

## H-2 ANTIGENS OF THE THYMUS DETERMINE LYMPHOCYTE SPECIFICITY\*

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The thymus is the site of T-lymphocyte differentiation. Stem cells for this differentiation pathway arise in the yolk sac, fetal liver, and bone marrow, and are carried by the blood stream to the thymus. After acquiring T-cell characteristics, the cells leave the thymus and migrate to the peripheral lymphoid organs. The reactivity of T cells in the periphery is influenced to a great extent by the expression of genes of the major histocompatibility complex (MHC)<sup>1</sup>. The MHC of the mouse is called the H-2 complex, and can be divided into regions which determine cell surface antigens: H-2K and H-2D are expressed on most cells, whereas H-2I antigens are differentiation antigens selectively expressed by certain cell types (1, 2).

It has been possible to show the influence of H-2 on T-cell specificity in three ways: (a) immune response (Ir) genes, (b) alloreactivity, and (c) H-2 restriction of T-cell function. H-2-linked Ir genes determine whether an animal will be a low or high responder to certain antigens. All Ir gene-controlled antigens are T-cell dependent, and the difference between strains is due to the ability or inability of T cells to respond (3, 4). Alloreactivity is a term which refers to the fact that a surprisingly large percentage (1–10%) of T cells proliferate in mixed lymphocyte culture (MLC) in response to stimulating cells expressing a foreign MHC (5). H-2 restriction refers to the phenomenon that after immunization against antigen X, murine T cells turn out to be specific not only for X, but for a self-H-2 antigen as well. In an F<sub>1</sub> mouse, e.g. H-2<sup>b</sup> × H-2<sup>d</sup>, the T cells are specific either for X-plus-H-2<sup>b</sup>, or for X-plus-H-2<sup>d</sup>. Helper T cells for B cells, and the T cells which respond to macrophage-bound antigen have proved to be H-2I region-restricted (6–8). Cytotoxic T lymphocytes (CTL), on the other hand, are H-2K- or D-restricted (9–11). CTL from the F<sub>1</sub> mouse above will lyse only those target cells that express X-plus-K<sup>b</sup>, X-plus-K<sup>d</sup>, X-plus-D<sup>b</sup>, or X-plus-D<sup>d</sup>. Two alternative explanations for H-2 restriction have been proposed. The dual recognition hypothesis, expressed by Katz (12, 13), proposes that each T cell has two receptors, one for self-H-2 and the other for antigen, whereas the "altered-self" hypothesis, suggested by Doherty et al. (10), proposes that T cells have only one receptor specific for a complex of antigen-plus-H-2.

It is valid to ask whether for any particular antigen an animal can respond better to antigen-plus-self-H-2 than to antigen-plus-foreign H-2. If such is the case, it would speak for either a genetic or a learned preference for self. Under natural conditions of immunization, animals are obviously stimulated only by antigen on syngeneic cells. If stimulated by cells bearing foreign non-H-2 and foreign H-2 antigens, animals still make no detectable response specific for the non-H-2 antigen. However, it may be that the large alloreactive response masks the presumed smaller response to non-H-2 antigens plus foreign H-2. That there is no genetic predisposition for self has been suggested by experiments in which H-2 homozygous stem cells (A) were used to repopulate lethally irradiated heterozygous animals (A × B). The strain A T cells that mature can respond to antigen-plus-B in addition to antigen-plus-A (14–17). There is disputed evidence against any learned preference for self also. Some groups have found that a population of mature (peripheral) A strain T cells depleted of clones capable of reacting to strain B can

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<sup>1</sup> Abbreviations used in this paper: ATXBM, adult thymectomized, irradiated, bone marrow-reconstituted mice; Con A, concanavalin A; CTL, cytotoxic T lymphocyte; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; NMS, normal mouse serum.

respond well to strain B cells expressing a new antigen (18, 19). However, other groups have found that this is not the case (20, 21). Arguing strongly in favor of a learned preference for self-H-2 in H-2 restriction are the studies of Bevan (22) and the more complete studies of Zinkernagel et al. (23–25). When H-2 heterozygous A × B stem cells were used to repopulate a lethally irradiated parental A mouse, CTL reactivity was detected only (23–25), or mainly (22) against antigen-plus-A cells, and no or little reactivity to antigen-plus-B was detected. Since the stem cells are heterozygous, this is not a genetic preference for a certain H-2 type, but rather a preference learned in the host environment.

