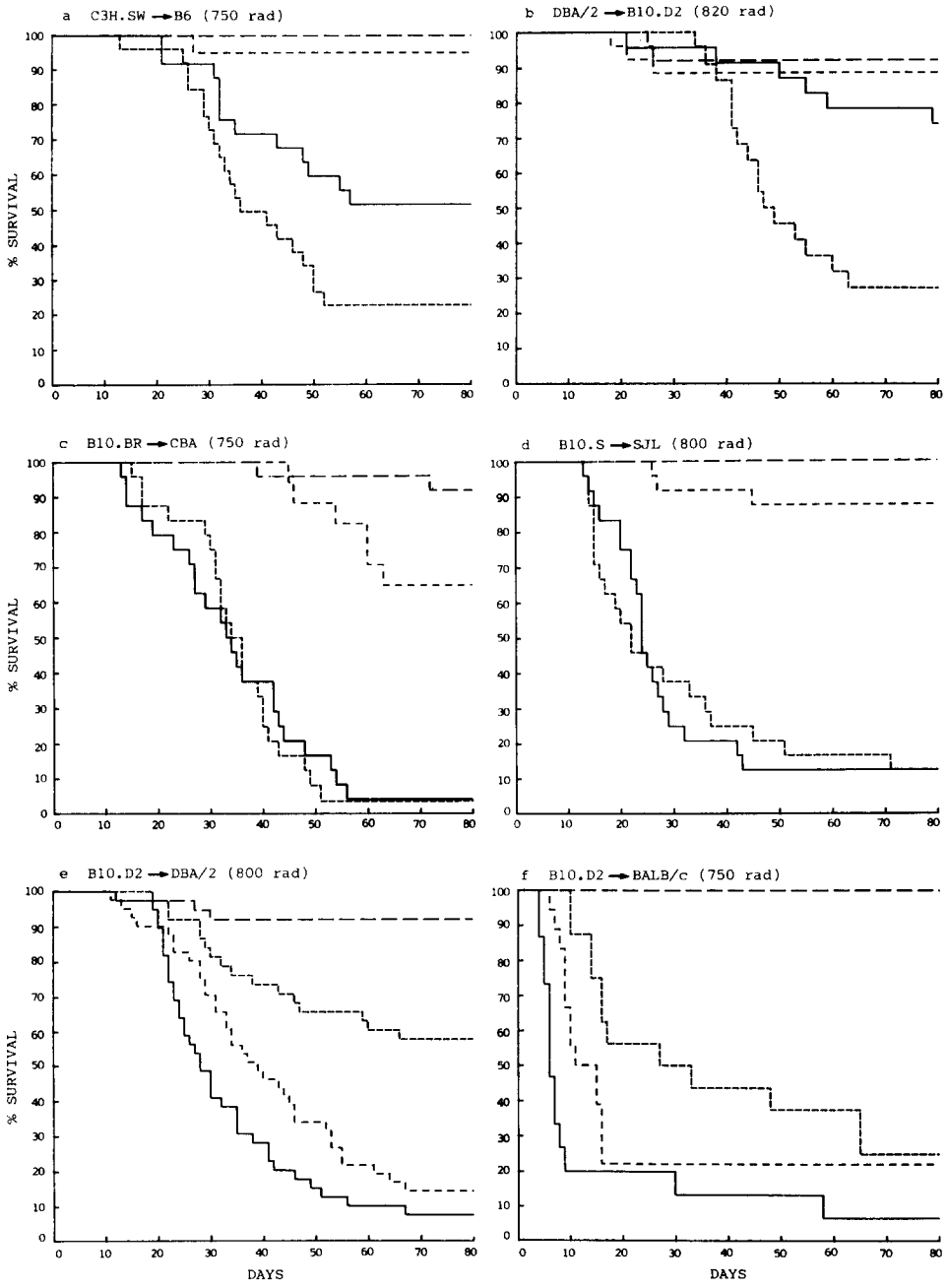

FIGURE 1. Lethal GVHD in H-2-compatible multiple minor H antigen–different strain combinations (*a–f*). Recipient mice were irradiated and injected intravenously with 4×10^6 donor ATBM alone (*long dash line*) or along with 10^6 donor whole T cells (*solid line*), purified L3T4+ cells (*medium dash line*), or Lyt-2+ cells (*short dash line*).

