THE LYMPHORETICULAR SYSTEM IN TRIGGERING VIRUS PLUS SELF-SPECIFIC CYTOTOXIC T CELLS: EVIDENCE FOR T HELP*

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The thymus seems to be the organ in which the capacity of T cells to recognize self-H-2 structures differentiates (1). This observation is compatible with the interpretation that T cells express two recognition sites. However, in these studies on the generation of virus-specific *H-2*-restricted cytotoxic T cells in chimeras, some results are unexplained. Why is the virus-specific cytotoxic activity of parent → F_1 chimeras always favoring the own parental *H-2* type (2, 3)? Why do $H\text{-}2^A \times H\text{-}2^B \rightarrow H\text{-}2^A \times H\text{-}2^C$ chimeras fail to generate virus-specific cytotoxicity associated with $H\text{-}2^C$ even though these chimeras have $H\text{-}2^C$ on their thymus cells and most other somatic cells, and therefore should be able to recognize C as self. Furthermore, why do *H-2I* incompatible stem cell chimeras that are *D* region compatible, fail to generate any measurable cytotoxic activity at all?

The literature contains ample evidence that *H-2* incompatible chimeras generate cell-mediated-immune responses poorly, except for transplantation reactions against grafts from unrelated donors (3–14). For example, neonatally thymectomized or nude mice later transplanted with allogeneic neonatal thymuses showed little reconstitution of immunocompetence in producing antibody against a T-cell-dependent antigen such as sheep erythrocytes; however, these animals' alloreactivity against unrelated grafts was surprisingly well developed (3–7, 11, 12). In a different model, mice were irradiated lethally and reconstituted with *H-2* incompatible bone marrow cells. Although these allogeneic irradiation bone marrow chimeras produced only small amounts of antibodies against T-cell-dependent antigens, they readily rejected unrelated tissue grafts (8–10, 13, 14).

Subsequently it was shown that this deficiency in syngeneic immunocompetence could be reconstituted, for example, by addition of B cells that were syngeneic with the chimeric host (14). It thus appears from published experiments and from our own chimera data, that for the phenotypic expression of

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syngeneic T-cell activities specific for a foreign antigen and self-H-2 structure, conditions must be given, which are lacking in some of the chimeras.

We attempted to analyze this question by determining the possible identity of the cells that present virus in an immunogenic fashion and the role of the *H-2* haplotype of these cells and that of the peripheral lymphocytes in triggering mature T cells. The approach we used was to prime lymphocytes from the various kinds of chimeras (A × B → A, A × B → A × C, recombinant A|B → recombinant A|C or A → B) in irradiated and virus infected recipients possessing infected immunogenic cells of appropriate *H-2* types.

In this paper we demonstrate that, although the thymus determines which range of H-2 antigens might be recognized as self, it is mainly the *H-2* of the lymphoreticular system's (LRS)[1] cells and not of other somatic cells that determines the actual, phenotypically expressed and measurable specificity for self-H-2. Thus if T cells of any *H-2* type learn to recognize $H\text{-}2^A$ as self, they cannot express their immunocompetence unless the same $H\text{-}2^A$ is expressed, at least partially, on the cells they interact with, i.e., the lymphocytes themselves and their host's LRS. Furthermore, these results indicate that *H-2I*-restricted T helper cells are essential for the generation of virus-specific cytotoxic T cells.

Materials and Methods

Chimeras. The mouse strains used and their origins have been described (1). The various chimeras were prepared as described by Sprent et al. and Zinkernagel et al. (15, 1). Chimeras were used 2 mo after reconstitution, except the few that were used after only 6 wk. The chimeras were H-2 typed and tested individually.

Neonatally Tolerant Mice. The A.AL (tolerant A.TL) mice, as described previously (16, 17), were rendered tolerant by the intravenous (i.v.) injection of about 2.5×10^7 (A.AL × A.TL)F_1 spleen cells plus bone marrow cells during the first 24 h after birth. Tolerance was tested by monitoring a skin graft of (A.AL × A.TL)F_1 origin, which was not rejected for more than 6 mo. The A (tolerant C57BL/6) were injected with (C57BL/6 × A)F_1 spleen cells intraperitoneally (i.p.). Chimerism was monitored by immunoglobulin allotype testing according to published methods (18).

Adoptive Transfers. Spleen and lymph node cells of chimeras or unmanipulated donors were transferred i.v. to recipients that had been irradiated with 850–900 rads and infected with about 10^7 plaque-forming units (PFU) of vaccinia virus 2 h before the transfer. Usually $3\text{–}5 \times 10^7$ live lymphocytes were transferred. Recipients were killed 6 days later, and their spleen cells were tested for cytotoxic activity. Cells from three or four recipients of lymphocytes from a single chimeric or normal donor were pooled before testing.

The *H-2* types of chimeras and tolerant mice were determined individually from the cells used for the transfer. Only fully reconstituted (i.e., >90–95% of reconstituting *H-2* and undetectable levels of recipient type) chimeras were used for further experimentation.

