ROLE OF T CELL SUBSETS IN LETHAL GRAFT-VERSUS-HOST DISEASE (GVHD) DIRECTED TO CLASS I VERSUS CLASS II H-2 DIFFERENCES

I. L3T4$^+$ Cells Can Either Augment or Retard GVHD Elicited by Lyt-2$^+$ Cells in Class I–different Hosts

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Bone marrow transplantation in man can cure certain diseases, but is frequently accompanied by severe graft-versus-host disease (GVHD)[1] (1). Although GVHD can be prevented by depleting the marrow inoculum of mature T cells (2), patients receiving T-depleted marrow cells often show an increased incidence of graft rejection (reviewed in reference 3). Moreover, in leukemic patients the incidence of leukemic relapse tends to be higher with transfer of T-depleted marrow cells than with untreated marrow (3). T depletion of marrow cells thus appears to be a double-edged sword, and some clinicians (e.g., see references 4, 5) doubt whether the benefits of this procedure are sufficient to outweigh the severe side effects. Although this viewpoint is tenable in the case of HLA-compatible marrow transplants, i.e., where GVHD if often nonfatal, patients given HLA-incompatible marrow followed by conventional immunotherapy generally die from GVHD unless the marrow is T depleted (6). Despite the increased risk of graft rejection, T depletion of the marrow thus appears to be near mandatory for patients given HLA-incompatible marrow.

In considering methods for improving the success rate of HLA-mismatched marrow transplantation, an obvious issue is whether the marrow has to be depleted of both T cell subsets, i.e., of both CD4$^+$ and CD8$^+$ cells, or of only one subset. Although the relative importance of CD4$^+$ and CD8$^+$ cells in GVHD has been debated for a number of years (reviewed in reference 7), recent studies in mice conditioned with heavy irradiation have shown that each T cell subset can cause lethal GVHD in H-2-incompatible recipients (7–9). Based on studies with mutant strains of mice expressing slight (1–3 amino acid) differences in class I or class II H-2 molecules, it has been found that lethal GVHD directed to class I differences is controlled almost exclusively by CD8$^+$ (Lyt-2$^+$) cells, whereas CD4$^+$ (L3T4$^+$) cells account for GVHD to class II differences (10). Similar

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[1] *Abbreviations used in this paper:* BM, bone marrow; GVHD, graft-versus-host disease; LN, lymph node; MST, mean survival time; STx, sham thymectomized; Tx, thymectomized.

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stringent specificity of Lyt-2$^+$ and L3T4$^+$ cells for class I and class II molecules, respectively, applies in a variety of other assay systems including the mixed lymphocyte reaction (11) and skin allograft rejection (10, 12).

Despite the evidence that L3T4$^+$ and Lyt-2$^+$ cells both participate in GVHD (implying that human marrow should be depleted of both subsets), the issue of whether the two T cell subsets cause the same or different patterns of disease has not received close attention. In particular, very little is known about the features of the lethal form of GVHD seen in heavily irradiated mice. Rolink et al. (13, 14) reported that L3T4$^+$ and Lyt-2$^+$ cells elicited different patterns of GVHD in nonirradiated recipients, but GVHD in this situation was generally nonlethal.

The main aim of the experiments in this paper was to seek detailed information on the capacity of Lyt-2$^+$ cells to mediate lethal GVHD in class I–different irradiated mice and, in particular, to assess whether GVHD mediated by Lyt-2$^+$ cells depends on help from L3T4$^+$ cells. The results suggest that Lyt-2$^+$ cells do mediate GVHD in the apparent absence of L3T4$^+$ cells, although under certain conditions the potency of Lyt-2$^+$ cells can be considerably enhanced by addition of small doses of L3T4$^+$ cells or their products (IL-2). Paradoxically, large doses of L3T4$^+$ cells do not augment GVHD but instead provide long-term protection.

Materials and Methods

Mice. C57BL/6Kh (B6), B6.C-H-2^{bm1} (bm1), B6.C-H-2^{bm12} (bm12) and F_1 hybrids between these strains were obtained from the breeding colony at the Research Institute of Scripps Clinic (SCRF). All experiments were performed at SCRF. Male mice were used in most experiments.

Irradiation. Mice were exposed to a single dose of whole body γ-[^{137}Cs] irradiation at 90 rad/min via a Gamma Cell 40 irradiator (Atomic Energy of Canada, Ottawa, Canada).

Media. As described elsewhere (11), RPMI 1640 or HBSS supplemented with either 5% FCS or gamma globulin–depleted horse serum were used.

Monoclonal Antibodies. Anti-L3T4 (GK1.5 or RL172, ascites fluid), anti-Lyt-2 (3.168, ascites fluid), anti-Thy-1.2 (J1j, ascites fluid) and anti-B (J11d, culture supernatant) and guinea pig serum as a source of C′ were used as described previously (11).

