FEATURES OF T CELLS CAUSING H-2-RESTRICTED LETHAL GRAFT-VS.-HOST DISEASE ACROSS MINOR HISTOCOMPATIBILITY BARRIERS*

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Unprimed T cells transferred to heavily irradiated H-2-compatible mice cause a high incidence of lethal graft-vs.-host disease (GVHD)[1] in certain strain combinations (1-3). GVHD in this setting is a consequence of mature post-thymic donor T cells responding to the multiple minor histocompatibility antigen (minor HA) differences of the host. With the combination of CBA and B10.BR (both $H\text{-}2^k$), doses of as few as 10^6 CBA T cells regularly kill close to 100% of irradiated B10.BR mice. Recent studies with this strain combination demonstrated that T cells eliciting lethal GVHD to minor HA are subject to H-2-restriction (4). To examine this question, CBA T cells were recirculated through irradiated mice of the B10 *H-2* congenic lines and then tested for their capacity to kill B10.BR mice. When CBA T cells were filtered from blood to lymph for 1 d through irradiated B10.BR or H-2-semisyngeneic (CBA × B10)F_1 mice, negative selection occurred, i.e., the filtered T cells failed to kill B10.BR mice on further transfer. Selection was not apparent, however, when CBA T cells were filtered through totally H-2-different irradiated mice, e.g., B10 ($H\text{-}2^b$), B10.D2 ($H\text{-}2^d$), or B10.S ($H\text{-}2^s$). Selection thus depended upon a sharing of H-2 determinants between the donor and host.

These findings raised a number of questions, including: (*a*) What is the relationship between T cells causing GVHD to minor HA and minor HA-specific cytotoxic lymphocytes (CTL)? (*b*) Do GVHD-inducing T cells, like CTL, comprise discrete subgroups of H-2K- and H-2D-restricted cells? (*c*) Are H-2I-restricted cells involved in GVHD to minor HA? (*d*) What cells present minor HA to T cells during negative selection? (*e*) Is antigen processing involved during negative selection? This paper attempts to provide answers to these questions.

Materials and Methods

Mice. CBA/J (CBA), B10.BR/SgSn (B10.BR), C57BL/10J (B10), C57BL/6J, (B6), B10.D2, and B10.A mice were purchased from The Jackson Laboratory, Bar Harbor, ME. B10.OL and B10.TL mice were kindly provided by C. David, Mayo Clinic, Rochester, MN. B10.S, A.TH, and B10.S(7R) mice were donated by Dr. B. Knowles, The Wistar Institute. (B10.A × B10.OL)F_1 mice were bred in our own colony. (B6 × CBA)F_1 mice were obtained from

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[1] *Abbreviations used in this paper:* GVHD, graft-vs.-host disease; minor HA, minor histocompatibility antigens; CTL, cytotoxic lymphocytes; C′, complement; BM, bone marrow; LN, lymph node; TDL, thoracic duct lymphocytes; MST, median survival time; IL-2, interleukin 2.

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Cumberland View Farms, Clinton, TN, and (B6 × C3H/He)F_1 mice were purchased from Simonsen Laboratories, Gilroy, CA. Male mice were used as cell donors, filtration hosts, and recipients.

Media. RPMI 1640 (Microbiological Associates, Walkersville, MD) supplemented with 2% fetal calf serum was used.

Injections. Unless stated otherwise, all cell suspensions were given intravenously via the tail vein in a volume of 0.5–1.0 ml.

Reagents. A.TH anti-A.TL antiserum (anti-Ia^k) and monoclonal anti-Lyt-1.1 antibody were purchased from Accurate Chemical & Scientific Corp, Westbury, NY. Monoclonal rat anti-mouse Lyt-2 antibody, which detects a monomorphic determinant, was produced in our laboratory from hybridoma cells kindly donated by F. Fitch, University of Chicago, Chicago, IL. Monoclonal anti-Thy-1.2 (Jlj) (IgM) antibody was made in this laboratory (5). Hybridoma 10-3.6 (IgG2a) detects a public I-A specificity (Ia.17), whereas hybridoma 11-5.2 (IgG2b) detects a private I-A specificity (Ia.2) (6). Hybridomas 10-3.6 and 11-5.2 were made available by the Herzenberg group, Stanford University School of Medicine, Stanford, CA. Hybridoma antibody CE636 (IgM) detects a public I-E specificity (Ia.7) and was kindly provided by F. W. Symington, University of Pennsylvania, Philadelphia, PA. (7). Antibody titers of the monoclonal reagents ranged from 10^4 to 10^5. Guinea pig and rabbit complement (C′) were prepared in our own laboratory.