H-2 Typing. The antisera from the National Institutes of Health collection or from Dr. J. Klein, Dallas, Tex. and the methods used are the same as previously reported (19, 1). Positive and negative lymphocyte populations were included in each test.

Target Cells and ^{51}Cr Release Assay. Published methods were used as described in the preceding report (1). Results are expressed as a percent of water released and are uncorrected for spontaneous release (20, 21).

Statistical Methods. Means and SEM of triplicate determinations were determined and compared by the Student's *t* test.

[1] Abbreviations used in this paper: LRS, lymphoreticular system; PFU, plaque-forming unit; PHA, phytohemagglutinin; TNP, trinitrophenol.

Results

Virus-Specific Cytotoxic Activity Generated in Parent → F_1 Irradiation Bone Marrow Chimeras. In a follow-up of our own studies and of others (1–3) we analyzed why irradiated, bone marrow reconstituted chimeric mice show such a marked preference of immunologic activity for the reconstituting parental *H-2* haplotype. Vaccinia virus infected A → (C57BL/6 × A)F_1 or C57BL/6 → (C57BL/6 × A)F_1 chimeras generated cytotoxic activity that was specific for the donor parental H-2 type only. In contrast, when we used C3H → (C3H × DBA/2) mice from the same group of mice used in our original study, this preference was less marked, and significant lysis of the nonreconstituting infected *H-2* target was detected Table I, exp. 1. Whether a C3H characteristic has something to do with this finding is unclear and will be discussed later; however, another chimera BALB/c → (BALB/c × C3H)F_1, which contains C3H, also less strictly preferred the reconstituting *H-2* in the virus-specific response (data not shown). Since, according to our evidence (1), parental stem cells learned to recognize both F_1 *H-2* haplotypes as self in the F_1 thymus, these results suggested that the actual expression of *H-2* associated virus-specific cytotoxicity depended on an additional factor. Since parent → F_1 chimeras express the incompatible parental *H-2* haplotype on all somatic cells except those destroyed by irradiation and reconstituted by one parent's lymphoreticular hemopoietic stem cells, it appeared as if this compartment essentially determined immunogenicity. This proposition was tested in the following experiments.

Primary Sensitization of F_1 Lymphocytes against Infected Parental Cells in an Adoptive Transfer Model. To assess the reaction potential of mature chimeric T cells we attempted to sensitize lymphocytes in acutely irradiated and infected recipient mice in a primary fashion (Table II). (C57BL/6 × A)F_1 normal spleen cells were transferred into recipients that had been irradiated, acutely infected with vaccinia virus, and were killed 6 days later. Irradiated and infected recipient mice which had not received any spleen cells did not generate measurable cytotoxicity (data not shown). Clearly, the F_1 cells were sensitized only against infected targets that possessed the same *H-2* haplotype as the donor and the intermediate infected recipient. No crosspriming occurred. Thus, in this model of acute adoptive transfer for primary sensitization, it is the *H-2* type of the irradiated recipient that determines the generation of virus plus *H-2*-specific cytotoxicity.

Potential of Parent → F_1 Chimeric T Cells to React with Virus in Association with Both Parental H-2 Haplotypes. Spleen cells from the symmetrical chimeras A → (C57BL/6 × A)F_1 and C57BL/6 → (C57BL/6 × A)F_1 were transferred into freshly irradiated and vaccinia virus-infected (C57BL/6 × A)F_1 recipients. The cytotoxic activities detected in their spleens 6 days later are listed in exp. 2 in Table I. Lymphocytes from both kinds of parent → F_1 chimeras responded to virus in association with both parental *H-2*. Thus, although the original infected parent → F_1 chimeras responded noticeably only against infected cells bearing the same *H-2* type as the reconstituting parent's cells, the chimeric T cells tested here could react comparably with virus plus the *H-2* type of the nonreconstituting parent. Both in the original chimeras and in the newly irradiated and infected recipients, most somatic cells were of the F_1 *H-2* type

TABLE I

*Virus-Specific Cytotoxicity of Parent → F Irradiated Bone Marrow Chimeras**

Donor → Recipient (H-2 types)‡ → Second recipient			Spleen cell to target cell ratio	^{51}Cr Release from vaccinia infected target cells§		
				D2(d)	L(k)	MC57G(b)
Experiment 1:						
1. A ($k\|d$)	→ C57BL/6 × A‡ ($b \times k\|d$)	None	40:1	[59]	[33]	22
			13:1	[40]	[16]	21
2. C57BL/6 (b)	C57BL/6 × A ($b \times k\|d$)	None	40:1	18	10	[46]
			13:1	24	9	[32]
3. C3H (k)	→ C3H × DBA/2 ($k \times d$)	None	40:1	[39]	[53]	23
			13:1	[32]	[27]	20
A ($k\|d$)	None		40:1	[70]	[101]	24
C57BL/6 (b)	None		40:1	29	12	[47]
C3H (k)	None		40:1	23	[54]	26
Experiment 2:‖						
(A ($k\|d$)	→ C57BL/6 × A ($b \times k\|d$)	→ C56BL/6 × A ($b \times k\|d$)	15:1		[60]	[39]
			5:1		[46]	[29]
(C57BL/6 (b)	→ C57BL/6 × A ($b \times k\|d$)	→ C57BL/6 × A ($b \times k\|d$)	15:1		[66]	[49]
			5:1		[54]	[30]
C57BL/6 (b)	None		15:1		30	[72]
A ($k\|d$)	None		15:1		[91]	22
Medium					26	20