In this study we show by thymus grafting that the thymus is the organ in which this learned preference occurs. Similar experiments and results have been reported (23). That is, F<sub>1</sub>(A × B) T cells that have matured in a homozygous A thymus graft in an otherwise wholly F<sub>1</sub> mouse respond to immunization by producing many more CTL that react with antigen-plus-A than with antigen-plus-B. We have extended this finding to show that the “thymus preference” is probably not due to peripheral suppression of clones capable of reacting with antigen-plus-B. The preference instead seems to reflect a bias in the number of committed precursor cells.

### Materials and Methods

*Mice.* BALB/c (C,H-2<sup>d</sup>), BALB.B (C,B,H-2<sup>b</sup>), the neonatal thymus donors C57BL/10 Sn (B10,H-2<sup>b</sup>) and B10.D2/nSn (H-2<sup>b</sup>), and the F<sub>1</sub> hybrids were all bred at the Center for Cancer Research, Massachusetts Institute of Technology. Strains C and C.B and strains B10 and B10.D2 are congenic pairs differing only in the chromosomal region bearing H-2.

*T-Cell Depleted Mice.* 5–7-wk-old female F<sub>1</sub>(B10 × B10.D2) animals were anesthetized with tribromoethanol, and thymectomized by the suction method of Sjodin et al. (26). 3–5 wk later, the thymectomized animals were administered 800 rads from a <sup>137</sup>Cs source, and reconstituted immediately by intravenous injection of 10<sup>7</sup> viable syngeneic bone marrow cells pretreated with anti-Thy1.2 serum plus complement. Such mice are referred to as ATXBM mice.

*Thymus Grafting.* 1–2 wk after irradiation, ATXBM mice were grafted subcutaneously (under the shoulder) with 1.5–3 lobes of 1-day-old thymuses of one or the other parental type. Some ATXBM animals were left ungrafted to serve as controls in the antiserum treatment and cytotoxicity assays described below. In our hands, grafting by this method is ≈60% successful, as judged by the presence of a healthy graft and by concanavalin A (Con A)-induced proliferation of spleen and lymph node cells.

*In Vivo Priming.* F<sub>1</sub>(B10 × B10.D2) ATXBM mice grafted with B10 or B10.D2 neonatal thymuses were primed ≈9 wk after grafting by intraperitoneal injection of 10<sup>7</sup> F<sub>1</sub>(C × C.B) spleen cells. The animals were therefore immunized against the minor (non-H-2) histocompatibility differences between the B10 and BALB backgrounds (27, 28).

*Adoptive Transfer Immunization.* Approximately 15 wk after grafting, 5–6 × 10<sup>7</sup> viable spleen and lymph node cells from unprimed F<sub>1</sub>(B10 × B10.D2) ATXBM mice that had been grafted with either B10 or B10.D2 thymuses were injected intravenously into irradiated (850 rads) F<sub>1</sub>(B10 × B10.D2) mice. Similarly, 1:1 mixtures (totalling 6 × 10<sup>7</sup> cells) of spleen and lymph node cells from the grafted ATXBM mice and spleen cells from normal F<sub>1</sub> animals were injected into irradiated F<sub>1</sub> mice. All adoptive animals were immunized the following day by intraperitoneal injection of 10<sup>7</sup> F<sub>1</sub>(C × C.B) spleen cells in balanced salt solution.

*MLC and Cytotoxicity Assay.* MLCs were set up with spleen and lymph node cells from ATXBM mice sacrificed ≈11 wk after grafting, and from adoptive hosts ≈3½ wk after injection of responder cells. Cells were cultured only from those ATXBM mice that retained a healthy thymus graft and that were determined by inspection to be completely thymectomized. Cultures were boosted with irradiated (1,000 rads) F<sub>1</sub>(C × C.B) spleen cells, and assayed 5 days later for cytotoxic activity directed against <sup>51</sup>Cr-labeled C, C.B, or F<sub>1</sub>(B10 × B10.D2) 2-day Con A blasts (29). Serial threefold dilutions of responder cells were added to a constant number of labeled targets, and the percent specific lysis after 4 h of incubation was calculated in the following manner:

$$\left( \frac{\text{cpm released in the presence of responders} - \text{cpm spontaneously released}}{\text{total cpm} - \text{cpm spontaneously released}} \right) \times 100.$$