Purification of T Cell Subsets. As described in detail elsewhere (11), ≥99% purified T cell subsets were obtained by subjecting pooled lymph node (LN) cells to mAb + C′ followed by positive panning on mAb–coated dishes. For example, Lyt-2$^+$ cells were made by (*a*) treating LN cells with anti-L3T4 + anti-B (J11d) mAb + C followed by (*b*) positive panning on anti-Lyt-2 mAb–coated plates.

GVH assay. Mice aged 3–10 mo were irradiated 1 d before transfer of lymphoid cells (injected intravenously) and were given standard food and water ad libitum without antibiotics. Mice were examined three to four times/week (usually daily for the first 3–4 wk) for 100 d or until death. Mice judged to be moribund, i.e., unable to take food or water, were killed.

Thymectomy. Mice aged 6–8 wk were thymectomized or sham thymectomized by the method of Miller (15).

rIL-2. Preparations of rIL-2 and mock rIL-2 were kindly provided by Cetus Corp. (Emeryville, CA) and were kept frozen until the day of use. Preparations were diluted in HBSS and given intraperitoneally in a volume of 0.2 ml.

Median Survival Time (MST). MSTs were calculated as described previously (8).

Results

In all of the experiments presented below, mice were exposed to irradiation 1 d before transfer of lymphoid cells. Unless stated otherwise, Lyt-2$^+$ and L3T4$^+$

TABLE I

Mortality in Heavily Irradiated (1,000 rad) (B6 × bm1)F_1 Mice Injected with B6 BM and Varying Doses of B6 Lyt-2$^+$ Cells

T cells transferred with B6 BM	T cells transferred	Experiments	Mortality			
			Dead/total	Dead	MST	Deaths occurring before 21 d
	n	*n*		%	*d*	%
B6 Lyt-2$^+$	2×10^7	1	5/5	100	38	20
	1×10^7	2	16/16	100	33	19
	5×10^6	2	11/13	85*	37	23
	3×10^6	5	33/34	97*	37	18
	2×10^6	3	19/19	100	39	0
	1×10^6	3	22/22	100	35	0
	3×10^5	3	22/22	100	35	0
	1×10^5	2	14/16	88*	52	0
	1×10^4	1	4/8	50*	87	0
B6 L3T4$^+$	1×10^7	2	0/12	0	>100	0
	3×10^6	4	1/22	5	>100	100
	1×10^6	5	1/25	4	>100	0
B6 BM only	—	11	3/60	5	>100	67

Mice given whole body irradiation 1 d before were injected intravenously with highly purified B6 Lyt-2$^+$ or L3T4$^+$ cells mixed with a uniform dose of 2×10^6 anti-Thy-1 + C-treated B6 BM. The recipients were inspected three to four times per week until death or for 100 d. The data were compiled from 11 different experiments, each experiment involving at least four mice per group.

* The surviving mice in these groups all showed prominent symptoms of chronic GVHD during the first 2 mo but then partially recovered; two mice subsequently died after 100 d.

cells were purified from LN cells by a combination of mAb + C treatment followed by positive panning (Materials and Methods). In most experiments, T cells were coinjected with donor-type marrow cells; marrow cells were pretreated with anti-Thy-1 mAb + C and injected in a dose of 2×10^6 cells/mouse. All cell suspensions were given intravenously. To avoid the problem of host-versus-graft reactions, parental strain T cells were transferred to F_1 hybrid mice.

Dose of Lyt-2$^+$ Cells Required to Induce Lethal GVHD in Class I–different Recipients. In previous studies (10), lethal GVHD developing in class I–different homozygous bm1 recipients of B6 Lyt-2$^+$ cells was examined with relatively high doses of Lyt-2$^+$ cells ($1.5–7.5 \times 10^6$) and with heavy irradiation of the host (1,000 rad). The pooled results of 11 different experiments in which varying doses of B6 Lyt-2$^+$ cells were transferred with B6 BM to heavily irradiated (1,000 rad) heterozygous (B6 × bm1)F_1 mice are shown in Table I. Two main points arise from the data. First, mortality rates approaching 100% were observed over a 200-fold range in the dose of Lyt-2$^+$ cells; 10^5 cells caused severe mortality and even as few as 10^4 cells caused 50% mortality. Second, irrespective of the dose

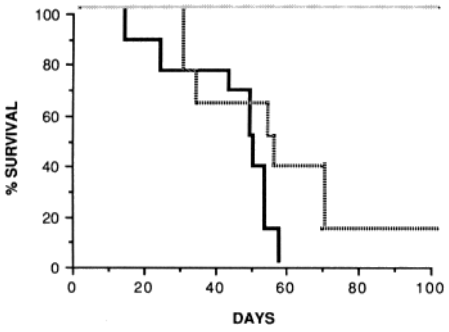

FIGURE 1. Mortality rates in heavily irradiated (1,000 rad) (B6 × bm1)F_1 mice given high vs. low doses of B6 Lyt-2^+ cells plus a constant dose of 2 × 10^6 T-depleted B6 BM (eight mice/group); Lyt-2^+ cells were injected in a dose of 10^7 cells (▬) (MST = 49 d) or 10^5 cells (▮▮) (MST = 54 d) per mouse. Control mice (▒▒) received irradiation and BM without Lyt-2^+ cells (five mice/group). Data are from a single experiment.