Preparation of Cells. Suspensions of bone marrow (BM) cells and lymph node (LN) cells (pooled from mesenteric, axillary, inguinal, and cervical nodes) were prepared as described previously (4). LN cells were treated with A.TH anti-A.TL antisera (1:4 final dilution) or monoclonal anti-Lyt-1.1 (1:20) in a two-step procedure involving incubation of $\leq 10^8$ cells with antibody in 2 ml for 30 min at 4°C, washed once, and incubated at 37°C for 40 min in 5 ml with a mixture of guinea pig (1:10) and rabbit (1:60) C′. Treatment of cells with anti-Lyt-2 (1:10), anti-Thy-1.2 (1:25) antibody, or with a mixture of anti-Ia antibodies [anti-Ia.17 (1:25), anti-Ia.2 (1:25), and anti-Ia.7 (1:50)] were performed in a single step in 5-ml vol at 37°C for 40 min with guinea pig C′ (1:5) or with a mixture of rabbit and guinea pig C′.

Irradiation. Mice were exposed to ^{137}Cs-γ-irradiation at a dose of ~100 rad/min.

Negative Selection to Minor HA. The filtration procedure was identical to that previously described (4). In brief, 1×10^8 to 2×10^8 LN cells were injected intravenously into irradiated (850 rad) hosts, and thoracic duct lymphocytes (TDL) were collected between 18 and 40 h later. TDL were pooled from two to three mice per group.

Mortality Assay for GVHD. As previously described (1, 4), 2- to 4-mo-old B10.BR mice were exposed to a midlethal dose of irradiation (750 rad) and then ~6 h later were injected intravenously with a mixture of T cell-depleted CBA BM (4×10^6 viable cells treated with anti-Thy-1.2 antibody and C′), together with the test population of CBA T cells (either TDL or normal LN). Recipients of marrow cells alone served as controls.

Statistical Analysis. Median survival times (MST) and comparison between individual groups were calculated as previously described (4).

Preparation of Bone Marrow Chimeras. As described elsewhere (8), chimeras were prepared by transferring 5×10^6 to 10×10^6 anti-Thy-1.2 plus C′-treated marrow cells intravenously into mice exposed to 750–1,100 rad 4–6 h previously. Double chimeras were prepared by reconstituting irradiated (1,100 rad) (B6 × CBA)F_1 mice with a mixture of 5×10^6 CBA marrow plus 10^7 B10 marrow (more of the latter cells were injected because B10 cells are subject to Hh resistance). In the case of H-2-incompatible combinations, appropriate anti-H-2 sera were used to establish the degree of lymphoid cell chimerism (8). Cage mates of the chimeras used for selection at 6–11 mo postreconstitution contained virtually no (<1%) host cells. Double chimeras contained 40–60% of each donor marrow population and no detectable host cells.

Results

Experimental Design. To induce negative selection, large doses of CBA LN cells were injected intravenously into heavily irradiated mice, and the donor cells were collected from TDL of the recipients 18–40 h post-transfer. Small doses of the filtered T cells (usually 10^6) were then transferred with anti-Thy-1.2-treated CBA marrow

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cells into lethally irradiated (750 rad) B10.BR mice (see Materials and Methods). Mortality of the recipients was studied over 80–100 d. Recipients of marrow cells but not T cells served as controls; scattered deaths occurred in these control groups within the first 4 wk post-transfer but were rare thereafter.

Separation of H-2K- and H-2D-restricted T Cells by Double Negative Selection. In previous studies (4), it was shown that a complete sharing of H-2 determinants between the donor T cells and the selection host was required to obtain negative selection (a failure of the filtered T cells to kill B10.BR mice). Thus, irrespective of whether CBA ($K^kI^kD^k$) (*kkk*) T cells were filtered through irradiated K/I-matched B10.A (*kkd*) mice or D-matched B10.OL (*ddk*) mice, the cells retained the capacity to kill close to 100% of B10.BR mice; effective selection occurred in (B10.A × B10.OL)F_1 mice. To explain these findings, it was suggested that CBA T cells might comprise a roughly 50:50 mixture of subsets of K- and D-restricted T cells; filtration of the cells through, e.g., B10.A mice, would remove the K-restricted cells but not the D-restricted cells and thereby reduce the GVHD-inducing potency of the cells by a factor of ~2, a difference that is beyond the sensitivity of the GVHD mortality assay.

