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Immunology

Influence of H-2 antigen expression on killer T cell specificity, differentiation, and induction

(radiation chimera/thymus/major histocompatibility complex/helper cells)

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ABSTRACT **Murine cytotoxic T lymphocytes (CTL) and helper cells are H-2 antigen restricted in their specificity: recognition of foreign antigen by these cells requires the concomitant recognition of self-H-2 molecules. Which H-2 antigens T cells treat as "self" is determined by the particular H-2 antigens expressed on radioresistant cells of the thymus in which these T cells mature. Using tetraparental [(P_1 + P_2) → F_1] radiation chimeras with *in situ* F_1 thymuses, we have found that the *H-2* genotype of the stem cells does not influence their H-2 restriction specificity. This has allowed us to use tetraparental chimeras that have been thymectomized and grafted with parental (P_1, P_2, or both) thymus lobes to study the requirements for H-2-restricted T–T interactions during CTL ontogeny and induction. In animals that have received thymus grafts of both parental origins, CTL display no preference for maturation within a syngeneic thymus graft, a finding that is not compatible with a suggested requirement for intrathymic H-2-restricted T–T interactions in the maturation of precursor CTL. We have also grafted thymectomized tetraparental radiation chimeras with thymus grafts from only one parent to compare the induction of P_1 and P_2 CTL in environments in which peripheral (extrathymic) T cell interactions are restricted to one H-2 haplotype. Again, we find no evidence for preferential induction of CTL precursors syngeneic to the thymus graft, contrary to expectation if CTL induction requires that T helper cells restricted to thymic H-2 antigens interact directly with precursor CTL. In those animals with one parental thymus graft, there is variability in the ratios of P_1 and P_2 cells induced with several antigens, a finding that may be indicative of an H-2-restricted suppression mechanism operating in the periphery.**

The *H-2* region, the murine major histocompatibility complex, influences the functions of T cells in several ways. Both helper cells and cytotoxic T lymphocytes (CTL) are H-2 restricted, which means that the activity of these cells depends not only on the recognition of the foreign antigen for which they are specific but also on the simultaneous recognition of the proper H-2 antigens on the presenting cell (1–6). Which particular allelic product of *H-2* a given T cell recognizes in conjunction with foreign antigen is not defined by the H-2 phenotype of the T cell itself, but is determined by the environment in which this T cell differentiates and meets antigen. Thymus grafting studies have shown that the thymic environment in which T cells differentiate can influence to a great extent their H-2 restriction specificity; in these cases "self" H-2 is defined by the H-2 antigens expressed by radioresistant cells of the thymus (7–9).

It is possible for T cells to learn a wholly allogeneic H-2 as self; CTL and helper T cells restricted to $H\text{-}2^B$ can be readily induced in [A → (A × B)] chimeras (10–12). In this situation, there is a partial H-2 matching between thymus and thymocyte: both express $H\text{-}2^A$. There has been some controversy over whether T cells can differentiate in a wholly allogeneic thymus. Zinkernagel and coworkers have documented the immunoincompetence of allogeneic [A → B] bone marrow radiation chimeras in various T cell assays, even after stimulation in the relevant F_1 environment (7, 11, 13, 14). This immunoincompetence could be explained by a requirement for an H-2-restricted interaction between T cells at some stage of CTL differentiation or induction. For example, the $H\text{-}2^A$ lymphocytes in an [A → B] chimera may require a positive signal from a helper cell. This hypothetical helper cell would itself have differentiated in the $H\text{-}2^B$ thymus, would be $H\text{-}2^B$ restricted, and therefore would be unable to recognize and help the $H\text{-}2^A$ CTL precursor. The immunoincompetence of *K–I* region mismatched chimeras and the realization of the importance for CTL triggering of viral antigen presentation by the lymphoreticular system led to the idea that helper cells for CTL are *H-2I* region restricted, as are helper cells for B cells (11).

Involvement of the *I* region in CTL induction has also been suggested by the immunogenetics of the CTL response to the male-specific antigen H-Y. By complementation analyses, the ability to mount a CTL response to H-Y has been mapped to two *H-2* regions; the immune response gene that maps to the *K-IA* region is thought to control the response of a helper cell required to induce CTL specific for H-Y (15–17).