TABLE II

Summary of GVHD Mortality after Transferring Purified T Cell Subsets Along With ATBM to Lethally Irradiated H-2-compatible Allogeneic Recipients

Donor		Recipient		Some of the known genetic differences between donor and recipient	80-d Mortality (dead/total) and MST (d) after transfer of donor cells:*			
Strain	H-2	Strain	Irradiation		Whole T	L3T4$^+$ T	Lyt-2$^+$ T	ATBM alone
Unprimed:			*rad*					
C3H.SW	H-2^b	B6	750	H-1,-3,-7,-8,-9,-13 Mls^b, Ly-1,2	12/25 (58)[1]	1/21 (>80)	20/26 (45)[2]	0/24 (>80)
DBA/2	H-2^d	B10.D2	820	H-1,-3,-4,-8,-13 Mls^b, Ly-1,2	6/23 (>80)[3]	3/26 (>80)	16/22 (53)[4]	2/25 (>80)
B10.BR	H-2^k	CBA	750	H-1,-3,-7,-8,-9,-12 Tla, Mls^d, Ly-1,2	23/24 (38)[5]	6/17 (73)	23/24 (39)[6]	2/24 (>80)
B10.S	H-2^s	SJL	800	H-1,-3,-7,-8,-9, -12,-13 Tla, Mls^c	21/24 (34)[7]	3/24 (>80)	21/24 (35)[8]	0/20 (>80)
B10.D2	H-2^d	DBA/2	800	H-1,-3,-4,-8,-13 Mls^a, Ly-1,2	36/39 (38)[9]	35/41 (45)[10]	16/38 (58)[11]	3/38 (>80)
B10.D2	H-2^d	BALB/c	750	H-1,-3,-4,-7,-8,-9,-13	14/15 (20)[12]	14/18 (24)[13]	12/16 (39)[14]	0/13 (>80)
Primed:								
C3H.SW	H-2^b	B6	750	H-1,-3,-7,-8,-9,-13 Mls^b, Ly-1,2	14/17 (37)[15]	5/25 (>80)	20/21 (48)[16]	2/24 (>80)

* By use of Mann-Whitney statistical analysis, significant differences ($p < 0.01$) were found for groups 12 vs. 13, 12 vs. 14, 13 vs. 14, and 15 vs. 16, whereas no significance differences ($p > 0.05$) were observed for groups 1 vs. 2, 3 vs. 4, 5 vs. 6, 7 vs. 8, 9 vs. 10, 9 vs. 11, and 10 vs. 11.

TABLE III

Cytotoxic Activity of Anti–minor H CTL Generated from LN of Thymectomized C3H.SW Mice Immunized with B6 Spleen Cells

Responder LN group	Percent specific lysis of B6 target cells at E:T ratios of:					
	25.0:1	12.5:1	6.3:1	3.2:1	1.6:1	0.8:1
Preimmunized ATX C3H.SW	71	58	44	31	21	10
ATX C3H.SW	18*	10	7	5	4	2
Preimmunized normal C3H.SW	83	78	60	41	25	13
Normal C3H.SW	0	0	0	0	0	1

CTL were generated and assayed as described in Materials and Methods. Why a low primary response was observed with the ATX C3H.SW mice but not with normal C3H.SW mice is obscure.