*Treatment with Antisera and Complement.* Anti-Thy1.2 serum was prepared by immunizing AKR/J mice with C3HeB/FeJ thymocytes as described previously (30). F<sub>1</sub>(C3H × DBA/2) anti-BALB.B (anti-H-2<sup>b</sup>) serum was prepared and absorbed with a mixture of BALB/c and BALB.K lymphoid cells. Anti-H-2<sup>d</sup> serum was BALB.B anti-B10.D2-absorbed with B10 cells. 3-day Con A-induced blasts of spleen and lymph node cells from normal mice and from grafted and ungrafted ATXBM mice were labeled with <sup>51</sup>Cr and treated with normal mouse serum (NMS) or serial dilutions of anti-Thy1.2, anti-H-2<sup>b</sup>, or anti-H-2<sup>d</sup> serum followed by agarose-absorbed guinea pig complement. Aliquots of responder cells were treated on day 5 of MLC with antisera followed by guinea pig or rabbit complement, before assaying cytotoxic activity against the appropriate target cells. The activity of antiserum-treated cells relative to NMS-treated cells was estimated according to the following formula:

$$\text{percent relative activity} = \left( \frac{\text{responder: target ratio of untreated cells giving same lysis as antiserum-treated cells}}{\text{responder: target ratio of untreated cells giving same lysis as NMS-treated cells}} \right) \times 100.$$

## Results

*Testing the ATXBM Mice.* Spleen and lymph node cells from F<sub>1</sub>(B10 × B10.D2) ATXBM mice that had been grafted with either a B10 or a B10.D2 thymus and immunized with F<sub>1</sub>(C × C.B) spleen cells were cultured in the presence of the T-cell mitogen Con A. Treated in the same manner were cells from a nongrafted ATXBM mouse and a normal F<sub>1</sub>(B10 × B10.D2), both of which had been previously primed for minor histocompatibility antigens with F<sub>1</sub>(C × C.B) spleen cells. To determine whether transplantation of neonatal thymuses resulted in co-transfer of contaminating parental T cells, <sup>51</sup>Cr-labeled 3-day Con A blasts were treated with either NMS, anti-Thy1.2, anti-H-2<sup>b</sup>, or anti-H-2<sup>d</sup> serum and complement, using <sup>51</sup>Cr release as a measure of the extent of lysis. In the normal F<sub>1</sub> control, anti-Thy1.2 and guinea pig complement released 86% of the <sup>51</sup>Cr released by anti-H-2<sup>d</sup> plus complement, with 79 and 75% relative anti-Thy1.2 killing of blasts from the B10-grafted and B10.D2-grafted mice, respectively. These percentages indicate that co-culturing with Con A resulted in the predicted enrichment for T cells. If contaminating parental T cells were present in significant numbers in the grafted mice, cells from the recipients of a B10 thymus would have been killed to a greater extent than normal F<sub>1</sub> cells by anti-H-2<sup>b</sup> relative to anti-H-2<sup>d</sup> serum and vice versa for the cells from mice with a B10.D2 thymus. However, there was no significant difference in anti-H-2<sup>b</sup> and anti-H-2<sup>d</sup> killing of Con A blasts from grafted ATXBM and normal mice. Thus, it appears that most of the T cells from these mice are of F<sub>1</sub> origin.

*Cytotoxicity Assay.* Cells from normal F<sub>1</sub> mice and grafted and ungrafted ATXBM mice were cultured in the presence of irradiated F<sub>1</sub>(C × C.B) spleen cells as stimulators. After 5 days in MLC, cells were assayed for cytotoxic activity on <sup>51</sup>Cr-labeled C, C.B, and syngeneic target cells. To determine the H-2 type of the effector cells, aliquots of 1–2 × 10<sup>6</sup> responder cells were removed for antiserum treatment, before target cell addition. The data in Fig. 1 reveal that the ATXBM mouse grafted with a B10 thymus killed C.B target cells ≈80 times more efficiently than C target cells. Similarly, the B10.D2-grafted mouse killed C target cells ≈15 times more efficiently than C.B. In the same experiment, normal F<sub>1</sub> cells primed in vivo and