The idea of a T helper cell for CTL generation is supported by much independent evidence (reviewed in ref. 18). What is as yet unclear is whether this helper cell–CTL interaction involves H-2-restricted antigen recognition for helper cell induction or delivery of the helper signal to the precursor CTL; is the helper cell–CTL interaction analogous to the helper cell–B cell interaction? Data from Bennink and Doherty (19) indicate this may not be the case. In this set of experiments, CTL were efficiently primed to vaccinia virus in a totally *I* region disparate environment, a finding incompatible with a requirement for self-*I*-region-restricted activation of a helper cell for CTL induction. In analogous experiments assaying humoral responses, one would expect no T helper cell priming (20). These authors conclude that the *I* region may not be involved in regulation of CTL induction, and they suggest that there is a requirement for *I* region-restricted help at some earlier (intrathymic?) stage of T cell ontogeny (19).

The severity of the immunoincompetence of wholly allogeneic chimeras has been called into question by several investigators measuring immune responses such as alloreactive, minor-H-specific (21) and Sendai-virus-specific (22–24) CTL, keyhole limpet hemocyanin-specific helper T cells (25), induction of graft-versus-host disease, response to T cell mitogens, generation of a secondary antibody response (26), and skin graft rejection (27). These responses have ranged from normal to well below normal, and the H-2 restriction specificity of the T cells

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Abbreviations: CTL, cytotoxic T lymphocytes; MLC, mixed lymphocyte culture; TNP, 2,4,6-trinitrophenyl; Con A, concanavalin A.

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involved has been found to vary from largely stem cell type (22, 24) to a mixture of stem cell and thymus type (21, 23), depending on the method of effector cell induction. A further problem in interpreting data from wholly allogeneic chimeras arises from the possibility that thymic learning is not absolute. P_2-restricted T cells can often be elicited from semisyngeneic [$F_1 \rightarrow P_1$] radiation chimeras (28, 29). If H-2-restricted T–T interactions are required for eliciting the various immune responses measured above, using wholly allogeneic chimeras may select for these nonthymic restricted T cells. The low viability often reported for allogeneic chimeras may add another level of selection pressure for those animals that are most immunocompetent. It has also been shown that alloreactive and 2,4,6-trinitrophenyl (TNP)-specific (but not minor-H-specific) CTL can be elicited from athymic *nude* mice (30–32), which adds a further complication to interpreting allogeneic chimera experiments in which low levels of immune response are measured.

We have designed an experimental system that is relatively free of these complications for studying H-2-restricted interactions in T cell ontogeny and CTL induction. Our system is an internally controlled one in which lymphoid stem cells are given a "choice" of a syngeneic or an allogeneic thymic environment in which to mature. We are able to quantitate the extent of maturation of CTL precursors in a syngeneic versus an allogeneic thymus. This allows us to determine whether intra- or extrathymic H-2-restricted interactions influence CTL differentiation in animals that have healthy immune systems.

MATERIALS AND METHODS

Mice. C3HeB/FeJ (C3H, H-2^k), C57BL/10Sn (B10, H-2^b), B10.D2nSn (H-2^d), B10.BrSgSn (H-2^k), (BALB/c × C57BL/6J)F_1 (CB6, H-2^d/H-2^b), and (AKR/J × DBA/2J)F_1 [(AKD2)F_1, H-2^k/H-2^d, Thy-1.1/Thy-1.2] mice were purchased from The Jackson Laboratory. (B10.Br × B10.D2)F_1, BALB/c (C, H-2^d), BALB.K (C.K, H-2^k), (C.K × C)F_1, (BALB.B × B10.Br)F_1 (H-2^b/H-2^k), and (AKR/J × B10.D2)F_1 (H-2^k/H-2^d) mice were bred at the Center for Cancer Research, Massachusetts Institute of Technology.