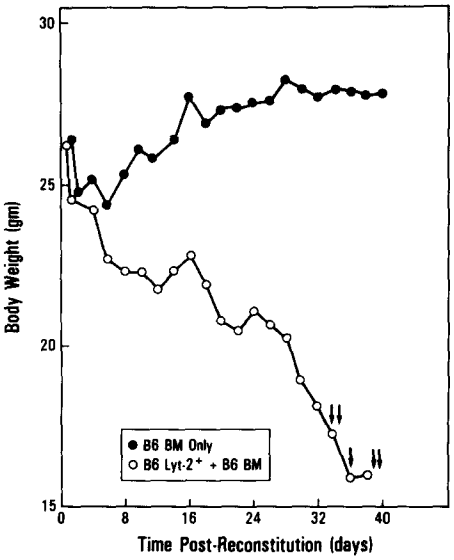

FIGURE 2. Mean body weights of heavily irradiated (1,000 rad) (B6 × bm1)F_1 mice given a mixture of 2 × 10^6 Lyt-2^+ cells plus B6 BM or B6 BM alone (five mice/group). The recipients were weighed every 2 d for 40 d or until death (*arrows*); the control mice survived for >100 d. In the particular experiment shown, the recipients of B6 Lyt-2^+ cells plus B6 BM became ill, with hunched posture and ruffled fur at day 6. The clinical condition of the mice improved after day 12 but deteriorated again at about day 24. Thereafter the condition of the mice worsened progressively until death.

of Lyt-2^+ cells injected, most of the recipients died from chronic GVHD, death rates in recipients of high (10^7) vs. low (10^5) doses of cells being remarkably similar (Fig. 1). Especially with high doses of Lyt-2^+ cells, the recipients became obviously ill at the end of the first week after transfer and showed weight loss and ruffled fur. Although a few mice deteriorated further and died over the next 2 wk (not seen with lower cell doses; Table I), most of the mice showed a temporary recovery; although weight gain was marginal (Fig. 2), ruffled fur became much less prominent at the end of the second week and the mice regained near-normal physical activity. At ~4 wk after transfer, the mice showed a sudden recrudescence of symptoms. The mice developed severe and progressive weight loss with diarrhea and hunched posture and most mice died over the next 1–2 wk; weight loss at the time of death was often extreme (>40%) (Fig. 2).

In accordance with previous findings (10), transfer of B6 L3T4$^+$ cells to irradiated class I–different (B6 × bm1)F_1 mice caused no mortality (Table I) or signs of ill health.

GVHD in Lightly Irradiated Recipients. As illustrated in Table II, varying the dose of irradiation given to (B6 × bm1)F_1 recipients of B6 Lyt-2^+ cells had surprisingly little effect on the severity of GVHD. Thus, over a 20-fold range in

TABLE II

Mortality in Irradiated (B6 × bm1)F_1 Recipients of B6 Lyt-2$^+$ Cells and B6 BM: Effect of Varying the Dose of Irradiation

B6 Lyt-2$^+$ cells transferred with B6 BM	Dose of irradiation given to recipients	Mortality		
		Dead/total	Mortality	MST
n	*rad*		%	*d*
5×10^6	1,000	6/8	75	43
	800	8/8	100	37
	600	8/8	100	33
2.5×10^5	1,000	8/8	100	33
	800	8/8	100	32
	600	8/8	100	33
BM only	1,000	0/5	0	>100
	800	0/5	0	>100
	600	0/5	0	>100

Data are from a single experiment.

TABLE III

Rapid Mortality in Heavily Irradiated (1,000 rad) (B6 × bm1)F_1 Mice Given B6 Lyt-2$^+$ Cells plus F_1 Host BM

Cells transferred to (B6 × bm1)F_1 mice given 1,000 rad	Mortality		
	Dead/total	Mortality	MST
		%	*d*
10^6 B6 Lyt-2$^+$ + B6 FM	8/8	100	43
10^6 B6 Lyt-2$^+$ + F_1 BM	8/8	100	12
10^6 B6 Lyt-2$^+$ + B6 BM + F_1 BM	8/8	100	47
B6 BM only	0/5	0	>100
F_1 BM only	0/5	0	>100
B6 + F_1 BM	0/5	0	>100

Each BM population was injected with a dose of 2×10^6 cells.

the dose of Lyt-2$^+$ cells, mortality rates were as severe in 600 rad irradiated recipients as in mice given 1,000 rad, and the MSTs showed little variation.