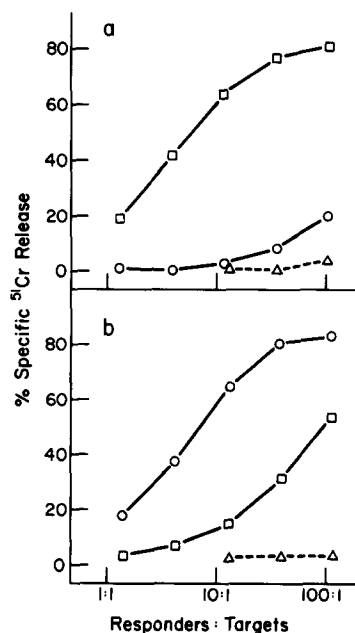


FIG. 1. H-2 restriction of CTL from  $F_1$  mice with homozygous thymus grafts. CTL from an  $F_1$ (B10  $\times$  B10.D2) ATXBM mouse grafted with (a), a B10 or (b), a B10.D2 thymus and immunized in vivo and in vitro with  $F_1$ (C  $\times$  C.B) cells.  $^{51}\text{Cr}$ -labeled target cells of the following types were used: C.B, (□, spontaneous release 23%); C, (○, spontaneous release 23%); and  $F_1$ (B10  $\times$  B10.D2), (△, spontaneous release 18%). In the same experiment, a normal  $F_1$ (B10  $\times$  B10.D2) mouse gave equal lysis of C.B and C target cells; 47% lysis of C.B and 48% lysis of C at a ratio of 10:1.

boosted in vitro with  $F_1$ (C  $\times$  C.B) cells killed both targets equally well. No cytotoxic activity was detected in the cultures made from the ungrafted ATXBM control, although treatment of Con A blasts with anti-Thy1.2 serum released  $\approx 42\%$  of the  $^{51}\text{Cr}$  released by anti-H-2<sup>d</sup> serum. The  $F_1$  nature of the effector cells from B10- and B10.D2-grafted mice is revealed by treatment with anti-H-2<sup>b</sup> and anti-H-2<sup>d</sup> sera (Table Ia). Thus, CTL derived from  $F_1$  stem cells maturing in a parental thymus exhibit a marked preference for targets bearing minor antigens and the thymic type H-2 antigens.

*Adoptive Transfer.* To test whether this target cell preference is due to the presence of suppressor cells capable of depressing a killer cell response to nonthymic H-2-type target cells, the following experiment was performed. Spleen and lymph node cells from normal  $F_1$ (B10  $\times$  B10.D2) and ATXBM mice bearing a homozygous thymus were mixed in a 1:1 ratio and injected intravenously into irradiated (850 rads)  $F_1$  hosts. The adoptive animals were primed with  $F_1$ (C  $\times$  C.B) spleen cells by intraperitoneal injection the following day, and were sacrificed 3–4 wk later. Determination of cytotoxic activity and H-2 type of the adoptively transferred cells was carried out as described above, with the exception that rabbit serum was substituted for guinea pig serum as the source of complement. As can be seen from Fig. 2d, the killing of C targets displaying H-2<sup>d</sup> plus minor antigens by adoptively transferred cells from a B10.D2-grafted ATXBM mouse was  $>200$ -fold that of C.B targets. The preference of adoptively transferred cells from a B10-grafted mouse for C.B over C targets appears absolute (Fig. 2b). Fig. 2c and e demonstrate that the cytotoxic activity of spleen and

TABLE I  
*The Effect of Treatment with Anti-H-2 Serum and Complement  
 on the Ability of CTL to Mediate Target Lysis\**