Radiation Chimeras. Two protocols were used for constructing thymus-grafted chimeras. In the first, 6-week-old female (AKD2)F_1 mice were thymectomized and allowed to rest for 2 weeks. They were then administered 950 rad (1 rad = 0.01 gray) from a ^{137}Cs source and injected with a mixture of C and C.K bone marrow cells totalling 1.5×10^7 cells in the ratios 1:1, 1.3:1, or 1:1.3. Bone marrow cells were treated prior to mixing and injection with a mixture of two monoclonal anti-Thy-1 reagents (13-4 and T24; see below) plus rabbit complement. Eight weeks later, these animals were grafted subcutaneously (under the shoulder) with several lobes of <24-hr-old C or C.K thymuses or both that had been given 800 rad. In this system, host [(AKD2)F_1] and donor (C and C.K) T cells can be readily distinguished on the basis of Thy-1 phenotype.

In the second protocol, 5- to 8-week-old (C.K × C)F_1 mice were thymectomized and 1 week later grafted with unirradiated neonatal thymus lobes from C or C.K donors or both. The following week, the animals (and their thymus grafts) were given 900 rad and 1.2×10^7 C plus C.K anti-Thy-1-treated bone marrow cells in the ratios 1:1 or 1:1.3.

Tetraparental chimeras (with *in situ* F_1 thymuses) were irradiated and stem cells were injected as described above.

Priming for Minor H Antigens. Animals were primed to minor H antigens 2–9 months after irradiation and bone marrow injection by intraperitoneal injection of 10^7 (AKR/J × B10.D2)F_1 or (B10.Br × B10.D2)F_1 spleen cells. Spleen cells used for priming were either untreated or anti-Thy-1 treated (13-4 plus T24) as indicated. We have found that viable spleen cells can present minor H antigens directly, without the need for reprocessing by the host presentation system. Thus, both P_1 and P_2 restricted CTL from [$P_1 \rightarrow F_1$] chimeras can be primed by injection with F_1 minor-H-different spleen cells (unpublished).

Antisera and Typing of Chimeras. Spleen and lymph node cells were labeled with sodium [^{51}Cr]chromate (New England Nuclear) and typed with anti-Thy-1, anti-H-2^k, anti-H-2^d, and a mixture of anti-H-2^d and anti-H-2^k antibodies plus rabbit complement in a two-step assay as described (33). CTL were treated with antiserum prior to incubation with target cells. As is evident in Figs. 1 and 2, under the conditions used for antiserum treatment, 100% of the CTL effector activity is eliminated from homozygous or heterozygous cells expressing the appropriate antigens. Effector-to-target ratios were calculated on the basis of initial responder cell number, prior to antiserum treatment. Sera used were: (BALB.B × B10.Br)F_1 anti-B10.D2 (anti-H-2^d) and CB6 anti-C3H (anti-H-2^k). Monoclonal antibodies were 22-1, 13-4 [anti-Thy-1.1 and anti-Thy-1.2, respectively (33)], and T24 [a rat antibody specific for Thy-1 (34)].

Induction and Assay of CTL. CTL were induced polyclonally by concanavalin A (Con A) for 3 days and assayed in the presence of phytohemagglutinin as described (35). Minor, allogeneic, and TNP-specific CTL were induced in 5-day mixed lymphocyte culture (MLC) as described (31). The killing activity mediated by serial 1: 3 dilutions of responder cells was measured by using a constant number of ^{51}Cr-labeled 2- to 3-day Con A blasts as target cells in a 4-hr assay as described (31). The % specific lysis was calculated as follows: 100 × [(cpm released by responders − cpm released by medium)/(cpm released by detergent − cpm released by medium)].

RESULTS

The *H-2* Genotype of T Cells Does Not Influence their H-2 Restriction Specificity. Tetraparental [(C + C.K) → (C.K × C)F_1] radiation chimeras were constructed as described in *Materials and Methods*. We have determined the H-2 phenotype of lymphoid cells and various CTL effector populations from six such chimeras. Spleen and lymph node cells from minor H-antigen-primed chimeras were typed with anti-H-2 and anti-Thy-1 reagents. Untreated lymphoid cells were cultured for 5 days with minor-H-different, allogeneic, or TNP-modified F_1 stimulator cells, and the resulting CTL effectors were typed prior to assaying for killer activity.