Influence of Donor-vs.-Host BM Cells. In all of the above experiments, Lyt-2$^+$ cells were transferred with syngeneic (B6) BM. When B6 Lyt-2$^+$ cells were transferred with host-type (F_1) BM to heavily irradiated (1,000 rad) recipients, all of the mice died within 2 wk (Table III). At death, the mice showed extreme tissue pallor and marked atrophy of the spleen, implying that death resulted from hematopoietic failure. Coinjecting B6 Lyt-2$^+$ cells with a mixture of donor and host marrow led to the typical pattern of chronic GVHD seen with transfer of donor marrow (Table III). This latter finding suggested that the donor Lyt-2$^+$ cells destroyed F_1 BM cells by direct cell contact rather than via a bystander effect.

Striking evidence on the capacity of donor Lyt-2$^+$ cells to destroy host-type hematopoietic cells is illustrated by the experiments shown in Table IV in which

TABLE IV

Rapid Mortality in Lightly Irradiated (500 rad) (B6 × bm1)F_1 Mice Given B6 Lyt-2$^+$ Cells without Marrow Cells

Cells transferred to (B6 × bm1)F_1 mice given 500 rad	B6 BM added	Experiments	Mortality		
			Dead/total	Dead	MST
		n		%	*d*
2×10^6 B6 Lyt-2$^+$	+	2	10/10	100	32
2×10^6 B6 Lyt-2$^+$	−	4	25/25	100	15
1×10^5 B6 Lyt-2$^+$	−	2	10/16*	63	59
—	+	2	0/8	0	>100
—	−	2	0/8	0	>100

* Of the six mice surviving >100 d, four mice died over the next 6 wk with typical symptoms of chronic GVHD.

B6 Lyt-2$^+$ cells were transferred to lightly irradiated (500 rad) (B6 × bm1)F_1 recipients ± donor-type BM. When 2×10^6 B6 Lyt-2$^+$ cells were transferred to 500 rad recipients with B6 BM, the recipients all died from chronic GVHD. However, when the Lyt-2$^+$ cells were transferred without BM cells, the mice suddenly died at 2 wk after transfer from hematopoietic failure associated with marked anemia and striking lymphoid atrophy. This finding was only observed with relatively high doses of cells (2×10^6); lower doses (10^5) produced chronic GVHD. No mortality was seen in control mice given 500 rad without Lyt-2$^+$ cells.

Are Lyt-2$^+$ Cells Helper Independent In Vivo? The conspicuous potency of Lyt-2$^+$ cells at causing lethal GVHD to class I differences raised the question whether Lyt-2$^+$ cells function autonomously in vivo or depend on minor degrees of help provided by L3T4$^+$ cells. Two approaches were used to address this question.

Thymectomy of the Host. It was mentioned earlier that recipients of class I–different Lyt-2$^+$ cells plus donor BM typically showed a sudden, lethal exacerbation of symptoms at about 4 wk after transfer. It seemed possible that this pattern of disease reflected bystander help provided by newly formed donor-derived L3T4$^+$ cells arising in the host thymus. This possibility was investigated by studying GVHD in thymectomized (Tx) recipients. Table V shows results compiled from five different experiments in which B6 Lyt-2$^+$ cells plus B6 BM were transferred to heavily irradiated Tx (B6 × bm1)F_1 mice; sham-thymectomized (STx) mice were used as controls. Heavy mortality occurred in both groups of mice: MSTs appeared to be slightly prolonged in ATx recipients but this difference was not statistically significant. A small proportion of the recipients survived for >100 d in both groups, but these mice all showed evidence of chronic GVHD (Table V, footnote). It is worth mentioning that the mice used in these experiments were somewhat older than in earlier experiments. Although we have not done a thorough study of anti–class I GVHD in old-vs.-young mice, older mice tend to die more slowly, perhaps because of their greater stores of body fat.

Injection of Anti-L3T4 mAb. The second approach for testing whether GVHD induced by Lyt-2$^+$ cells requires help from L3T4$^+$ cells was to transfer B6 Lyt-2$^+$ cells plus B6 BM to heavily irradiated (B6 × bm1)F_1 mice and then inject the

TABLE V

Mortality in Thymectomized Irradiated (1,000 rad) (B6 × bm1)F_1 Mice Given B6 Lyt-2$^+$ Cells plus B6 Marrow

T cells transferred with B6 BM	Thymectomy (Tx) or sham thymectomy (STx) of recipients	Experiments	Mortality			
			Dead/total	Dead	MST	*p* values
		n		%	*d*	
5 × 10^6 B6 Lyt-2$^+$	STx	2	8/8	100	57	>0.1
	Tx	2	7/8	88	69	
3 × 10^6 B6 Lyt-2$^+$	STx	2	14/16	88	39	>0.1
	Tx	3	17/21	81	57	
3 × 10^5 B6 Lyt-2$^+$	STx	1	4/5	80	52	>0.1
	Tx	1	4/6	67	98	
B6 BM only	STx	3	0/13	0	>100	
	Tx	4	1/12	8	>100	