* Recipient mice were irradiated with 900–950 rads and transfused with 1.5–2.0 × 10^7 anti-θ + C-treated bone marrow cells. Chimeras were infected with about 10^7 PFU of vaccinia virus.

‡ The typing results were for:

Chimera 1		*Chimera 2*		*Chimera 3*	
Anti-K^k (K-603)	>95%	Anti-K^k (K-603)	<5%	Anti-K^k (K-603)	>95%
Anti-K^b (D-33)	<5%	Anti-K^b (D-33)	>95%	Anti-K^d (D-31)	<5%
Control	<5%	Control	<5%	Control	<5%

§ Means of triplicate determinations; the SEM <3%. The test duration was: 16 h. The lymphocytes did not cause any significant lysis when tested against uninfected target cells. Statistically significant values ($P < 0.01$) are boxed.

‖ Spleen and lymph node cells were from completely reconstituted chimeras transferred to freshly irradiated (850 rads) and with vaccinia virus infected (10^7 PFU) recipient mice. These second recipients were killed 6 days later and the spleen cells were tested for virus-specific cytotoxicity.

but the cells of their LRS differed with respect to *H-2* type. In the LRS compartment, the chimeras expressed mainly reconstituting parental *H-2*, whereas the irradiated intermediate recipients expressed *both* parent's *H-2*. Since this difference appeared to correlate with the generation of measurable virus-specific activity associated with the nonreconstituting *H-2*, these experiments indicate that the predominant determiner of immunogenicity are infected cells in the LRS and not other somatic cells.

TABLE II

*Vaccinia Virus-Specific Cytotoxicity in Irradiated Recipients Infected with Vaccinia Virus and 2 h later Transfused with 5 × 10^7 Adult Spleen Cells**

Donor ——→ Recipient		Ratio of lymphocytes to target cells	^{51}Cr Release from vaccinia infected target cells‡	
			L(k)	MC57G(b)
C57BL/6 × A →	C57BL/6	40:1	33	[101]
($b \times k \mid d$)	(b)	13:1	34	[78]
C57BL/6 × A →	A	40:1	[84]	17
($b \times k \mid d$)	($k \mid d$)	13:1	[64]	–
C57BL/6 × A →	C57BL/6 × A	40:1	[80]	[98]
($b \times k \mid d$)	($b \times k \mid d$)	13:1	[65]	[67]
Medium			36	21

* Parental of F_1 recipient mice were irradiated with 850 rads before i.v. infection with about 10^7 PFU of vaccinia virus. Recipients were transfused with 5 × 10^7 adult donor spleen cells. These animals were killed 6 days later and then spleens were tested for cytotoxicity.

‡ Means of triplicate determinations; SEM <3%. The duration of the test was 16 h. No activity was detected on uninfected target cells. Values were compared with medium controls, and significant results ($P < 0.01$) are boxed.

Priming of F_1 → Parent Chimeric or Neonatally Tolerant Lymphocytes in Infected F_1 Recipients. When, as in the previous experiments, F_1 → parent chimeric lymphocytes were transferred to irradiated, and infected F_1 recipients, cytotoxicity was generated only in association with the *H-2* type of the chimeric recipient (data not shown). Since in F_1 → parent chimeras the parental thymus determines which *H-2* is recognized as self, it is not surprising that this specificity for "self" cannot be changed in these priming mice. Both in the original chimera F_1 → parent and in the sensitizing F_1 recipients the LRS that expresses immunogenic viral antigens is of F_1 type.

Neonatally tolerant mice were tested for their potential to be sensitized against infected tolerated targets by transfer into the respective irradiated and infected sensitizing F_1 hybrids. Cytotoxic activity was measured 6 days later in their spleens. Activity was directed virtually exclusively to virus plus the *H-2* type of the tolerant mouse. These results support the notion that tolerance alone is not sufficient for immunocompetent T cells to become reactive to virus and the tolerated *H-2* (Table III).