In Fig. 1*A* is shown the H-2 typing of minor-H-specific CTL from a representative tetraparental chimera. This animal generated approximately equal cytotoxicity against B10.Br (H-2^k, upper panel) and B10.D2 (H-2^d, lower panel) targets. Both activities were roughly 50% decreased by pretreatment of effector cells with either anti-H-2^d or anti-H-2^k antiserum, in contrast to the complete reduction in activity of normal (C.K × C)F_1 CTL by similar treatment (Fig. 1*C*). The effector CTL population from the tetraparental chimera is therefore an approximately 1:1 mixture of C and C.K cells, reflecting the composition of the total lymphocyte pool. These data demonstrate that the H-2 type of parental thymocytes differentiating in a semisyngeneic F_1 thymus does not influence their H-2 restriction specificity. These results are similar to those found previously for helper cells (10; 36, 37). This invariance allows us to use fluctuations in the H-2 phenotype of restricted CTL in animals with parental thymus grafts as a clue to intra- and extrathymic interactions promoting CTL induction.

There Is No Preferential Maturation of CTL Syngeneic to the Thymus in Tetraparental Chimeras with Both Types of Parental Thymus Grafts. By H-2 typing CTL effectors in thymectomized [(C + C.K) → F_1] animals receiving both C and C.K thymus grafts, we can ask whether H-2^d CTL precursors preferentially mature in an H-2^d or an H-2^k thymus, identifying the thymus in which the CTL differentiate according to restric-

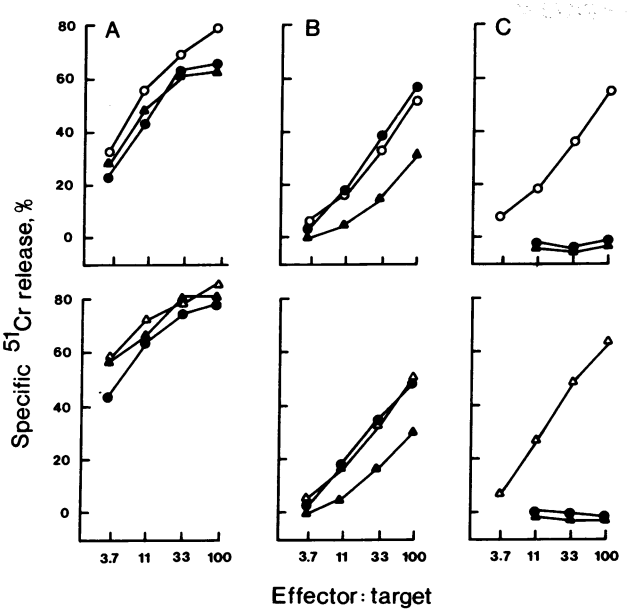

FIG. 1. H-2 type of minor-H-specific CTL from tetraparental chimeras with F_1 thymuses or with both types of parental thymus grafts. Responders are: [(C + C.K) → (C.K × C)F_1] tetraparental chimera with an *in situ* F_1 thymus (*A*), [(C + C.K) → (C.K × C)F_1] tetraparental chimera with both C and C.K thymus grafts (*B*), and normal (C.K × C)F_1 (*C*). All animals were primed *in vivo* with anti-Thy-1-treated (B10.Br × B10.D2)F_1 spleen cells. Responder spleen and lymph node cells were boosted *in vitro* with irradiated (B10.Br × B10.D2) F_1 spleen cells. CTL were assayed in the upper panels on ^{51}Cr-labeled B10.Br Con A blasts (○, H-2^k, spontaneous release 19%), and in the lower panels on B10.D2 (△, H-2^d, spontaneous release 21%). No lysis of (C.K × C)F_1 targets was seen with any responder cells. Responders were treated prior to assay with normal mouse serum (open symbols), anti-H-2^d (●), or anti-H-2^k (▲) antiserum plus rabbit complement. Spleen and lymph node cells from animal *A* were typed before MLC as: 48% C, 48% C.K, 4% F_1. Lymphoid cells from animal *B* were 34% C, 63% C.K, 3% F_1. CTL specific for TNP-C.K, TNP-C, and B10 from all three animals were also examined, with results similar to those shown here for minor-H-specific CTL.

tion specificity. That this is a valid criterion of thymus maturation is evident from the thymus-type restriction specificity of the single grafted chimeras discussed below. Furthermore, the generation of minor-H-specific CTL requires a functioning thymus: in eight of eight thymectomized, irradiated, stem cell reconstituted animals tested, no minor-H-antigen-specific response could be induced (data not shown).