neally with 2×10^7 B6 spleen cells. After another 3 wk the recipients were sacrificed, and their lymph nodes were pooled for T cell subset preparations. Stimulation of unseparated lymph node cells with B6 spleen cells in culture led to typical secondary CTL responses (Table III), indicating that, at least in the case of CTL generation, the T cells were indeed primed. For GVHD induction, the potency of whole T cells was appreciably higher with preimmunized cells (82% incidence, MST of 37 d) (Fig. 2) than with unprimed cells (48% incidence, MST of 58 d) (Fig. 1*a*). Preimmunization increased the potency of Lyt-2$^+$ cells in terms of mortality rate (95% for primed cells, 73% for unprimed cells) but had little effect on the MST (48 vs. 45 d). Preimmunization did cause a slight increase in the mortality rate with L3T4$^+$ cells, 5 of 25 mice dying between days 34 and 80, though none of the surviving mice exhibited any symptoms suggestive of chronic GVHD.

Extent of Hematopoietic Chimerism. To examine the extent of donor reconsti-

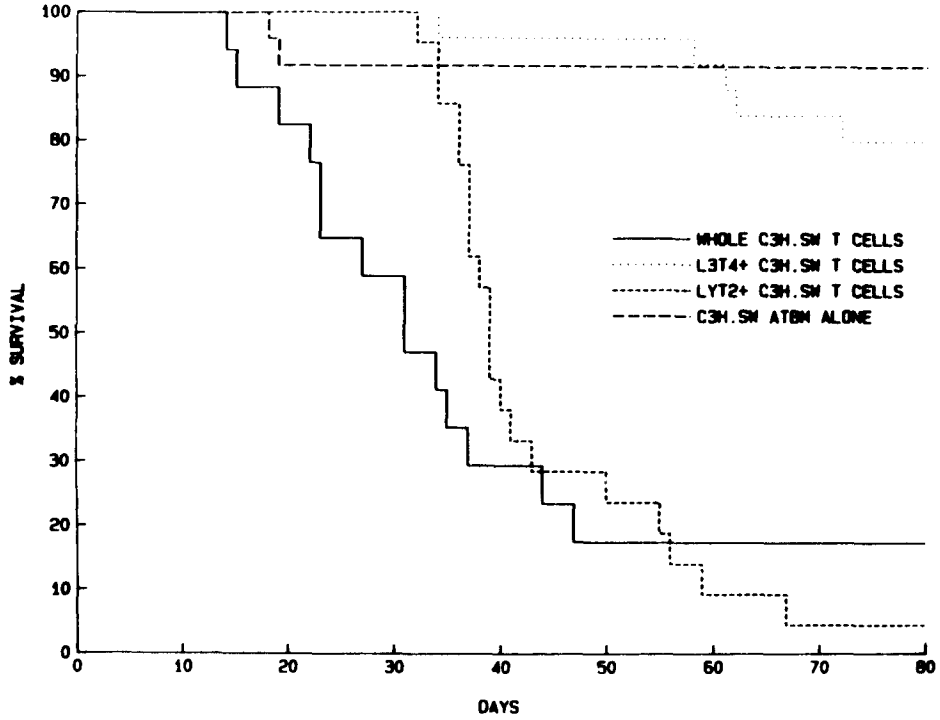

FIGURE 2. Lethal GVHD in irradiated (750 rad) B6 mice injected intravenously with 4 × 10^6 C3H.SW ATBM alone or along with either 10^6 presensitized C3H.SW whole T cells, purified L3T4$^+$ cells, or Lyt-2$^+$ cells.

tution of irradiated B6 mice given either unprimed or preimmunized C3H.SW L3T4$^+$ T cells plus donor ATBM, randomly selected mice were sampled after 80 d for lymphocyte expression of the Lgp100 determinant; this marker is expressed on a high proportion (>80%) of C3H.SW lymphocytes, but is not expressed on B6 cells (Table IV). It is apparent from Table IV that all of the mice tested showed near complete repopulation with donor-derived cells.