The Potential of Lymphocytes from (A × B) → (A × C) or (A\|B) → (A\|C) Chimeras to React to Virus Plus H-2. A → C3H (exp. 1, Table IV) or B10.A(2R) → B10.A (exp. 2, Table IV) chimeric lymphocytes were transferred to the respective infected and irradiated F_1 recipients. 6 days later virus-specific cytotoxic activity was tested on targets that distinguished between the various virus plus *H-2* specificities. A($H\text{-}2^{k\mid d}$) → C3H ($H\text{-}2^{k\mid k}$) lymphocytes transferred into A × C3H F_1 ($H\text{-}2^{k\mid d \times k\mid k}$) were positive only for infected $H\text{-}2^k$ targets. Similarly, B10.A(2R) ($H\text{-}2^{k\mid b}$) → B10.A ($H\text{-}2^{k\mid d}$) lymphocytes transferred into B10.A × B10.A(2R) ($H\text{-}2^{k\mid d \times k\mid b}$) were active for the K^k compatible infected targets, and for D^d targets but not for the D^b targets.

In another experiment (exp. 2, Table IV) (BALB/c × C57BL/6) ($H\text{-}2^{d \times b}$) →

TABLE III

*Spleen Cells from Neonatally Tolerant Mice Fail to React to Virus and the Tolerated H-2 when Transferred into Irradiated Virus Infected F_1 Recipients**

Donor → Recipient			Spleen cell to target cell ratio	^{51}Cr Release from vaccinia infected target cells‡	
				L(k)	MC57(b)
Experiment 1					
A(Tolerant C57BL/6)	→	(C57BL/6 × A)	40:1	[79]	13
(k|d)		(b × k|d)	13:1	[98]	14
			4:1	[61]	13
C57BL/6 × A	→	C57BL/6 × A	40:1	[104]	[51]
(b × k|d)		(b × k|d)	13:1	[73]	[29]
			4:1	[67]	[28]
Normal C57BL/6				21	12
				L(k)	B10.S(s)
Experiment 2:					
A.AL(Tolerant A.TL)	→	A.AL × A.TL	15:1	[51]	35
(K^k|D^d Tolerant K^s|D^d)		(K^k|D^d × K^s|D^d)	5:1	[37]	43
A.TL	→	A.TL	15:1	17	[72]
K^s|D^d		K^s|D^d	5:1	19	[61]
Medium				17	40

* Neonatally tolerant mice were made as described in Materials and Methods. Tolerance was controlled by determining Ig-allotype of donor origin (exp. 1) or by skin transplantation (exp. 2). H-2 typing failed to reveal measurable chimerism (i.e., < 5%). The lymphocytes were sensitized in irradiated (850 rads) and virus-infected recipients for 6 days.

‡ Uncorrected means of triplicate determination · SEM < 5%. Statistical significance was determined against medium release or release by normal cells; significant values ($P < 0.01$) are boxed.

(BALB/c × C3H) ($H\text{-}2^{d \times k}$) chimeric lymphocytes were transferred into irradiated and infected F_1 recipients of both kinds. The virus-specific activity detectable 6 days later in second recipients that were BALB/c × C57BL/6 ($H\text{-}2^{d \times b}$) was positive for infected $H\text{-}2^d$ targets only, but not for infected $H\text{-}2^k$ or $H\text{-}2^b$ targets; however, activity from BALB/c × C3H ($H\text{-}2^{d \times k}$) second recipients was positive for infected $H\text{-}2^d$ and $H\text{-}2^k$ targets but still negative for infected $H\text{-}2^b$ targets.

Thus, Table IV illustrates both principles that have emerged from this and the foregoing report. (A × B) → (A × C) or (A|B) → (A|C) chimeras learn to recognize the *H-2* self markers that are expressed in the chimeras' thymus. However, this potential to recognize self together with virus is expressed and thus becomes detectable only if the relevant cells that express viral antigens immunogenically, *i.e.*, cells in the LRS also express these self markers.

Analysis of the Immunoincompetence of H-2 Incompatible or H-2K, I Incompatible Chimeras. We showed that infected *H-2* incompatible or *H-2K, I* incompatible chimeras do not generate measurable cytotoxic T-cell activity (Zinkernagel et al. 1978. *J. Exp. Med.* 147:882). If the LRS alone is the critical factor in expressing antigen immunogenically, then lymphocytes from *H-2* or

TABLE IV

*Determination of the Potential to React with Virus and H-2 of Lymphocytes from "Partially Histocompatible" Chimeras**

Donor → Recipient → Second recipient	Spleen cell to target cell ratio	%^{51}Cr Release from vaccinia infected target cells‡		
		L(k)	$D_2(d)$	MC57G(b)
Experiment 1:				
1. (A ($k\|d$) → C3H (k/k)§ → C3H × A ($k\|k \times k\|d$)	40:1	[95]	25	–
	13:1	[67]	22	–
A ($k\|d$) → None → C3H × A ($k\|k \times k\|d$)	40:1	–	[88]	–
	13:1	–	[59]	–
C3H ($k\|k$) → None → (C3H × A) ($k\|k \times k\|d$)	40:1	[71]	–	–
	13:1	[54]	–	–
Normal A	40:1	20	22	–
Experiment 2:				
2. (B10.A(2R) ($k\|b$) → B10.A) ($k\|d$) → B10.A × B10.A(2R) ($k\|d \times k\|b$)	40:1	[80]	[64]	27
	13:1	[65]	[69]	26
	4:1	[45]	37	27
B10.A(2R) → None → None	40:1	[60]	38	[36]
3. (BALB/c × C57BL/6) ($d \times b$) → (BALB/c × C3H)] ($d \times k$) → BALB/c × C57BL/6 ($d \times b$)	40:1	15	[91]	15
	13:1	16	[75]	16
→ BALB/c × C3H ($d \times k$)	40:1	[80]	[100]	13
	13:1	[37]	[60]	15
BALB/c × C57BL/6 ($d \times b$) → None → None	40:1	20	[102]	[68]
	13:1	22	[98]	[59]
Medium		16	31	12