Source of cytotoxic cells	Treatment‡	Target	Specific lysis	Activity
			at maximum responder: target ratio	relative to NMS control
			%	%
(a) Normal F <sub>1</sub> (B10 × B10.D2)	NMS	BALB/c	56	100
	anti-H-2 <sup>b</sup>		30	29
	anti-H-2 <sup>d</sup>		18	13
Normal F <sub>1</sub> (B10 × B10.D2)	NMS	BALB.B	63	100
	anti-H-2 <sup>b</sup>		32	21
	anti-H-2 <sup>d</sup>		16	7
ATXBM with B10 thymus	NMS	BALB.B	67	100
	anti-H-2 <sup>b</sup>		28	13
	anti-H-2 <sup>d</sup>		20	9
ATXBM with B10.D2 thymus	NMS	BALB/c	72	100
	anti-H-2 <sup>b</sup>		33	10
	anti-H-2 <sup>d</sup>		18	7
(b) Normal F <sub>1</sub> (B10 × B10.D2)	NMS	BALB/c	49	100
	anti-H-2 <sup>b</sup>		9	4
	anti-H-2 <sup>d</sup>		8	4
Normal F <sub>1</sub> (B10 × B10.D2)	NMS	BALB.B	43	100
	anti-H-2 <sup>b</sup>		5	13
	anti-H-2 <sup>d</sup>		5	13
ATXBM with B10 thymus	NMS	BALB.B	21	100
	anti-H-2 <sup>b</sup>		3	12
	anti-H-2 <sup>d</sup>		4	13
ATXBM with B10.D2 thymus	NMS	BALB/c	56	100
	anti-H-2 <sup>b</sup>		23	6
	anti-H-2 <sup>d</sup>		20	5

\* Cytotoxic cells immunized against F<sub>1</sub>(C × C.B) cells. In (a) the cells were immunized conventionally (see Fig. 1), in (b) they were immunized in adoptive transfer (see Fig. 2).

‡ In (a) sera were used at a dilution of 1:3 and guinea pig complement at 1:9. In (b) sera were used at 1:6 and a selected rabbit serum at 1:15.

lymph node cells derived from adoptive hosts injected with mixtures of cells from normal and grafted mice fell within the range for normal F<sub>1</sub> cells, with approximately equal killing of both C and C.B targets. The antisera treatments (data in Table Ib) demonstrate that responder cells exhibiting "non-F<sub>1</sub>-like" target cell preference were actually F<sub>1</sub> in origin. Thus, it appears that the "training" of F<sub>1</sub> stem cells in terms of preference for target cells displaying the thymic H-2 type is not due to the presence of cells which suppress the activity of cells otherwise capable of responding to antigens in association with the nonthymic H-2 type.