We have determined the H-2 type of lymphoid cells and various CTL effector populations from seven double thymus grafted animals [two (AKD2)F_1 hosts and five (C.K × C)F_1 hosts]. The data from five such animals are presented in Table 1. The CTL titration curves for chimera no. 3 (Table 1) are shown in Fig. 1*B*. Equal H-2^k-restricted and H-2^d-restricted activities were generated from this double thymus grafted chimera. Treatment of the CTL just prior to the ^{51}Cr release assay with anti-H-2^k serum plus complement resulted in a reduction to one-third in activity against both H-2^k and H-2^d minor-H-different targets. Anti-H-2^d serum plus complement had no demonstrable effect (Fig. 1*B*). It is thus concluded that both the k-restricted and the d-restricted CTL in this chimera are about two-thirds C.K. and one-third C, a composition similar to that of the total lymphoid cells from this animal. These data indicate that there is no selective advantage or disadvantage for pre-CTL to mature in a syngeneic versus an allogeneic thymus graft.

In Table 1, only in the case of the H-2-restricted CTL from animal no. 1 is there an indication of preferential maturation within a syngeneic thymus. This syngeneic preference is pronounced only in the CTL population specific for the minor-H-different H-2^d target, 90% of which are C and 10% of which are C.K. In all other animals tested that display approximately equal d-restricted and k-restricted CTL activity, no pronounced syngeneic or allogeneic preference is seen. The CTL from animal no. 4 exhibit a 15-fold preference for lysis of H-2^d targets; this animal may therefore have had only a functioning BALB/c graft. The H-2 type of the H-2^d-restricted CTL population in this chimera mirrors that of the whole lymphoid population, whereas the H-2^k-restricted population is largely H-2^d in phenotype. The only other double-grafted animal examined that shows a skewed ratio of H-2-restricted CTL activity (in this case favoring the H-2^k target) also displays an allogeneic preference, again only in the CTL population specific for the "weak" target. We have no explanation for this surprising finding.

Our data show that there is no preference for CTL to mature within a syngeneic thymus in tetraparental radiation chimeras.

Tetraparental Chimeras with a Thymus Graft from Only One Parent Show No Syngeneic Preference but Often Show an Allogeneic Preference. In order to distinguish between in-

Table 1. Genotype of CTL from tetraparental chimeras with thymus grafts from both parents

Chimera no.	Specific lysis of target cells,* % (d + X)	(k + X)		H-2 type of cells† Lymphocytes	d-restricted CTL	k-restricted CTL	Alloreactive CTL‡
1	70	64	% H-2^d	52	90	33	
			% H-2^k	46	10	67	ND
	[1×]		% F_1	2			
2	60	43	% H-2^d	35	75	33	50
			% H-2^k	64	25	67	50
	[2×]		% F_1	1			
3	47	49	% H-2^d	34	35	35	50
			% H-2^k	63	65	65	50
	[1×]		% F_1	3			
4	72	30	% H-2^d	38	40	100	60
			% H-2^k	56	60	0	40
	[15×]		% F_1	6			
5	44	22	% H-2^d	34	40	40	66
			% H-2^k	53	60	60	33
	[3×]		% F_1	13			