In Vitro Proliferation Responses of L3T4$^+$ Cells. In view of the surprising finding that unprimed L3T4$^+$ cells caused acute GVHD in the B10.D2 → DBA/2 and B10.D2 → BALB/c groups, we examined the capacity of B10.D2 L3T4$^+$ cells to mount primary proliferative responses to DBA/2 vs. BALB/c stimulators in vitro in an assay involving all six of the donor–recipient combinations described above (as shown in Table V). It is apparent that L3T4$^+$ cells responded poorly, if at all, in the C3H.SW anti-B6, DBA/2 anti-B10.D2, B10.S anti-SJL, and B10.D2 anti-BALB/c combinations; anti–allo-H-2 control responses were strong. B10.BR anti-CBA and B10.D2 anti-DBA/2 combinations gave high proliferative responses, but these responses were probably directed to Mls determinants (Mls^d and Mls^a being strongly stimulatory for unprimed T cells). It is apparent therefore that the ability of L3T4$^+$ T cells to proliferate in vitro does not correlate with GVHD induction. The point to emphasize is that unprimed B10.D2 L3T4$^+$ cells caused severe GVHD in BALB/c mice yet gave virtually no proliferative response to BALB/c in vitro.

TABLE IV

Extent of Chimerism in Irradiated B6 Mice Transplanted with C3H.SW ATBM Alone or in Addition to C3H.SW L3T4$^+$ T Cells

Exp.	Priming of donor L3T4$^+$ cells	Donor group	Percent of cells positive for Lgp100*
Normal C3H.SW LN		—	82.9
Normal B6 LN		—	0.0
C3H.SW → B6	−	L3T4$^+$ (a)	82.5
	−	L3T4$^+$ (b)	82.6
		ATBM (a)	96.5
		ATBM (b)	84.8
		ATBM (c)	96.7
C3H.SW → B6	+	L3T4$^+$ (a)	95.2
	+	L3T4$^+$ (b)	100.1
	+	L3T4$^+$ (c)	94.2
		ATBM (a)	83.5
		ATBM (b)	87.8

* LN cells from individual experimental mice were tested for the expression of the Lgp100 determinant by FACS analysis using secondary staining with FITC-conjugated goat anti-rat IgG (Fc) antibody. The percent positive values are relative to the normal positive control.

TABLE V

Proliferation Responses of L3T4$^+$ T Cells From Donor Strains

Responder strain	Stimulator strain	Stimulus*	[^{3}H]Thymidine incorporation on:			Severity of GVHD by L3T4$^+$ cells‡
			Day 4	Day 5	Day 6	
			cpm			
C3H.SW	C3H.SW	—	925	613	1,286	
	B6	MHA + Mls^b	636	1,063	1,570	Very weak
	DBA/2	H-2^d + MHA + Mls^a	51,660	33,635	13,696	
DBA/2	DBA/2	—	1,590	1,825	2,024	
	B10.D2	MHA + Mls^b	797	679	1,005	Very weak
	CBA	H-2^k + MHA + Mls^d	8,148	13,859	28,365	
B10.BR	B10.BR	—	666	575	675	
	CBA	MHA + Mls^d	41,111	12,251	8,883	Weak
	DBA/2	H-2^d + MHA + Mls^a	47,917	10,830	15,870	
B10.S	B10.S	—	1,185	1,339	1,079	
	SJL	MHA + Mls^c	408	393	376	Very weak
	DBA/2	H-2^d + MHA + Mls^a	24,355	19,539	16,361	
B10.D2	B10.D2	—	610	301	477	
	DBA/2	MHA + Mls^a	6,638	1,947	1,091	Strong
	BALB/c	MHA	759	833	832	Strong
	CBA	H-2^k + MHA + Mls^d	22,002	36,399	18,644	

* See Table II for details of minor H antigens (MHA) and other known differences.
‡ See Table II for mortality data.