* Recipient mice were irradiated with 900 or 925 rads and transfused with 1.5–2 × 10^7 anti-θ + C-treated bone marrow cells or fetal liver cells. Chimeras were killed, typed for H-2, and their lymphocytes transferred to irradiated and virus-infected recipients at the following times after reconstitution: exp. 1: 2½ mo., exp. 2: 6 mo.

‡ Results are uncorrected means of triplicate determinations, the SEM was smaller than 5%. Statistical comparison was made with the highest of the values of medium release, release by normal cells or immune H-2 incompatible cells. Statistically significant values ($P < 0.01$) are boxed. The lymphocytes were all tested on the respective uninfected target cells and did not cause any significant lysis.

§ Chimeras were H-2 typed as described in Materials and Methods.

Chimera 1	*Chimera 2*	*Chimera 3*
Anti-D^d (D-4) >90%	Anti-D^b (D-2) >90%	Anti-K^k (K-603) <10%
Anti-D^k (D-32) <5%	Anti-D^d (D-4) <10%	Anti-K^b (D-33) >95%

H-2K, I incompatible A → B chimeric lymphocytes might generate cytotoxic T cells when transferred to freshly irradiated and infected recipients expressing A and B. In two experiments, first when (C3H → BALB/c) chimeric lymphocytes were transferred into irradiated and infected (BALB/c × C3H)F_1 and second when BALB/c → A were sensitized in (BALB/c × A)F_1, no virus-specific activity was measurable (Table V). Although the number of these *H-2* incompatible chimeric cells tested in this way is still small, we conclude that allogeneic chimeras are not triggered properly, even when the LRS expresses an *H-2* type that transferred cells learned to recognize in the thymus of the chimera.

Discussion

The thymic epithelium apparently determines which H-2 structures are recognized as "self" by T cells in the process of *H-2* restriction (1). When the full

TABLE V

H-2 and H-2K, I Incompatible Chimeras' Lymphocytes Failure to get Sensitized to Virus Expressed on Cells of the LRS that is H-2 or H-2K, I Compatible with the Educating Thymus of the Chimeras

Donor (H-2 typing)	→ Recipient* chimera	→ Second recipient	Ratio of lymphocytes to target cell	^{51}Cr Release from vaccinia infected target cells‡	
				L	D_2
1. (C3H	→ BALB/c)	→ BALB/c × C3H	40:1	11	30
(*k*)	(*d*)	(*d* × *k*)	13:1	11	31
			4:1	10	30
2. (BALB/c	→ A)	→ BALB/c × A	40:1	11	–
(*d*)	(*k*|*d*)	(*d* × *k*|*d*)	13:1	12	–
			4:1	10	–
Controls:					
BALB/c × C3H			40:1	60	62
(*d* × *k*)			13:1	40	45

* Recipient mice were irradiated with 950 rads and reconstituted with anti-θ-treated bone marrow. The chimeras were killed 3 mo later and H-2 typed. The spleen and lymph-node cells of one donor were transferred to freshly irradiated and infected recipient (3×10^7 per recipient) 6 days later these second recipients were killed and their spleen cells tested for cytotoxic activity. The typing results were for:

Chimera 1		*Chimera 2*	
Anti-K^k (K-603)	>95%	Anti-K^d (D-31)	>95%
Anti-K^d (D-31)	<10%	Anti-K^k (K-603)	<5%

‡ Results are means of duplicate or triplicate determinations, the SEM were smaller than 5%. Statistically significant results ($P < 0.01$) are boxed. The lymphocyte's activity on the respective uninfected target cells was not significant.

spectrum of restriction specificities is not observed, the LRS of the chimeric host being tested has imposed an additional constraint on immune responsiveness. This second paper provides the formal demonstration of the LRS effect. The data summarized in Table VI can be restated in general as follows: (*a*) chimeras of the type parent → F_1 generate killer T cells that lyse only those targets carrying the *H-2* type common to both donor and parent (Table I); none the less, parent → F_1 chimeras also carry splenic T cells which, upon transfer into an infected, irradiated F_1, can give rise to killer T cells with activity specific for the other parental *H-2* type. (*b*) T cells from chimeras (A × B) → A or from A (neonatally tolerant to B), whose responsiveness was restricted to A-type H-2 antigens (16), upon transfer to infected, irradiated F_1 animals, do not generate killer T cells that are specific for the targets of B type. (*c*) Chimeric cells of the type (A × B)F_1 → (A × C)F_1, which generated killer T cells specific for the A type H-2 antigens, but not B or C H-2 antigens (1), however, do generate killer T cells restricted to the second recipient's parental H-2 C antigens when that recipient of transfer is an infected and irradiated (A × C)F_1 animal. However, the similar transfer of chimeric T cells into infected, irradiated (A × B)F_1 recipients raises no killer T cells that are specific for infected targets of B type. Similarly, $H\text{-}2K^A|D^B$ → $H\text{-}2K^A|D^C$ chimeras do not express either D^B or D^C-restricted specificities. However, upon transfer of these chimeric spleen cells to