To seek direct support for this interpretation, we examined the effects of double negative selection through B10.OL and B10.A mice (Fig. 1). CBA LN cells were recirculated through large numbers of B10.OL mice and divided into two groups. One-half of the cells were recirculated through B10.A mice, whereas the other one-half were recirculated for a second time through B10.OL mice. As shown in Fig. 1, the capacity of CBA T cells to kill B10.BR mice after double selection through B10.OL mice was similar to that of control cells filtered once through syngeneic CBA mice. By contrast, CBA T cells filtered first through B10.OL (*ddk*) mice and then through B10.A (*kkd*) mice failed to cause GVHD. The mortality rate with these cells was no higher than in mice given T cells filtered through (B10.A × B10.OL)F_1 mice or in control mice given marrow cells but no T cells; in each of these three groups there were no deaths after day 35. These data imply that non-cross-reactive subsets of K- and D-restricted T cells are the principal effector cells for GVHD induction.

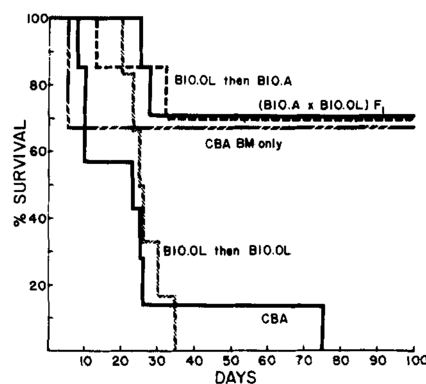

FIG. 1. Separation of H-2K- and H-2D-restricted T cells by double negative selection. The data show mortality in irradiated (750 rad) B10.BR mice given T cell-depleted CBA BM plus 10^6 CBA (*kkk*) T cells, recirculated once through B10.OL (*ddk*) mice and then once through B10.A (*kkd*) mice (B10.OL then B10.A) or twice through B10.OL mice (B10.OL then B10.OL, MST 24.5 ± 1.0 d). Control T cells were filtered once through CBA (MST 14.9 ± 1.4 d) or (B10.A × B10.OL)F_1 mice. Data from a single experiment involving six to seven mice per group.

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Failure of I-restricted T Cells to Mediate GVHD. In the case of T helper cells, exposure to antigen during recirculation through H-2I-matched irradiated hosts is an effective method for producing negative selection of I-restricted T cells (9). The fact that CBA T cells retain their GVHD potency after filtration through I-A/B/J/E-matched B10.A mice (which should remove K- and I-restricted cells but not D-restricted cells) (4, see above) argues against the view that minor HA-specific, I-restricted T cells play an obligatory role in GVHD induction, e.g., by acting as T helper cells (see Discussion).

The possibility that I-restricted T cells themselves might mediate GVHD, however, has not been excluded. Testing this possibility necessitates determining whether T cells can mediate GVHD after prior removal of the K/D-restricted subsets. In theory, this could be done by filtering CBA T cells through B10 congenic mice of the $K^kI^xD^k$ haplotype. Because mice of this haplotype have not been described, we turned to a different H-2-compatible minor HA-disparate strain combination, viz., A.TH and B10.S(7R) (both $K^sI^sD^d$).

As for the combination of CBA and B10.BR mice, A.TH T cells filtered through syngeneic A.TH mice caused severe mortality when transferred to irradiated B10.S(7R) mice (Table I). Lethal GVHD in B10.S(7R) mice also occurred with passage of A.TH (*ssd*) T cells through K/I-matched, D-mismatched B10.S (*sss*) mice or through D-matched, K/I-mismatched B10.D2 (*ddd*) mice (for obscure reasons, GVHD was less severe with filtration through B10.S mice than with filtration through B10.D2 mice). Significantly, A.TH T cells filtered through K- and D-matched, I-mismatched B10.TL (*skd*) mice, which would be expected to remove K/D-restricted T cells but not I-restricted cells, failed to cause GVHD; with these cells there were no deaths after day 30.