Discussion

There are two main findings in this paper. First, highly purified populations of Lyt-2$^+$ cells caused a high incidence of lethal GVHD in all six H-2-compatible minor H–different strain combinations tested. Second, the capacity of L3T4$^+$ cells to cause GVHD across minor H barriers was highly variable: GVHD was virtually absent in two strain combinations, weak in one combination, and very strong in two other combinations.

The finding that purified Lyt-2$^+$ cells were able to elicit GVHD to minor H differences in the apparent absence of L3T4$^+$ cells is consistent with our previous finding that depleting T cells of Ia-restricted T cells or Lyt-1$^+$ T cells failed to inhibit GVHD (5, 6). The data are also in line with the evidence that transfer of purified Lyt-2$^+$ cells to heavily irradiated (1,100 rad) thymectomized mice (11) or nude mice (15) leads to rejection of H-2 class I–different skin allografts. These and other data (13, 16–23) strongly suggest that, at least in certain situations, Lyt-2$^+$ cells are able to function in the apparent absence of L3T4$^+$ cells, i.e., cells capable of producing exogenous help (IL-2). It does not necessarily follow, however, that the response of Lyt-2$^+$ cells is IL-2 independent. Lyt-2$^+$ cells themselves are able to produce IL-2 (16–21), and it is quite possible that the function of Lyt-2$^+$ cells at a population level depends upon IL-2 release from a minor subset of helper-independent Lyt-2$^+$ cells (22, 24). In support of this idea it has been found that GVHD to minor H differences can be inhibited by injection of anti–IL-2-R antibody (25) and that injection of IL-2 potentiates GVHD directed to class I (but not class II) differences (9).

Because the present experiments used only intermediate doses of irradiation (750–820 rad; higher doses led to unacceptable losses in the ATBM control group), the possibility exists that IL-2 produced by radioresistant host L3T4$^+$ cells provided significant levels of help for the injected Lyt-2$^+$ cells. Even if host L3T4$^+$ cells could be removed, e.g., by preconditioning the recipients with anti-L3T4 antibody (26), there is still the objection that the "de novo" generation of L3T4$^+$ cells from donor stem cells could lead to bystander IL-2 production. We are currently investigating this possibility by using thymectomized mice as recipients.

The limited capacity of L3T4$^+$ T cells to cause GVHD across minor H barriers in four of the six strain combinations tested is interesting in light of the finding that L3T4$^+$ cells are very potent mediators of GVHD directed to H-2 class II differences but not to class I differences (11, 27, 28); by the same token, L3T4$^+$ cells give high primary MLR only to class II and not to class I differences. Since MLR to minor H differences are generally poor, one could argue that the capacity of L3T4$^+$ cells to mediate GVHD is simply a reflection of precursor frequency. In this respect we anticipated that the marked variability in the capacity of L3T4$^+$ cells to mediate GVHD across minor H barriers would correlate with primary proliferative responses to the non-H-2 differences involved. No such correlation was found. For example, L3T4$^+$ cells gave high MLR to strains expressing Mls^a (B10.D2 → DBA/2) and Mls^d (B10.BR → CBA/J) differences, but L3T4$^+$-mediated GVHD was prominent in only one of these two situations (B10.D2 → DBA/2). Although Mls differences might potentiate GVHD (29), the most intense form of L3T4$^+$-mediated GVHD was seen in the

B10.D2 → BALB/c combination, i.e., an Mls-identical combination. In the case of MLR, however, B10.D2 L3T4$^+$ cells gave virtually no response to BALB/c stimulators, implying that primary proliferative responses in vitro are conspicuously unhelpful in predicting the severity of GVHD. It should be mentioned that B. L. Hamilton (personal communication) has also seen a high incidence of GVHD in irradiated BALB/c recipients of B10.D2 L3T4$^+$ cells (anti-Lyt-2 + C–treated spleen cells).