Animal no. 1 is a [(C + C.K) → (AKD2)F_1] chimera with C and C.K thymus grafts, primed with whole (B10.Br × B10.D2)F_1 spleen cells. Animals 2–5 are [(C + C.K) → (C.K × C)F_1] chimeras with C and C.K thymus grafts, primed with anti-Thy-1-treated (B10.Br × B10.D2)F_1 spleen cells. All animals were used 8–9 months after irradiation. Responder cells were boosted *in vitro* with irradiated (B10.Br × B10.D2)F_1 or B10 spleen cells. Animals 2 and 3 (the latter shown in Fig. 1*B*) were also boosted with TNP-modified (C.K × C)F_1 spleen cells, with CTL profiles similar to those shown here for minor-H-antigen-specific CTL. In all experiments, normal (AKD2)F_1 or (C.K × C)F_1 animals were also tested; anti-H-2^d and anti-H-2^k pretreatment of F_1 responders removed all CTL activity. ND, not determined.

* Data for an effector-to-target ratio of 50:1. Minor-H-specific CTL were assayed on ^{51}Cr-labeled B10.D2 (d + X) and B10.Br (k + X) target cells. Numbers in brackets represent the ratio of killing on B10.D2 cells to that on B10.Br cells. This ratio varied no more than 3-fold with F_1 responder cells.

† Lymphocytes were a mixture of spleen and lymph node cells and were typed fresh from the animal. CTL were generated in a 5-day MLC. Cytotoxic effector cells surviving antiserum treatment were assayed for killing activity on the appropriate target cells. Numbers represent percent H-2^d, H-2^k, or F_1 lymphocytes or effector cells.

‡ Anti-H-2^b CTL were induced and assayed on B10 target cells.

trathymic H-2-restricted T–T interactions and those that may occur in the periphery, we compared animals that received both types of parental grafts with those reconstituted with thymic lobes from a single parent. H-2-restricted interactions that permit CTL induction in the periphery would be uncompromised in double-grafted animals, because the T cells mediating these interactions can be the products of either parental graft; intrathymic interactions would still be blocked. In tetraparental chimeras with thymus grafts from only one parent, even peripheral H-2-restricted T–T interactions would be restricted to only one H-2 type. To eliminate the possibility of providing helper T cells in the spleen cells injected as immunogen, all (C.K × C)F_1 recipients were primed with anti-Thy-1-treated (B10.Br × B10.D2)F_1 spleen cells.

Nine single-thymus-grafted animals were examined. Data from six such animals are compiled in Table 2, and representative titration curves from two experimental animals are depicted in Fig. 2. The minor-H-specific CTL induced in all single-grafted animals examined show a strong thymic preference in H-2 restriction specificity. Animal no. 1 (Fig. 2A) received C thymus grafts and the CTL generated against (B10.Br × B10.D2)F_1 cells lyse mainly B10.D2 ($H\text{-}2^d$) targets. This d-restricted CTL activity is reduced approximately 90% by treatment with anti-$H\text{-}2^k$ serum plus complement and therefore consists of 90% C.K. effector cells. This is an example of an allogeneic restriction preference, because only 31% of the total lymphoid cells and only 35% of Con A-induced CTL are C.K. (Table 2). Animal no. 6 (Fig. 2B) received C.K. thymus grafts and generated mainly k-restricted CTL activity. The composition of this CTL population is quite similar to that of the total lymphocyte pool from this animal (Table 2).

Table 2. Genotype of CTL from tetraparental chimeras with thymus grafts from one parent

Chimera no.	Specific lysis of target cells,* % (d + X)	(k + X)		H-2 type of cells: Lymphocytes*	Minor-H CTL†	Con A-induced CTL‡	Allo-reactive CTL*
			BALB/c thymus graft				
1	64	9	% $H\text{-}2^d$	59	10	65	
			% $H\text{-}2^k$	31	90	35	ND
	[>60×]		% F_1	10			
2	68	0	% $H\text{-}2^d$	51	0		
			% $H\text{-}2^k$	40	100	ND	ND
	[>80×]		% F_1	9			
3	65	9	% $H\text{-}2^d$	32	60		50
			% $H\text{-}2^k$	59	40	ND	50
	[>80×]		% F_1	9			
4	35	0	% $H\text{-}2^d$	51	33		50
			% $H\text{-}2^k$	43	60	ND	50
	[>40×]		% F_1	6			
			BALB.K thymus graft				
5	0	29	% $H\text{-}2^d$	66	90		
			% $H\text{-}2^k$	29	10	ND	ND
	[<0.04×]		% F_1	5			
6	19	66	% $H\text{-}2^d$	48	55	70	
			% $H\text{-}2^k$	49	45	30	ND
	[<0.04×]		% F_1	3			