These data suggest that I-restricted T cells per se do not induce GVHD. Studies on the Lyt phenotype of GVHD-inducing T cells, considered below, support this view.

Lyt Phenotype of T Cells Causing Anti-Minor HA GVHD. It is generally accepted that I-restricted T cells have the Lyt-1^+2^- phenotype, whereas K/D-restricted T cells are either Lyt-1^-2^+ or Lyt-1^+2^+ (10, 13). From the above findings (Table I), it would therefore follow that pretreatment of T cells with anti-Lyt-2 antibody and complement

TABLE I

Failure of A.TH T Cells to Kill H-2-compatible Irradiated B10.S(7R) Mice after Filtration through K/D-matched, I-mismatched B10.TL Mice

Selection host for A.TH T cells	*H-2* haplotype								Mortality in B10.S(7R) mice*		
		I									
	K	*A*	*B*	*J*	*E*	*C*	*S*	*D*	Dead/ total	Percent dead	MST (± SE)
											d
A.TH	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*d*	*10/12*	*83*	*24.2 ± 1.2*
B10.S(7R)	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*d*	*2/12*	*17*	*>100.0*
B10.TL	*s*	*k*	*k*	*k*	*k*	*k*	*k*	*d*	*2/12*	*17*	*>100.0*
B10.D2	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*6/6*	*100*	*25.8 ± 1.2*
B10.S	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*6/9*	*67*	*59.8 ± 1.3*
BM alone									*2/12*	*17*	*>100.0*
Irradiation alone									*3/3*	*100*	*16.5 ± 1.1*

* Irradiated (750 rad) B10.S(7R) mice given 5 × 10^6 T cell-depleted A.TH BM cells plus 10^6 A.TH T cells filtered from blood to lymph for 1 d through irradiated (900 rad) mice of various strains. Data were pooled from two separate experiments.

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TABLE II
Surface Antigen Phenotype of T Cells Causing Anti-Minor HA GVHD

Pretreatment of CBA LN cells in vitro	Number of viable CBA LN cells transferred to irradiated B10.BR mice ($\times 10^{-6}$)	Mortality in B10.BR mice*		
		Dead/total	Percent dead	MST ± SE
				d
None	20	10/10	100	18.2 ± 1.2
	5	29/31	94	21.9 ± 1.1
	2	18/18	100	29.3 ± 1.1
	1	4/6	67	51.8 ± 1.9
αLyt-2 + C′	20	2/12	18	>100.0
	5	7/28	25	>100.0
	2	4/18	22	>100.0
αLyt-1.1 + C′	5	13/13	100	19.5 ± 1.1
	2	5/6	83	29.3 ± 1.7
	1	3/6	50	85.7 ± 2.3
αLyt-1.1 + C′ (double treatment)	5	14/20	70	58.0 ± 1.1
αIa^k (A.TH αA.TL antiserum) + C′	5	8/8	100	17.1 ± 1.1
	2	6/6	100	17.3 ± 1.8
αIa^k (mixture of αIa.2, αIa.17 and αIa.7) + C′	5	15/15	100	22.3 ± 1.1
	2	6/6	100	16.7 ± 1.2
	1	6/9	67	38.5 ± 1.7
αThy-1.2 + C′	20	1/9	11	>100.0
αThy-1.2 − C′	20	3/12	25	>100.0
Marrow alone		4/27	15	>100.0
Irradiation alone		18/22	82	18.7 ± 1.2

* Irradiated (750 rad) B10.BR mice given T cell-depleted CBA BM together with normal CBA LN cells (50–60% Thy-1.2$^+$) treated with alloantibody and C′ in vitro. Cytotoxic indices with respect to T (Thy-1.2$^+$) cells were anti-Lyt-2, 20–40%; anti-Lyt-1.1, ≥95% (for both single and double treatment); anti-Ia, ≤5%. Data were pooled from four experiments. All antibodies used were monoclonal (hybridoma) reagents unless stated otherwise.