In trying to account for the variable capacity of L3T4$^+$ cells to mediate GVHD across minor H barriers, it is of interest that certain minor H antigens are immunodominant in terms of Lyt-2$^+$ CTL recognition (30–33). Similar immunodominance might apply to L3T4$^+$ cells. Thus it is conceivable that L3T4$^+$ cells might be able to recognize only a very limited number of minor H antigens, perhaps far fewer than Lyt-2$^+$ cells. If so, it is possible that only certain minor H–different strain combinations, e.g., B10.D2 → BALB/c, display the particular antigens that are immunogenic for L3T4$^+$ cells. In this respect it is intriguing that B10.D2 L3T4$^+$ cells express high Ia-restricted CTL activity for BALB/c target cells after priming in vivo followed by secondary stimulation in vitro in the presence of IL-2 (34). The obvious question is whether the intensity of L3T4$^+$ CTL responses to minor H differences in vitro show any correlation with the severity of GVHD. We are currently investigating this possibility. We caution that, at present, the nature of the target antigens for L3T4$^+$-mediated GVHD in the B10.D2 → BALB/c and B10.D2 → DBA/2 combinations is completely unknown. Indeed, there is no direct evidence that the target antigens are minor H antigens per se rather than other non-MHC antigens. It should also be stressed that the crucial issue of whether GVHD reflects CTL activity is still unsettled.

Because of the difficulty of preparing highly purified T cell subsets in large numbers, relatively low doses of T cells (10^6) were used in the present studies. It could be argued, therefore, that in the three strain combinations in which L3T4$^+$ cells failed to elicit GVHD to minor H differences, significant GVHD might have been observed with higher doses of L3T4$^+$ cells. Although we cannot rule out this possibility, previous studies showed that even very high doses (2×10^7) of anti-Lyt-2 + C–treated lymph node cells failed to elicit significant GVHD in the CBA → B10.BR combination. Paradoxically, very high doses of L3T4$^+$ cells can sometimes inhibit GVHD. Thus, whereas small doses (10^5–10^6) of B6 L3T4$^+$ cells regularly cause close to 100% mortality in irradiated class II–different bm12 mice, doses of 2×10^7 L3T4$^+$ cells often result in only low mortality rates (our unpublished data).

Defining which particular minor H antigens (or other non-MHC antigens) act as GVHD targets for L3T4$^+$ cells is of potential importance for HLA-matched marrow transplantation in humans. On the basis of studies with the CBA → B10.BR combination (where L3T4$^+$ cells did not cause GVHD) we suggested previously that depleting marrow inocula of CD8$^+$ (Lyt-2$^+$ equivalent) cells but leaving the CD4$^+$ cells intact might be beneficial in terms of facilitating engraftment and leading to rapid restoration of T-dependent antibody production (35). However, with the present appreciation that L3T4$^+$ cells can cause severe GVHD to non-MHC differences in certain situations, the rationale for not depleting human marrow of CD4$^+$ cells obviously needs to be reconsidered. An important

goal in the future will be to devise methods for predicting whether $CD4^+$ cells are likely to be injurious to the HLA-matched recipient.

Summary

Highly purified populations of $L3T4^+$ and Lyt-2^+ T cell subsets were compared for their capacity to cause lethal GVHD in six different H-2-compatible, multiple minor histocompatibility antigen–different murine strain combinations. In four of these combinations (C3H.SW → B6, DBA/2 → B10.D2, B10.BR → CBA, and B10.S → SJL), lethal GVHD appeared to be caused almost entirely by Lyt-2^+ cells; the injection of $L3T4^+$ cells resulted in low mortality even when these cells were presensitized to the recipient antigens. In the remaining two combinations (B10.D2 → DBA/2 and B10.D2 → BALB/c), $L3T4^+$ T cells were able to cause a high incidence of GVHD and were more potent than the Lyt-2^+ cells. The implications of these findings are discussed.

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