Host mice were used at 4–8 wk after Tx or STx for most experiments and at 9 mo for one experiment. Of the total of seven Tx mice that survived for >100 days, all of these mice showed symptoms of chronic GVHD and only three of the mice survived beyond 150 d; of the three survivors in the STx group, two of the mice survived beyond 150 d.

recipients repeatedly with large amounts of anti-L3T4 (GK1.5) mAb (Table VI, footnote). Control experiments established that a course of anti-L3T4 mAb injections was highly effective at inhibiting anti–class II GVHD, i.e., GVHD developing in irradiated (B6 × bm12)F_1 recipients of class II–different B6 L3T4$^+$ cells (Table VI, Exp. A, groups 4 and 5). Significantly, injection of anti-L3T4 mAb had little if any effect in prolonging the survival of irradiated (B6 × bm1)F_1 mice given class I–different B6 Lyt-2$^+$ cells (Table VI, Exp. A, groups 1 and 2). Likewise, repeated injection of anti-L3T4 mAb failed to overcome the pattern of rapid death from hematopoietic failure seen when B6 Lyt-2$^+$ cells were transferred to (B6 × bm1)F_1 mice with host-type marrow cells (Table VI, Exp. B, groups 2 and 3).

Effects of Supplementing Lyt-2$^+$ Cells with Small Doses of L3T4$^+$ Cells or rIL-2. Although the above two approaches showed little or no evidence that GVHD mediated by Lyt-2$^+$ cells required the presence of L3T4$^+$ cells, studies in which Lyt-2$^+$ cells were supplemented with small doses ($\leq 10^6$) of donor-type L3T4$^+$ cells led to a pattern of acute GVHD and early death (Table VII). Interestingly, this effect was much more prominent with relatively high doses of Lyt-2$^+$ cells (5 × 10^6) than with lower doses (2 × 10^6).

It seemed highly likely that the capacity of small doses of L3T4$^+$ cells to exacerbate GVHD mediated by Lyt-2$^+$ cells reflected local release of IL-2. To

TABLE VI

Mortality in Irradiated (1,000 rad) (B6 × bm1)F_1 Mice Given B6 Lyt-2$^+$ Cells and Repeated Injections of Anti-L3T4 mAb

Exp.	Group	Cells transferred to irradiated mice	Irradiated recipients	Injection of anti-L3T4 mAb	Mortality		
					Dead/total	Dead	MST
						%	*d*
A	1	1×10^6 B6 Lyt-2$^+$ + B6 BM	(B6 × bm1)F_1	−	8/8	100	50
	2			+	8/8	100	55
	3	B6 BM only	(B6 × bm1)F_1	−	0/5	0	>100
	4	1×10^6 B6 L3T4$^+$ + B6 BM	(B6 × bm12)F_1	−	8/8	100	10
	5			+	1/8	13	>100
	6	B6 BM only	(B6 × bm12)F_1	−	0/4	0	>100
B	1	2×10^6 B6 Lyt-2$^+$ + B6 BM	(B6 × bm1)F_1	−	5/5	100	43
	2	2×10^6 B6 Lyt-2$^+$ + F_1 BM	(B6 × bm1)F_1	−	5/5	100	13
	3			+	5/5	100	11
	4	B6 BM only	(B6 × bm1)F_1	−	0/5	0	>100
	5	F_1 BM only	(B6 × bm1)F_1	−	0/5	0	>100

Mice were injected intraperitoneally with undiluted GK1.5 ascites fluid in doses of 0.2 ml/mouse on days 1, 3, and 7 and then 0.1 ml at weekly intervals thereafter; for (B6 × bm12)F_1 recipients, the injections were stopped after 6 wk. Others (16) have demonstrated that GK1.5 mAb is highly effective at removing L3T4$^+$ cells in vivo. The MSTs in group 1 vs. 2 in Exp. A were not significantly different ($p > 0.1$).