would abolish the capacity of the surviving cells to mediate GVHD. This was indeed found to be the case. CBA LN cells, surviving treatment with monoclonal anti-Lyt-2 antibody plus C′, failed to cause GVHD in B10.BR mice, even with cell doses as high as 2×10^7 viable cells (Table II). Interestingly, pretreatment of LN cells with monoclonal anti-Lyt-1.1 antibody plus C′, under routine conditions (Materials and Methods), had little effect on their GVHD potency; such treatment killed ≥95% of the Thy-1$^+$ cells in the cell suspension. However, subjecting the cells to two consecutive treatments with anti-Lyt-1.1 antibody plus C′ did cause an appreciable reduction in potency, manifested by lower mortality and increased MST. Table II also shows that treating CBA LN cells with anti-Ia antibody, either broad spectrum anti-Ia^k (A.TH anti-A.TL) antiserum or a mixture of monoclonal anti-I-A^k (anti-Ia.2 + anti-Ia.17)

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and anti-I-E^k (anti-Ia.7) antibodies, failed to reduce the incidence of GVHD. No GVHD was observed with anti-Thy-1.2-treated cells, even when the cells were incubated with antibody in the absence of C′. The effector cells thus have the Thy-1^+ Lyt-$1^{\pm}2^+$ Ia^- phenotype; there is no apparent requirement for Lyt-1^+2^- cells or Ia^+ cells.

Collectively, the data in Tables I and II suggest that I region-restricted cells do not participate demonstrably in GVHD to minor HA and thus favor the view that the K/D-restricted subsets are the sole effector cells.

Nature of Cells Presenting Minor HA during Negative Selection. Studies on the generation of anti-viral cytotoxic responses in vivo have suggested that H-2-restricted presentation of antigen is controlled by marrow-derived cells (11). To examine whether marrow-derived cells control the presentation of minor HA, negative selection was studied in three types of H-2-semiallogeneic BM chimeras: (*a*) supralethally irradiated (1,000–1,100 rad) (B6 × CBA)F_1 mice reconstituted with T cell-depleted B10 marrow (B10 → F_1 chimeras); (*b*) irradiated (B6 × CBA)F_1 mice injected with a mixture of CBA and B10 marrow cells (CBA + B10 → F_1 chimeras), and (*c*) irradiated B6 mice reconstituted with [B6 × C3H/He (*H-2^k*)]F_1 marrow (F_1 → B6 chimeras). To ensure maximum repopulation with donor-derived cells, the chimeras were left for 6–12 mo after reconstitution and then re-irradiated (850 rad) for use as selection hosts for CBA T cells. (It should be noted that in terms of GVHD elicited by CBA T cells, B6 and B10 are indistinguishable, although these strains differ by at least one minor H antigen [1]).

As shown in Fig. 2, the capacity of CBA T cells to kill B10.BR mice was unimpaired after filtration through B10 mice but abolished after filtration through (B6 × CBA)F_1 mice. In marked contrast to this complete selection observed in normal (B6 × CBA)F_1 mice, no selection occurred when CBA T cells were filtered through B10 → F_1 chimeras; selection was complete, however, with filtration through F_1 → B6 chimeras. These findings imply that marrow-derived cells play a mandatory role in presenting minor HA during T cell selection.

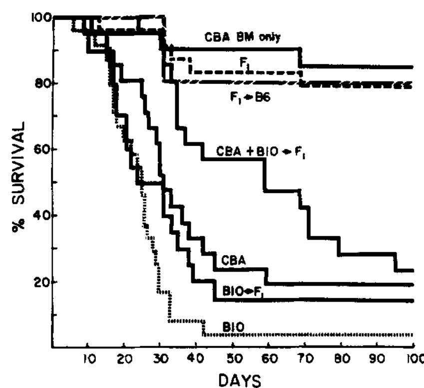

FIG. 2. Nature of cells presenting minor HA during negative selection. The data show mortality in irradiated B10.BR mice given T cell-depleted CBA BM plus 10^6 CBA T cells filtered through various BM chimeras (F_1 → B6, B10 → F_1, CBA + B10 → F_1) (see text) or through normal mice (CBA, B10, F_1). MST induced by the filtered T cells were CBA, 33.2 ± 1.1 d; B10, 22.3 ± 1.1 d; B10 → F_1, 25.5 ± 1.1 d; CBA + B10 → F_1, 56.1 ± 1.1 d ($P < 0.01$, cf. CBA). The data were pooled from four separate experiments involving a total of 20–24 mice per group.