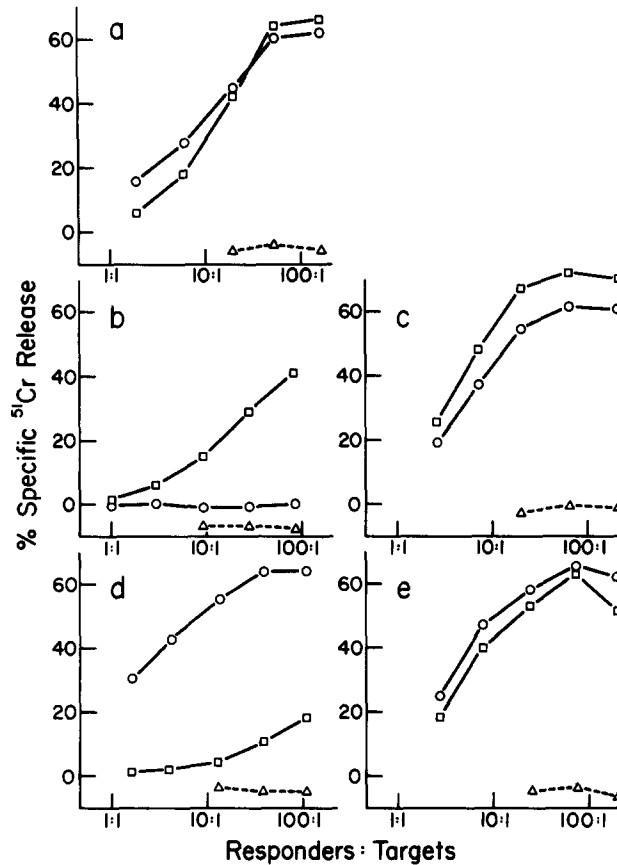


FIG. 2. Evidence against a suppressor mechanism in the thymus-grafted mice. (a), Cytotoxic activity of lymphoid cells from F<sub>1</sub>(B10 × B10.D2) adoptive hosts 25 days after irradiation and injection of  $5-6 \times 10^7$  lymphoid cells from a normal F<sub>1</sub> mouse. Cells were primed in the adoptive hosts and boosted in culture with F<sub>1</sub>(C × C.B) spleen cells. <sup>51</sup>Cr-labeled targets were: C.B. (□, spontaneous release 14%); C, (○, spontaneous release 10%); and F<sub>1</sub>(B10 × B10.D2), (△, spontaneous release 15%). (b), As in (a) with adoptive transfer of cells from an ATXBM mouse grafted with a B10 thymus. (c), As in (a) with adoptive transfer of a 1:1 mixture of cells from a normal F<sub>1</sub> and the same B10-grafted ATXBM mouse used as a donor in (b). (d), As in (a), with adoptive transfer of cells from a B10.D2-grafted ATXBM mouse. (e), As in (a) with adoptive transfer of a 1:1 mixture of cells from a normal F<sub>1</sub> and the same B10.D2-grafted ATXBM mouse used as a donor in (d).

### Discussion

The simplest interpretation of the results presented here and elsewhere (22-25) is that the H-2 type of the thymus determines the specificity of T cells which mature there and go on to make up the peripheral T-cell pool. Thus, in a normal A × B mouse, where T cells mature in an A × B thymus, about half of the peripheral CTL are restricted to seeing antigen-plus-H-2<sup>B</sup>. On the other hand, an F<sub>1</sub> ATXBM mouse with a grafted A thymus has a large preponderance of CTL restricted to recognizing antigen-plus-H-2<sup>A</sup>. We do not favor the more complicated interpretations of these results, which involve H-2-specific suppression or help. A suppressor model, for example, might predict that the peripheral CTL population in an F<sub>1</sub> mouse with a homozygous A thymus is very similar to that in a normal F<sub>1</sub>, but the cells with

receptors for B-plus-X are prevented from responding by suppressor cells which recognize determinants on an anti-B receptor. Such suppressor cells might have been induced in the thymus when maturing T cells expressed an anti-B receptor. Indeed, we tested for a suppressor mechanism in the thymus-grafted mice (Fig. 2), and found no evidence for its existence. As yet, very little is known about how this thymic "tutoring of self" is achieved. We do know that the cells that are responsible for this training can be transferred experimentally by thymus grafting alone, and therefore reside in the thymus. In addition, data indicating that this "tutoring" occurs in radiation chimeras (22-25) and in animals receiving irradiated thymus grafts (23) reveal that these cells are radiation-resistant.

Radiation chimeras in which heterozygous stem cells have been forced to mature in a parental thymus ( $A \times B \rightarrow A$ ) have been shown by Zinkernagel et al. (23-25) to respond only to virus-plus-A and not at all to virus-plus-B. Most of our ( $A \times B \rightarrow A$ ) chimeras have responded weakly to antigen-plus-B (22 and this paper). But these chimeras were all constructed with T-cell-depleted bone marrow as a source of stem cells, and in line with the work of Zinkernagel et al. (23-25), we have found recently that 3/7 ( $A \times B \rightarrow A$ ) animals made with fetal liver stem cells had no detectable reactivity with antigen-plus-B. Furthermore, with bone marrow ( $A \times B \rightarrow A$ ) chimeras, it has been shown in restimulation experiments that the CTL reactive with antigen-plus-B are not a subset of the cells reactive to the same antigen-plus-A (P. Fink and M. Bevan, unpublished observations). These observations underline the possibility that committed T cells may contaminate bone marrow preparations treated with anti-Thy1.2 plus complement, and account for the weak reactivity we detect against antigen-plus-B.

An absolute preference to react only with antigen-plus-self-H-2 (where self H-2 is defined by resident cells in the thymus) can be taken as supporting a dual recognition model for CTL specificity (23-25). According to this model, stem cells are selected for on the basis of expression of an anti-self receptor and they retain this receptor when they become mature peripheral cells. The alternative model proposes that stem cells are selected for on the basis of anti-self reactivity in the thymus, but this reactivity with pure self is lost before the cells mature and populate the secondary lymphoid organs. As Zinkernagel et al. have pointed out (23), it is difficult to explain by the latter model why in an  $F_1(A \times B)$  stem cell population maturing in an A-type thymus, a repertoire of receptors derived from anti-A receptors should not react at all with X-plus-B. Even adhering to the two-receptor model, it is not unreasonable to expect some anti-self A receptors to cross-react to some extent with self B. Incorporated into this model must be some means whereby the cross-reactive receptors are not expressed or the cells bearing them are either specifically removed or are simply not selected for in the thymus.