TABLE VII

Mortality in Irradiated (1,000 rad) (B6 × bm1)F_1 Mice Given B6 Lyt-2$^+$ Cells plus B6 Marrow: Effects of Adding B6 L3T4$^+$ Cells

Group	Dose of B6 Lyt-2$^+$ cells given with B6 BM	Dose of B6 L3T4$^+$ cells added to B6 Lyt-2$^+$ cells	Experiments	Mortality			
				Dead/total	Dead	MST	Reduction of MST
			n		%	*d*	%
1	5×10^6	—	3	13/13	100	50	
2		1×10^6	3	13/13	100	17	66
3		0.2×10^6	1	5/5	100	16	68
4	3×10^6	—	3	24/24	100	50	
5		1×10^6	3	24/24	100	24	52
6	2×10^6	—	2	14/14	100	37	
7		1×10^6	2	12/12	100	33	11
8	2×10^6	—	2	13/13	100	40	
9		3×10^7*	2	0/10	0		

* For preparing L3T4$^+$ cells in large doses, the (tedious) positive panning step was omitted, i.e., LN cells were pretreated with J11d + anti-Lyt-2 mAb + C but were not panned on anti-L3T4 mAb–coated plates. p values: groups 1 vs. 2, $p < 0.02$; groups 4 vs. 5, $p < 0.02$; groups 6 vs. 7, $p > 0.1$.

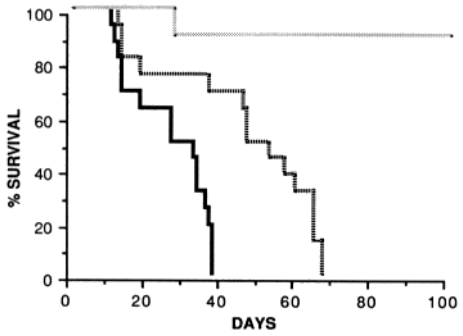

FIGURE 3. Capacity of rIL-2 to accelerate mortality in heavily irradiated (1,000 rad) (B6 × bm1)F_1 mice given 2×10^6 B6 Lyt-2^+ cells plus B6 BM (▬). Intraperitoneal injections of rIL-2 (5,000 U/injection) were given every 2 d for a total of six injections beginning on day 8 after irradiation (MST = 29 d, 16 mice). Control mice received either: (*a*) Lyt-2^+ cells plus B6 BM plus mock rIL-2 (▥) (MST = 49 d, 16 mice) or (*b*) B6 BM plus rIL-2 (▒) (MST = >100 d, 10 mice). Pooled data from two experiments. The accelerated mortality induced by the rIL-2 injections in recipients of Lyt-2^+ cells was statistically significant ($p < 0.02$).

assess this possibility, heavily irradiated (B6 × bm1)F_1 mice were given B6 Lyt-2^+ cells plus B6 BM and then injected repeatedly with rIL-2 (six doses of 5,000 U given every 2 d beginning at 1 wk after T cell transfer). As shown in Fig. 3, injection of rIL-2 caused the same pattern of accelerated mortality seen with transfer of small doses of L3T4$^+$ cells. Control mice given rIL-2 without B6 Lyt-2^+ cells showed little or no mortality.

Effect of Supplementing Lyt-2$^+$ Cells with Large Doses of L3T4$^+$ Cells. Although the capacity of small doses of L3T4$^+$ cells to augment anti–class I GVHD was a constant finding (provided that Lyt-2^+ cells were transferred in high doses; Table VII), transfer of larger doses of L3T4$^+$ cells ($2–5 \times 10^6$) led to inconsistent results (data not shown); potentiation of GVHD tended to be less prominent and, paradoxically, some mice showed little or no evidence of GVHD, implying that higher doses of L3T4$^+$ cells might be protective. To assess this idea, Lyt-2^+ cells were supplemented with comparatively massive doses of L3T4$^+$ cells (3×10^7 cells). The striking finding, observed in two separate experiments, was that heavily irradiated (B6 × bm1)F_1 mice given 2×10^6 B6 Lyt-2^+ cells plus B6 BM together with a dose of 3×10^7 B6 L3T4$^+$ cells showed no mortality (Table VII). Except for weight loss in the first two weeks and a mild retardation in weight gain thereafter, the mice showed no symptoms of GVHD.

Effect of Injecting Anti-Thy-1 mAb. The typical pattern of chronic GVHD mediated by Lyt-2^+ cells plus syngeneic BM in the absence of L3T4$^+$ cells (e.g., Fig. 1) raised the question whether the Lyt-2^+ cells mounted a prolonged attack against the host, or conversely, attacked the host for only a brief period, the consequences of this attack being slow to develop. To seek information on this question, heavily irradiated (B6 × bm1)F_1 recipients of B6 Lyt-2^+ cells plus B6 BM were given a single injection of anti-Thy-1 mAb at 3 wk after transfer. As shown in Fig. 4, injection of anti-Thy-1 mAb was clearly protective, although 50% of the recipients eventually died.