TABLE VI

Summary of Experiments Described in Tables I through V

Table	Donor ⟶ Recipient		Thymus of chimeras	Sensitizing second recipients	Lysis of virus infected target cells A, B, C
I	A	A × B	A × B	–	A
II	A × B	–	–	A	A
	A × B	–	–	B	B
	A × B	–	–	A × B	A, B
I	A	A × B	A × B	A × B	A, B
	A × B	A	A	A × B	A
III	A (tolerant B)	–	–	A × B	A
IV	$K^AI^A\|D^B$	$K^AI^A\|D^C$	$K^AI^A\|D^C$	$K^AI^A\|D^B \times K^AI^A\|D^C$	A, C
	A × B	A × C	A × C	A × C	A, C
	A × B	A × C	A × C	A × B	A
V	A	B	B	A × B	None
	$K^BI^B\|D^A$	$K^CI^C\|D^A$	$K^CI^C\|D^A$	$K^BI^B\|D^A \times K^CI^C\|D^A$	None

a $(D^B \times D^C)F_1$ mouse that has been infected and irradiated, the resulting killer T cells show the *H-2D*^{C}, but not the *H-2D*^{B} restriction. Of course at both stages, the *H-2K*^{A}-specific restricted responses of the chimeras and the second recipients were detectable. (*d*) Spleen cells from chimeras made between fully *H-2* incompatible strains, or strains incompatible for *H-2K, I* but previously shown to be incapable of generating cytotoxic T-cell activity, do not recover detectable activity when sensitized in *H-2* compatible (i.e., F_1) infected irradiated recipients.

A general rule then emerges from these experiments: in long-term irradiation bone marrow chimeras the LRS has been replaced by cells derived from the reconstituting stem cells. Cells of the LRS determine which *H-2K* and *H-2D* or *H-2I*-specific restricted T-cell precursors will be activated to become effector killer or helper T cells during an immunologic challenge. This effect of the LRS must be viewed as purely selective, since when chimeric spleen cells developed in the presence of a particular set of *H-2* specificities associated with the thymic epithelium, only those *H-2* restrictions were observable either in the chimera or in the second irradiated recipient. In contrast to long-term irradiation bone marrow chimeras, in *acutely* irradiated and infected sensitizing recipient mice the LRS is still intact and can trigger T cells.

On the Role of the Lymphoreticular System. In chimeras of $(A \times B)F_1 \rightarrow (A \times C)F_1$ type only anti-A restricted virus-specific killer cells were found; however, upon transfer of these cells to $(A \times C)F_1$ animals that had been infected and irradiated, it was possible to recover C restricted virus-specific killer T cells. The interpretation follows, for example, in the $A \times B \rightarrow (A \times C)F_1$ chimera, that the lymphoreticular cells carried only the H-2 antigens of A and B, not C; therefore, no C-specific anti-virus response was triggered even though T cells were present which, when transferred, could mount an anti-virus plus C-specific response as predicted by the presence of an $(A \times C)F_1$ thymic epithelium. The stimulator cells responsible for generation of virus plus C-specific killer T cells could therefore not simply be cells that bear *H-2*^{C} antigens plus viral antigens because the chimera was formed in a $(A \times C)F_1$ host. Thus,

it was important for the T cells at some stage to "see" *H-2*^{C} plus virus on radiosensitive cells of the LRS in order to develop this killer activity. These data are in agreement with studies that many viruses infect cells of the LRS (21), and with many examples demonstrating the selective stimulation of F_1 T-cell activity specific for one parental *H-2* type when antigen presenting stimulator cells of this one parent were used (22, 23).

Since the cells of the LRS, and thymus epithelial cells, are apparently the only ones that express *I* coded structures, our findings on the crucial role of LRS cells to present antigen in an immunogenic way are compatible with the interpretation that *I* region-specific T helper cells are involved in the generation of virus-specific cytotoxic T cells. Obviously the data do not exclude the possibility that K and D structures on cells of the LRS differ not only quantitatively but also qualitatively from those on other somatic cells. However, it is more likely that once T helper and/or T killer cells are triggered by infected cells of the LRS, cytotoxic T cells or memory cells derived from them may be stimulated to proliferate further by other infected somatic cells. Cytotoxic T cells against allogeneic H-2K or H-2D can be generated by spleen cells of A type against B provided the stimulator cells are from the LRS (24–25); monocytes and macrophages often being an optimal source of stimulators. However, allogeneic K^B, D^B antigens must be different from viral antigens, because recognition of allogeneic K, D antigens by the T-cell receptor seems to be sufficient to induce lymphocyte proliferation; as documented in the A → (A × B)F_1 chimera antivirus recognition alone was insufficient to trigger available chimeric lymphocytes with specificity for self-B. Thus, I-specific T help seems necessary for cytotoxic T-cell generation in an antiviral but less so for an alloreactive-immune response.