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Interestingly, intermediate results were obtained when CBA T cells were filtered through CBA + B10 → F_1 chimeras. Although T cells filtered through these chimeras did produce high mortality in B10.BR mice, the MST of the recipients was appreciably prolonged (56 d vs. 33 d for syngeneic passaged cells, $P < 0.01$). Based on previous studies (1, 4), this prolongation of MST probably signifies a 5- to 10-fold reduction in the potency of the filtered T cells. The significance of this finding will be discussed later.

Target Cells for GVHD. To examine whether marrow-derived cells present antigen during the effector stage of GVHD, T cells were transferred to re-irradiated CBA → B10.BR and B10.BR → CBA chimeras. The chimeras were prepared by transferring 10^7 anti-Thy-1-treated marrow cells into 750–820 rad irradiated mice. When tested at 4–8 mo post-reconstitution, the content of host-derived cells in such chimeras ranges from 0 to 10% (1). To examine their susceptibility to GVHD at 6 mo post-reconstitution, the chimeras were given a second dose of irradiation (750 rad) and then injected with normal CBA or B10.BR T cells (LN cells) plus T cell-depleted marrow cells.

As shown in Table III, GVHD was minimal or undetectable when CBA T cells were transferred to re-irradiated B10.BR → CBA chimeras, or B10.BR T cells were transferred to CBA → B10.BR chimeras; note that in both situations only the lymphohematopoietic system and not the radio-resistant host stroma were allogeneic with respect to the donor T cells. In the reciprocal situation, severe GVHD was observed, i.e., with CBA T cells transferred to CBA → B10.BR chimeras or B10.BR T cells to B10.BR → CBA chimeras. The implications of these findings will be discussed.

Discussion

The data on the effects of double negative selection (Fig. 1) would seem to argue strongly that discrete subgroups of K- and D-restricted (or K- and D-end-restricted)

TABLE III

Lethal GVHD in Re-Irradiated Chimeras after Transfer of Donor or Host LN Cells plus Marrow

Cells transferred to re-irradiated chimeras*		Re-irradiated chimeras	Mortality	
LN	BM		Dead/total	MST ± SE
				d
—	—	CBA → 750 rad B10.BR	7/7	13.6 ± 1.1
—	CBA	CBA → 750 rad B10.BR	1/6	>100.0
—	B10.BR	CBA → 750 rad B10.BR	0/3	>100.0
CBA	CBA	CBA → 750 rad B10.BR	5/6	17.6 ± 1.6
B10.BR	B10.BR	CBA → 750 rad B10.BR	3/12	>100.0
—	B10.BR	B10.BR → 750 rad CBA	1/6	>100.0
CBA	CBA	B10.BR → 750 rad CBA	1/6	>100.0
B10.BR	B10.BR	B10.BR → 750 rad CBA	6/6	12.2 ± 1.1
B10.BR	B10.BR	B10.BR → 820 rad CBA	6/6	19.4 ± 1.0
—	B10.BR	CBA → 750 rad CBA	1/9	>100.0
B10.BR	B10.BR	CBA → 750 rad CBA	9/9	15.9 ± 1.1

* Chimeras were prepared by transferring 5×10^6 anti-Thy-1.2 + C′-treated marrow cells into irradiated (750 or 820 rad) mice and then left for ≃6 mo. At this stage the chimeras were exposed to 750 rad and then received BM cells (anti-Thy-1.2 + C′-treated) ± LN cells (10^7 viable cells) 6 h later.

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T cells are the main effector cells for the production of lethal GVHD to minor HA. Two approaches suggested that I-restricted T cells per se cannot elicit lethal GVHD: (*a*) T cells filtered through minor HA-different K/D-matched, I-mismatched hosts failed to cause GVHD (Table I), and (*b*) no GVHD was observed with T cells depleted of Lyt-2^+ cells (Table II). In terms of H-2 restriction and Lyt phenotype, the T cells that cause GVHD to minor HA in vivo appears to be indistinguishable from CTL responding to minor HA in vitro (12, 13). Hence, it is tempting to conclude that CTL are the effector cells for GVHD. To prove this point, however, would require demonstrating that cloned CTL can elicit GVHD.