Discussion

Because of the expense and long-term nature of GVHD experiments, the principle aim of the present studies was not to examine GVHD in a wide variety of class I–different strain combinations but to seek comprehensive information on a single combination, i.e., B6 → bm1. Although some of the data, e.g., the protective effect of L3T4$^+$ cells, have been confirmed in the reciprocal bm1 → B6 combination (data not shown), detailed information on other combinations is

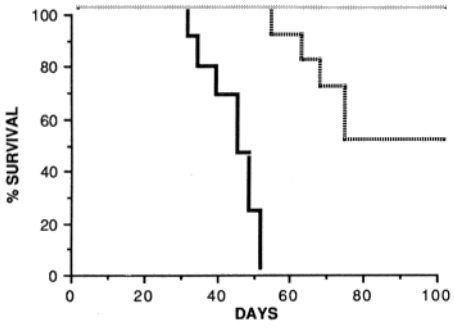

FIGURE 4. Mortality rates in heavily irradiated (1,000 rad) (B6 × bm1)F_1 mice given 2×10^6 B6 Lyt-2^+ cells plus B6 BM followed 21 d later by a single intraperitoneal injection of T24 anti-Thy-1 mAb (▬) (MST = 87 d, 10 mice); T24 anti-Thy-1 mAb (0.5 ml of undiluted ascites fluid/mouse) has known opsonizing activity in vivo (see reference 10). Control mice received Lyt-2^+ cells plus BM without anti-Thy-1 mAb (▥) (MST = 44 d, 9 mice) or BM cells only (▩) (MST >100 d, 8 mice). The enhanced survival seen in the recipients of anti-Thy-1 mAb was statistically significant ($p < 0.02$).

not yet available. It should be emphasized that the B6 anti-bm1 response is particularly strong (10, 11), and conclusions based on this strain combination (see below) might not necessarily apply to weaker class I differences. However, it is worth mentioning that a preliminary study in which purified B6 Lyt-2^+ cells were transferred to irradiated bm9 mice led to severe GVHD (Mowat, A., and J. Sprent, unpublished data); this combination leads to only weak responses in vitro in the absence of added help (11, 17).

The experiments in this paper indicate that the particular pattern of GVHD seen in irradiated (B6 × bm1)F_1 mice given class I–different B6 Lyt-2^+ cells depends critically on two factors: (*a*) the origin (donor vs. host) of the BM cells used for stem cell reconstitution and (*b*) the presence or absence of donor-derived L3T4^+ cells.

When Lyt-2^+ cells were transferred to irradiated class I–different recipients without BM or with *host* BM, the mice died rapidly from hematopoietic failure (Tables III and IV). Since this syndrome was not observed in mice given a mixture of donor and host BM, hematopoietic failure presumably did not reflect a bystander effect, i.e., release of toxic lymphokines, but rather direct destruction of class I–different host stem cells by antigen-specific Lyt-2^+ cells. In all probability this form of GVHD (perhaps better termed a graft-vs.-host reaction) is controlled by classic cytotoxic lymphocytes.

Early deaths were rare when class I–different mice were given purified Lyt-2^+ cells supplemented with *donor* BM. In this situation the recipients developed typical chronic GVHD, with most deaths occurring only after 1 mo; this syndrome was observed with 1,000-fold variation in the dose of Lyt-2^+ cells and with either heavy (1,000 rad) or light (500 rad) irradiation of the hosts. Except for persistent weight loss, symptoms of GVHD were not usually apparent before 3–4 wk after transfer. At this stage the mice became overtly ill with prominent ruffled fur, diarrhea, and increasing weight loss. Thereafter, the condition of the mice deteriorated progressively over a 2–3-wk period. This syndrome of chronic GVHD was almost invariably fatal; occasional mice made a partial recovery (more prominent with older recipients), but these isolated survivors rarely regained full health.

To account for the sudden, late onset of the symptoms which heralded chronic GVHD, we initially considered the possibility that clinical GVHD was precipitated by interaction of Lyt-2^+ cells with newly derived L3T4^+ cells developing in the host thymus. By responding to environmental antigens and releasing IL-2, we

speculated that these L3T4$^+$ cells augmented the effector function of Lyt-2$^+$ cells and thus promoted a rapid wave of tissue damage. This scenario was not supported by the finding that Tx and STx mice showed no significant difference in their susceptibility to GVHD. Moreover, repeated injection of anti-L3T4 mAb had little or no effect in inhibiting GVHD. Collectively, these data suggest that, as for short-term assays in vitro (11, 17), protracted in vivo responses of Lyt-2$^+$ cells do not require exogenous help from L3T4$^+$ cells (see also references 10, 12). Parenthetically, one might ask whether mice with GVHD are capable of sustaining *de novo* formation of L3T4$^+$ cells. On this point we have found only negligible numbers (1–2%) of L3T4$^+$ cells in spleen and LN of (non-Tx) mice with chronic GVHD (unpublished data). This finding is not unexpected since mice with chronic GVHD usually display marked thymic atrophy.

The protracted clinical course of chronic GVHD raises the question whether the donor Lyt-2$^+$ cells attack the host continuously from the time of injection or, alternatively, mount a brief attack which has delayed consequences. This second possibility is not supported by the finding that hosts given a single injection of anti-Thy-1 mAb at 3 wk after transfer of Lyt-2$^+$ cells showed a much milder pattern of GVHD (Fig. 4). The survival time of the recipients was considerably prolonged and only 50% of the mice died. These data favor the view that the donor Lyt-2$^+$ cells attack the host for a period exceeding 3 wk. Whether this attack occurs continuously or in repeated waves, however, is unclear.