From the combined use of chimeras and priming recipients, we conclude that anti-self-H-2 specificities are selected for exclusively in the thymus and, from this pre-existing repertoire, immunologically reactive cells are selected according to the H-2 antigens expressed on LRS cells (but not on other cells) that finally present the foreign (viral) antigen to the precursors of killer T cells. The results also indicate that on effector T cells, the anti-self-H-2 specificity is distributed clonally, as has been postulated and shown previously (3, 20).

Helper T-Cell Activity Required for the Induction of Killer T Cells is H-2I Restricted. The requirement for helper T-cell activity in B-cell induction and in the generation of killer T-cell responses against alloantigens has been documented (24, 25, 30). Although our results do not formally prove the existence of T helper cell for the generation of virus-specific cytotoxic T cells, they are compatible with this interpretation and show that the *H-2I* region is of critical importance. *H-2I*-specific T helper cells play a significant part in adoptively transferred sensitization within *H-2K, I* compatible, or incompatible chimeras (Table IV and V). The detailed mapping required to delineate which sub-regions of I are critical remains to be completed. From the preliminary data, the *I-A* region seems to be the most crucially involved since K^k IA^k compatible chimeras B10.A(4R) → B10.A generate good cytotoxic responses to K^k plus virus. Therefore, apparently T-cell help for B cells and T cells are both *H-2IA* restricted (26).

In contrast, lymphocytes from *H-2K*, *I* incompatible chimeras could not be sensitized to react against any of the infected target cells that were of donor or recipient *H-2K* or *D* type. In *I* region incompatible chimeras, such T helper cells are generated but they are specific for an *I* region that is *not* expressed by the lymphocytes of this chimera. The principle is apparent from the following experiments. For example, T helper lymphocytes from $K^AI^AD^B \rightarrow K^CI^CD^B$ have learned to recognize the thymic I^C as self but the pre-killer T cells or the B cells of these chimeras express only the I^A (and the K^A and D^B) self markers. Therefore, the putative T helper cells that are specific for I^C cannot help them. In this paradoxical situation of *I* region incompatible chimeras, no cooperation can take place; therefore, no virus-specific cytotoxic T cells or antibody producing B cells can be triggered (8–12). Thus, these experiments demonstrate that both types of thymus self-H-2 structures, namely those coded in *K*, *D*, and those coded in *I*, must be recognized as self in a selection process.

Comparison with Other Published Experiments. Our results here differ from data published earlier on the virus-specific cytotoxic activity generated in parent $\rightarrow F_1$ chimeras (2, 3). There, significant cytotoxicity was detected for the parental haplotypes of the nonreconstituting parent; however, this activity was always markedly less than that against infected targets of donor parental *H-2* types. In the present experiments using a higher dose (i.e., supralethal) of irradiation most $P \rightarrow F_1$ chimeras, particularly those of C57BL origin, generated activity exclusively associated with the reconstituting parental *H-2* type. Since the earlier chimeras were also reconstituted completely, at least as assessed by serological typing, this difference is most likely explained by persistence of a minor LRS-compartment of the host.

The rules established in these two reports are generally compatible with most of the data available on *H-2* restriction. Yet; they contrast with some results obtained for T cells sensitized against trinitrophenol (TNP)-modified syngeneic lymphocytes. Tolerance, achieved by in vivo filtration, is adequate to allow generation of TNP-specific cytotoxic T cells against modified targets bearing the tolerated *H-2* type (27). However, these results were not confirmed when tolerance was achieved by suicide in vitro (28), and this discrepancy has not been explained.

The idea that, when forced to differentiate and/or cohabitate with allogeneic cells in a chimeric environment, lymphocytes could *learn* to interact and get along with each other was first formulated by Katz and Benacerraf (26). They proposed that in chimeras B cells and T cells differentiate "adaptively" so that via cell interaction structures they can interact with the cells that make up their environment. The results presented here support this idea in general, and are compatible with similar speculations put forward by Waldmann (29). They specify the role of the thymus in the differentiation of the anti-self specificity. This explains the exclusive influence of the chimeric host on the specificity for self and suggests that only T cells express this receptor for thymus-self-H-2; this would be in agreement with the general *H-2* restriction of T cells in contrast to the *H-2* unrestrictedness of B-cell activities.