Animals 1, 2, 5, and 6 are [(C + C.K) → (AKD2)F_1] chimeras with C or C.K thymus grafts, as indicated, primed with (B10.Br × B10.D2)F_1 whole spleen cells. Animals 3 and 4 are [(C + C.K) → (C.K × C)F_1] chimeras with C thymus grafts primed with anti-Thy-1-treated (B10.Br × B10.D2)F_1 spleen cells. All animals were used 7–10 months after irradiation. Responder cells were boosted *in vitro* with irradiated (B10.Br × B10.D2)F_1 or B10 spleen cells. The data for animal no. 1 are represented in Fig. 2A, and for animal no. 6 in Fig. 2B. A total of six C-grafted and three C.K-grafted chimeras was examined. F_1 control animals were included in each experiment. ND, not determined.

* As in Table 1.

† CTL specific for minor H antigens plus the thymic H-2 type.

‡ CTL assayed on tumor targets in the presence of phytohemagglutinin were polyclonally activated by culturing for 3 days with Con A.

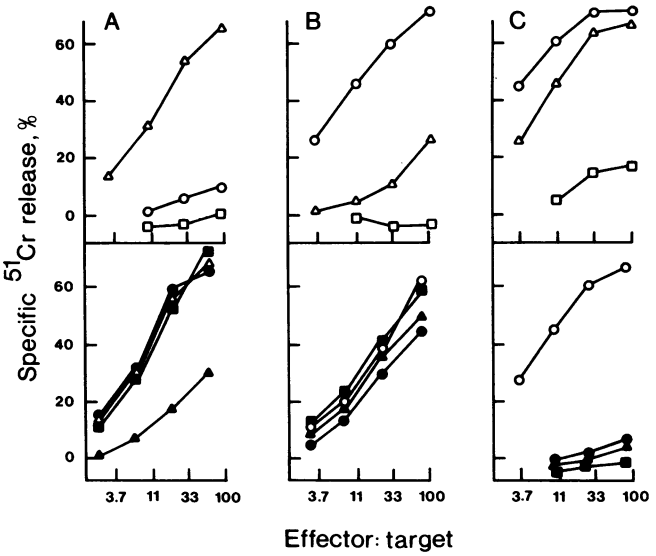

FIG. 2. H-2 type of minor-H-specific CTL from tetraparental chimeras with single parental thymus grafts. Responders are: [(C + C.K) → (AKD2)F_1] with a C thymus (*A*), [(C + C.K) → (AKD2)F_1] with a C.K thymus (*B*), and normal (AKD2)F_1 (*C*). All responders were primed in vivo and boosted in vitro with (B10.Br × B10.D2)F_1 spleen cells. ^{51}Cr-Labeled target cells were 2-day Con A blasts from B10.Br (○, spontaneous release 19%), B10.D2 (△, spontaneous release 16%), and (AKD2)F_1 (□, spontaneous release 19%). Responder cells are either untreated (upper panels) or (lower panels), treated with normal mouse serum (symbols as above), anti-$H\text{-}2^d$ serum (●), anti-$H\text{-}2^k$ serum (▲), or anti-Thy-1.1 monoclonal antibodies (■) plus rabbit complement. In *A*, antiserum-treated responder cells were tested on B10.D2 targets, and in *B* and *C*, on B10.Br targets. In animal *A*, spleen and lymph node cells were typed as 59% C, 31% C.K, and 10% F_1. Con A-induced CTL were typed as 65% C and 35% C.K. In animal *B*, spleen and lymph node cells were 48% C, 49% C.K, 3% F_1. Con A-induced CTL were 70% C and 30% C.K.