Despite the inability of I-restricted T cells per se to elicit GVHD, the possibility that these cells might be required to provide helper function deserves careful consideration. In the case of in vitro responses, I-restricted T helper cells or their products play a crucial role in governing CTL generation, probably by releasing the growth-promoting factor interleukin 2 (IL-2) (14, 15). Whether such a function is required in vivo is unclear. In the present context, the question centers on whether T cells depleted of I-restricted cells can cause GVHD. At face value, two pieces of evidence suggest that depletion of such cells does not cause a demonstrable reduction in GVHD potency. Firstly, it was shown previously (4) that T cells retain strong GVHD reactivity after filtration through I-matched, K/D-mismatched minor HA-different hosts; others have shown (16) that selection under such conditions does indeed remove T helper cells (cells governing CTL generation in vitro). Secondly, T cells exposed to a single treatment of monoclonal anti-Lyt-1 antibody and C′, which would be expected to remove T helper cells, retained strong GVHD reactivity, despite the fact that this treatment lysed ≥95% of Thy-1^+ T cells (Table II). It is of interest that double treatment with anti-Lyt-1 antibody did cause some reduction in GVHD reactivity. However, this finding is not surprising when it is borne in mind that monoclonal antibodies detect at least small amounts of the Lyt-1 antigen on virtually all Thy-1^+ cells (17).

Despite this evidence that depletion of I-restricted T cells fails to reduce GVHD reactivity, it does not necessarily follow that K/D-restricted T cells in vivo function in the absence of T cell help. For example, one might argue that the removal of I-restricted T cells in the experiments mentioned above was not total. Alternatively, help (IL-2) might be provided by cells responding to other antigens, e.g., to environmental antigens; this possibility fails to explain the strong GVHD reactivity of anti-Lyt-1-treated cells, i.e., a population presumably depleted of I-restricted reactivity to environmental antigens. Finally, one might entertain the simple notion that help for K/D-restricted T cells in vivo is not provided exclusively by I-restricted cells but can also be expressed by a subset of K/D-restricted cells. This explanation fits the data and is supported by recent in vitro studies.[2]

In the case of I-restricted T cells, it is well accepted that antigen is presented in association with Ia molecules on a specialized class of "accessory" cells (19). By contrast, comparatively little is known about the mechanism of antigen presentation to K/D-restricted T cells. In the case of minor HA, on *a priori* grounds, one might expect that any cell expressing foreign minor HA plus self-H-2 could induce T cell

[2] Raulet, D. H., and M. J. Bevan. Helper T cells for cytotoxic lymphocytes need not be I-region-restricted. Manuscript submitted for publication. This notion is also supported by Widmer and Bach (18), who have reported a helper cell-independent cytotoxic clone.

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selection. However, the finding that negative selection of CBA T cells to minor HA was complete in $F_1 \rightarrow$ B6 chimeras but not detectable in B10 $\rightarrow F_1$ chimeras (Fig. 2) would seem to provide strong evidence that selection reflects H-2-restricted contact with antigen on marrow-derived cells. This finding complements the observations of Zinkernagel (11) on anti-viral CTL responses in $a \rightarrow (a \times b)F_1$ chimeras: whereas only a-restricted CTL were generated in the chimeras themselves, which lack b-bearing marrow-derived cells, sensitization of the chimera T cells on adoptive transfer to normal F_1 mice generated both a- and b-restricted CTL. The identity of the cells presenting antigen in these two systems is unknown. Macrophages might be involved, but this has yet to be proven. Although the presence of Ia^+ macrophage-like cells is needed during CTL generation in vitro (20–22), one could argue that the main function of Ia^+ cells here is to present antigen to T helper cells and thereby lead to IL-2 production.