Although Lyt-2$^+$ cells appeared to be capable of eliciting GVHD in the apparent absence of L3T4$^+$ cells, supplementing Lyt-2$^+$ cells with small doses of L3T4$^+$ cells led to a pattern of acute GVHD with most deaths occurring at 2–3 wk after transfer; similar findings applied with mice given repeated injections of rIL-2. These data suggest that, in contrast to chronic GVHD, the expression of acute GVHD by Lyt-2$^+$ cells is under the strict control of L3T4$^+$ cells and/or their products. It should be mentioned that the capacity of IL-2 to potentiate GVHD has also been observed by two other groups (18, 19); these studies involved transfer of unseparated lymphoid cells rather than purified Lyt-2$^+$ cells.

The capacity of L3T4$^+$ cells to augment GVHD mediated by Lyt-2$^+$ cells only applied when L3T4$^+$ cells were injected in small doses. Thus when Lyt-2$^+$ cells were transferred to class I–different mice together with very large doses of L3T4$^+$ cells, symptoms of GVHD were relatively mild and no mortality occurred. This paradoxical finding raises the issue of the pathogenesis of GVHD. Since GVHD is relatively mild in mice kept in a germ-free environment (20, 21), severe forms of GVHD are presumably the end result of infection. In view of the propensity of activated T cells to migrate to mucosal surfaces, e.g., the gut (22, 23), one can envisage that infectious organisms enter the body as the result of local damage to the gut or respiratory tract; such damage might reflect direct cell-mediated lympholysis or local release of toxic lymphokines, or both. The notion that lethal forms of GVHD are mainly the result of infection is in keeping with the finding that GVHD is particularly severe in immunoincompetent hosts, e.g., in irradiated mice (20). The obvious corollary here is that overcoming the immune deficit in irradiated mice, e.g., by adding mature lymphoid cells lacking host alloreactivity, would lessen the severity of GVHD. By this line of reasoning, the protective effects of large doses of L3T4$^+$ cells are easily explained.

Although the precise mechanism whereby L3T4$^+$ cells protect mice against anti–class I GVHD is still unclear, the explanation we favor is that L3T4$^+$ cells interact with B cells in the marrow suspension (or with the progeny of these cells) and thus enable the host to make humoral responses against invading organisms. Since marrow cells per se contain relatively few mature B cells, it would follow that injection of a mixture of donor L3T4$^+$ cells and mature B cells, e.g., T-depleted donor spleen, would augment the protective function of L3T4$^+$ cells. Preliminary work has shown that this is indeed the case (our unpublished data).

Since L3T4$^+$ cells generally ignore class I differences (10, 11), the capacity of L3T4$^+$ cells to protect mice against GVHD might be expected to apply only in class I–different combinations. Thus, in class II–different hosts, the strong reactivity of donor L3T4$^+$ cells against host alloantigens would tend to nullify any protective (T helper) function of these cells. In fact, the sequel to this paper will present evidence that, although small doses of L3T4$^+$ cells do cause 100% mortality and rapid death in class II–different irradiated hosts, large doses of L3T4$^+$ cells lead to only limited mortality. The protective function of L3T4$^+$ cells thus seems to apply with either class I or class II disparity. The clinical implications of this finding remain to be explored.

Summary

Detailed information was sought on the capacity of purified Lyt-2$^+$ cells to mediate lethal graft-versus-host disease (GVHD) directed to class I H-2 differences. When B6 Lyt-2$^+$ cells were transferred to irradiated class I–different (B6 × bm1)F_1 mice, three different patterns of lethal GVHD were observed. First, rapid death from hematopoietic failure occurred when Lyt-2$^+$ cells were transferred together with host-type marrow cells; this form of GVHD probably reflected direct destruction of stem cells by Lyt-2$^+$ cytotoxic cells. Second, a pattern of late-onset, chronic GVHD resulting in death only after 4–6 wk occurred when Lyt-2$^+$ cells were supplemented with donor marrow. This syndrome developed in the apparent absence of L3T4$^+$ cells and was observed with either high or low doses of Lyt-2$^+$ cells and with either light or heavy irradiation of the host. Third, an acute form of GVHD resulted when Lyt-2$^+$ cells plus donor marrow cells were supplemented with exogenous help, i.e., by adding small doses of donor L3T4$^+$ cells or injecting the hosts with rIL-2.

Although L3T4$^+$ cells potentiated GVHD when injected in small doses, supplementing Lyt-2$^+$ cells with large doses of L3T4$^+$ cells paradoxically led to marked protection; symptoms of GVHD were mild and no deaths occurred.

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