Miller and Osoba (30), Feldman and Globerson (5), Leuchars et al. and Davies (7, 31) and more recently Kindred and Kindred and Loor (11, 12) showed that

neonatally thymectomized, ATxBM mice of nude mice that were reconstituted by grafts of H-2 incompatible thymuses regained immunocompetence only rarely and incompletely. Although these mice produced poor antibody responses to T-cell-dependent antigens, in many cases they could reject unrelated tissue grafts or react to phytohemagglutinin (PHA). These results and those obtained with thymuses transplanted in diffusion chambers (30) have been discussed in terms of whether the thymus influenced T-cell differentiation by hormonal factors only, or also via direct cell interaction. Our interpretation of these phenomena involves the concept that T cells are selected for to recognize "self"-H-2 structures that are present on thymic epithelial cells, but are absent on the lymphoid cells themselves. This constitutes a biological paradox when T cells are taught self-H-2 that is in fact not self!

The notion that T cells are selected for to recognize self whereby this self-spectrum would constitute the domaine of "thymic tolerance to self-H-2" whereas peripheral tolerance to H-2 is comparable to other forms of tolerance is supported by the results from experiments with neonatally tolerant mice. Based on the initial observation that histoincompatible T cells did not restore the missing helper T cells in nude mice, Kindred demonstrated that neonatally tolerant allogeneic T cells also failed to restore the nude mice's responses against a T-cell-dependent antigen (32); similarly we demonstrate here that neonatally tolerant mice could not be sensitized to lyse infected targets of the tolerated *H-2* type (16). The absence of the tolerizing *H-2* from the thymic epithelium excludes that this *H-2* type can be regarded as self.

Since alloreactivity may not depend upon anti-self recognition but only upon recognition of an alloantigen, T cells from H-2 incompatible chimeras can express alloreactivity or other similar reactivities, such as PHA stimulation, that do not rely on self-recognition. We can extend previous explanations (26) of experiments by Gengozian et al. and Urso and Gengozian (8, 14) who first described lethally irradiated mice reconstituted with *H-2* incompatible bone marrow cells. These mice and similar but germ-free mice (9, 10) failed to mount an adequate immune response against T-cell-dependent antigens. Such irradiation allogeneic bone marrow chimeras were tested in a very elegant study for their capacity to react against alloantigens and their spleen cells were fully capable of generating strong cytotoxic T-cell responses against unrelated alloantigens (10). In contrast, these spleen cells were unable to generate a measurable antibody response against the same alloantigens. The explanation for these results is the same as for mice without T cells that are given an allogeneic thymus; thus, in A → B irradiation allogeneic bone marrow chimeras, the precursor T cells learn to recognize B as self-H-2, but since the T cells and the rest of the LRS are made up from cells expressing A exclusively, no associative antigen recognition or cell interactions can occur.

In conclusion, the concept that T cells differentiate in the thymus specificity for H-2 self-markers, independently from anti-X recognition and that the effector specificity is selected further by the antigen expressing cells of the LRS has profound theoretical and practical implications. It has been impossible to discuss these tissues other than in a summary form and other aspects such as implications on our understanding of Ir gene function or H-2 polymorphism could not be dealt with because of shortage of space; some of them have been

raised previously (33–37). Obviously, many questions remain open and await the biochemical analysis of T-cell recognition structures.

Summary

The thymus determines the spectrum of the receptor specificities of differentiating T cells for self-H-2; however, the phenotypic expression of T cell's specificity for self plus virus is determined predominantly by the H-2 type of the antigen presenting cells of the peripheral lymphoreticular system. Furthermore, virus specific helper T cells are essential for the generation of virus-specific cytotoxic T cells. For cooperation between mature T cells and other lymphocytes to be functional in chimeras, thymic epithelial cells and lympho-hemopoietic stem cells must share the *I* region; killer T-cell generation also requires in addition compatibility for at least one *K* or *D* region.

These conclusions derive from the following experiments: A → (A × B)F_1 chimeric lymphocytes do produce virus-specific cytotoxic T-cell activity for infected A but not for infected B cells; when sensitized in an acutely irradiated and infected recipient (A × B)F_1 these chimeric lymphocytes respond to both infected A and B. Therefore the predominantly immunogenically infected cells of chimeras are the radiosensitive and by donor stem cells replaced lymphoreticular cells. In this adoptive priming model ($K^AI^A|D^B \rightarrow K^AI^A|D^C$) chimeric lymphocytes could be sensitized in irradiated and infected F_1 against K^A and D^C but not against infected D^B targets. In contrast $K^BI^B|D^A \rightarrow K^CI^C|D^A$ chimeras' lymphocytes could not be sensitized at all in appropriately irradiated and infected F_1 recipients. Thus these latter chimeras probably lack functional *I*-specific T helper cells that are essential for the generation of T killer cells against infected *D* compatible targets. If T cells learn in the thymus to recognize *H-2I* or *K*, *D* markers that are not at least partially carried themselves in other cells of the lymphoreticular system immunological interactions will be impossible and this paradox situation results in phenotypic immune incompetence in vivo.

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