The finding that CBA T cells underwent partial selection to minor HA with filtration through CBA + B10 $\rightarrow F_1$ chimeras is of some interest. Perhaps the simplest explanation is that, as a consequence of normal cell breakdown in the chimeras, the minor HA on the B10 cells were continuously "processed" by a class of CBA cells, e.g., macrophage-like cells; as the result of processing, the B10 minor HA became aligned with $H\text{-}2^k$ K/D molecules and thereby became immunogenic for CBA T cells. This interpretation rests on the assumption that processed antigen is indeed immunogenic for K/D-restricted T cells. The literature on this point is inconclusive. We (23) and others (24, 25) have concluded that recognition of processed antigen ("cross priming") is involved in priming or selection of T cells involved in anti-minor HA CTL responses. However, the key question of whether such recognition applies to K/D-restricted T cells rather than simply to I-restricted T helper cells has yet to be resolved. In the case of GVHD-inducing T cells, one might predict that if processed antigen is indeed immunogenic for K/D-restricted T cells, the failure of CBA T cells to undergo selection to antigen in H-2-different B10 mice could be overcome by adding CBA marrow-derived cells during selection. This approach has been conspicuously unsuccessful, despite the injection of even massive doses of cells (3×10^8 spleen cells 1 d before filtration) (unpublished data). Although trivial explanations for this finding, e.g., poor homing of the injected spleen cells, have not been excluded, it might be relevant that the time available for processing in the latter situation is comparatively short (1–2 d). By contrast, an indefinite period is presumably available for processing in CBA + B10 $\rightarrow F_1$ chimeras. It is conceivable, therefore, that processing of minor HA in the context of K/D molecule association is either relatively ineffective or occurs rather slowly. Further work on this subject is needed.

As for other forms of cellular immunity, GVHD to minor HA presumably reflects contact with antigen at two different stages, first, during T cell induction and second, during the effector phase. As mentioned earlier, the fact that CBA T cells failed to undergo negative selection to minor HA in B10 $\rightarrow F_1$ chimeras implies that, in the induction phase, T cells ignore antigen presented on nonhematopoietic cells. During the effector phase, however, contact with minor HA on nonhematopoietic cells appears to be crucial. Thus, no GVHD was observed when CBA T cells were transferred to re-irradiated B10.BR $\rightarrow$ CBA chimeras (Table III), a situation where the target minor HA were expressed solely on marrow-derived cells.

This latter finding bears on the issue of the pathogenesis of GVHD. Perhaps the

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simplest view is that lethal GVHD is largely a reflection of invasion by pathogens as the result of damage to the gut and respiratory tract (this being the most likely explanation for the resistance of germ-free mice to GVHD) (26, 27). Gut damage could either be a consequence of direct destruction of epithelial cells by effector T cells or, alternatively, reflect a "bystander effect," i.e., indirect tissue damage resulting from destruction of other cells in the gut, e.g., marrow-derived cells (28). The fact that CBA T cells killed CBA → B10.BR but not B10.BR → CBA chimeras is against the latter possibility.

In summary, the available evidence suggests that minor HA-specific T cells are induced at the level of marrow-derived cells and then, at the effector phase, cause lethal GVHD by attacking minor HA-bearing nonhematopoietic cells. At face value, this interpretation might seem inconsistent with the finding that severe GVHD occurred when CBA T cells were transferred to CBA → B10.BR chimeras. How did T cell induction occur in these chimeras, i.e., how did the cells encounter the requisite association of B10 minor HA plus H-2^k on marrow-derived cells? Two points should be made. First, unlike the H-2-semiallogeneic chimeras considered earlier, these chimeras were prepared with only ≤820 rad and hence were probably not totally devoid of host-type marrow-derived cells, i.e., cells expressing B10 minor HA plus H-2^k. Second, even if host cells were absent in such chimeras, processing of host-type minor HA by the donor CBA marrow cells might be sufficient to lead to T cell induction.

Summary

Evidence is presented that T cells that produce lethal graft-vs.-host disease (GVHD) to minor histocompatibility antigens (minor HA) comprise discrete subgroups of H-2K- and H-2D-restricted T cells; double negative selection of T cells in irradiated *H-2* recombinant mice was used to separate these two subgroups. No evidence could be found that I-restricted T cells contributed to GVHD, either as effector cells or helper cells. The (unprimed) precursor cells for GVHD expressed the Thy-1^+, Lyt-$1^{\pm}2^+$, Ia^- phenotype.

Studies in which H-2-semiallogeneic bone marrow chimeras were used as hosts for negative selection suggested that presentation of minor HA to T cells during the induction phase is controlled by marrow-derived cells; indirect evidence was obtained that these latter cells can "process" minor HA presented on H-2 different cells and thereby render the antigens immunogenic.

Studies in which minor HA-different, H-2-compatible chimeras were re-irradiated and then injected with donor-vs.-host T cells suggested that the effector phase of lethal GVHD involves contact of antigen on non-marrow-derived cells.

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