The CTL from all animals with C.K. thymus grafts that were tested show neither a syngeneic nor an allogeneic preference, with the exception of a weak allogeneic preference observed in the H-2-restricted CTL of animal no. 5. In the (C.K × C)F_1 hosts reconstituted with BALB/c grafts, for example, animals 3 and 4 in Table 2, neither an allogeneic nor a syngeneic preference is apparent. On the other hand, the striking allogeneic preference shown in Fig. 2A is seen in all (AKD2)F_1 animals with BALB/c thymus grafts. Although the procedures used for constructing and priming thymus-grafted (AKD2)F_1 recipients differ from those using (C.K × C)F_1 recipients, there is no obvious relationship between these differences and the presence of an allogeneic preference in (AKD2)F_1 recipients of C (and not C.K) thymus grafts. The possible implications of preferential induction of CTL that are allogeneic to the thymus will be discussed below.

In no single-thymus-grafted animal was a strong syngeneic preference seen, an observation incompatible with the suggestion that helper T cells for CTL are required to recognize the H-2 antigens of the CTL precursor in order to deliver an induction signal.

DISCUSSION

The experiments reported here were motivated by a desire to test for the existence of H-2-restricted interactions between T cell subpopulations promoting the differentiation and induction of CTL. The system employed avoids the complications of wholly allogeneic chimeras and represents a relatively "artifact-free" way of assessing the requirement for such interactions. We have constructed tetraparental radiation chimeras that allow us to quantitate the maturation of CTL precursors in an H-2-compatible versus an H-2-incompatible thymic environment. Using these animals, we have found that the H-2 genotype of CTL does not influence their H-2 restriction specificity. There is no indication of preferential maturation of CTL in an H-2-compatible thymus. In addition, we have no evidence for peripheral H-2-restricted helper cell–CTL interactions. Our results rule out those helper cell models that require recognition of H-2 on the CTL surface for delivery of a helper signal. Intra- or extrathymic enhancement of CTL differentiation mediated by non-H-2-restricted soluble factors is still a possibility.

In our system, single-grafted animals have shown that stem cells differentiate in the thymus graft and give rise to CTL specific for a variety of foreign antigens plus the particular H-2 antigens expressed by the irradiated thymus grafts. Demonstrating thymic influence in this system is essential for studying the requirement for H-2-restricted T–T interactions during CTL differentiation; one must be able to predict the H-2 restriction specificity of the proposed helper cells. Our experimental system would fail to detect helper cells if the cells mediating this helper function are not H-2 restricted at the CTL level, or if their restriction specificity is not defined by the thymus. However, if this were the case, the main in vivo evidence for H-2-restricted helper cells for CTL (the results of von Boehmer and Zinkernagel and their colleagues) would not be accommodated.

We see some variability in the H-2 phenotype of CTL from single-thymus-grafted animals. This fluctuation could be explained by the small number of stem cells that can seed an irradiated thymus (38) or the possibly low number of precursors that give rise to CTL specific for a given antigen. These explanations are less likely, given our results with tetraparental chimeras with *in situ* F_1 thymuses. Here the CTL populations specific for at least three different antigens have the same H-2 profile, which reflects that of the whole lymphoid population.

The preferential induction of H-2 restricted CTL allogeneic to the thymus graft in four of nine single-grafted animals does not occur in animals with both parental thymus grafts. This finding suggests that the allogeneic preference is generated in the periphery, not in the thymus. This type of allogeneic preference could be the result of an H-2-restricted mechanism for suppressing CTL activation. Such suppression would not be detected in unmanipulated F_1 or parental animals, in tetraparental chimeras with F_1 thymuses, or in double-thymus-grafted chimeras.

With regard to the controversy over whether or not allogeneic chimeras are immunocompetent, one could suggest that CTL differentiation is promoted by the release of soluble helper factors by helper cells. This release could be triggered in an H-2-restricted way, whereas the factors, once released, could act in a nonrestricted manner. If thymic tutoring of self in H-2 restriction is preferential rather than absolute, [A → B] chimeras may have low levels of helper factor production because of mismatching between the learned "self-H-2" and the peripheral antigen presenting cells. The results presented here show that there is no restriction at the CTL level in the delivery of helper signals.

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