Whichever model for CTL specificity is more correct, the original selection in the thymus for cells which express anti-self receptors seems to determine the antigen-binding specificity of the mature T-cell population. This relationship is well illustrated in the proliferative T-cell response to insulin studied by Rosenthal et al. (31) and Barcinski and Rosenthal (32). In this system, strains A and B and their  $F_1$  cross respond to insulin. But the  $F_1$  T-cell response to insulin on A macrophages involves binding a site on the insulin molecule different from that in the response to insulin-plus-B. With antigens under MHC-linked Ir gene control, it has generally been found

that T cells from  $F_1$  (nonresponder  $\times$  responder) animals respond only when the Ir gene-controlled antigen is presented on responder cells and not when it is presented on nonresponder cells (33, 34). Another remarkable example is the mouse T-cell response to the terpolymer glutamine-lysine-phenylalanine. In a cross between two nonresponders, the  $F_1$  T cells respond only to antigen on  $F_1$  macrophages and not to antigen on macrophages of either parental type (35). In each of these cases, the T-cell binding activity for antigen seems to be dependent upon its binding activity for self H-2. Findings such as these can be readily explained if T cells have one receptor that was originally picked out of the repertoire on the basis of binding self and then mutated to a receptor capable of binding self-plus-X. But again, they could be encompassed in a model involving two separate receptors if at the level of the antigen-presenting cell there is a requirement for H-2 and the foreign antigen to physically interact, such that insulin on a B-strain cell, for example, oriented differently from insulin on A-strain cells.

The issue of whether mature T cells do or do not have anti-self receptors is as yet unresolved. The evidence that has been accumulated so far can be interpreted, either with ease or with more difficulty, according to either the one- or two-receptor model for CTL specificity.

### Summary

After immunization, normal H-2 heterozygous mice (for example  $H-2^b \times H-2^d$ ) generate two populations of cytotoxic effector T cells, one specific for target cells expressing  $H-2^b$ -plus-antigen and the other specific for  $H-2^d$ -plus-antigen. With a multideterminant antigen, these two populations have about the same activity. We show here that the H-2 type of resident cells in the thymus determines the H-2 preference of cytotoxic T lymphocytes.  $F_1$ (B10  $\times$  B10.D2) ( $H-2^b \times H-2^d$ ) mice were thymectomized, lethally irradiated, and reconstituted with T-cell-depleted syngeneic hematopoietic cells. Groups of such ATXBM mice were grafted subcutaneously with neonatal thymus lobes from parental mice, either B10 ( $H-2^b$ ) or B10.D2 ( $H-2^d$ ). 2-3 mo later, the mice were immunized against the minor histocompatibility antigens on  $F_1$ (BALB/c  $\times$  BALB.B) cells and assayed for cytotoxic T-cell activity.  $H-2^b \times H-2^d$  ATXBM mice with  $H-2^b$  thymus grafts responded to antigen-plus- $H-2^b$  much better than to antigen-plus- $H-2^d$ , and vice versa for the mice with  $H-2^d$  thymus grafts. As judged by antiserum treatment, the effector cells were of  $F_1$  origin.

To explore the possibility that the "thymus preference" may have been due to suppression of T-cell activity, nonimmune spleen and lymph node cells from normal  $H-2^b \times H-2^d$  mice and cells from  $H-2^b \times H-2^d$  mice bearing a homozygous thymus were mixed 1:1 and immunized in adoptive transfer. The mixture responded to antigen-plus- $H-2^b$  and antigen-plus- $H-2^d$  equally well, demonstrating that the cells that showed a "thymus preference" could not suppress a response to antigen in association with the nonthymic H-2 type. We conclude from these and other experiments that H-2 antigens present on resident cells of the thymus determine the spectrum of specificity of T cells which mature in that thymus and eventually make up the peripheral T-cell pool.

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