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Immunology

T cells respond preferentially to antigens that are similar to self H-2

(alloreactivity/thymus/major histocompatibility complex)

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ABSTRACT **We have constructed bone marrow irradiation chimeras to investigate the influence of self antigens on the specificity of the T lymphocyte receptor repertoire. Bone marrow cells from (A × B)F_1 mice heterozygous for the major histocompatibility genes were allowed to mature into T cells in irradiated parent A or parent B strains. More than 8 weeks after irradiation, when the lymphoid system had regenerated from the F_1 stem cells, the degree of T cell reactivity to mutant major histocompatibility antigens, A′, was assessed. It was found that T cells that had matured in the irradiated A mice, [F_1 → A] chimeras, responded better to A′ antigen than did T cells from the [F_1 → B] chimeras. Because the mutant histocompatibility antigen A′ is very similar in structure to A, differing only by one or a few residues, this suggests that the T cell repertoire in [F_1 → parent] chimeras reacts preferentially with foreign antigens that are slight variants of the self antigens expressed on radiation-resistant cells—probably cells in the thymus.**

The major histocompatibility complex (MHC) codes for cell membrane glycoproteins, which are recognized by thymus-derived (T) lymphocytes during their induction and the performance of their effector function. The classical MHC transplantation antigens expressed on all adult cells, exemplified by the murine H-2 K and D and the human HLA-A and B antigens, are involved in cytotoxic T lymphocyte (CTL) recognition. Differentiation antigens, which are coded by the *I* region in mice and the *D* region in humans, are expressed mainly on B cells and macrophages and are recognized by helper T cells (1, 2). Thus, in the mouse H-2 K and D antigens are said to restrict the recognition of antigen by CTL. CTL from an H-2^b (K^bD^b) mouse immunized with antigen X recognize X-plus-self H-2 antigens and do not recognize the same antigen on cells with different K and D alleles—e.g., *H-2*^{d} or *H-2*^{k} cells. The phenomenon of the H-2 restriction of T-helper and T-killer function came to light in the years 1973 and 1974 (reviewed in refs. 1–3). This series of discoveries was a significant breakthrough in our understanding of the function of the MHC.

Experiments with hematopoietic radiation chimeras suggest that the population of mature T cells has a preference to react with X-plus-self H-2 antigens—i.e., fewer clones of T cells can respond to X-plus-foreign H-2 than to X-plus-self H-2. This conclusion from the chimera experiments was reached and made possible because the H-2 antigens that act as self H-2 are not those expressed by lymphoid cells but are the ones expressed by the radiation-resistant thymic epithelial cells (4–8). For example, when H-2 heterozygous *b/d* stem cells mature in an irradiated parental *b* mouse ([F_1 → parent] chimera), then most of the maturing T cells become b-restricted and very few become d-restricted. A normal heterozygous *b/d* animal usually generates equal numbers of b-restricted and d-restricted T cells in response to any complex antigen. Yet if the only site for T cell maturation is a grafted, homozygous *b/b* thymus, then most of the T cells are b-restricted. Such positive selection for the restriction specificity of T cells by the H-2 antigens on the resident thymus cells was interpreted as supporting the idea that T cells have two separate binding sites on their surface, one for self-H-2 and another for foreign antigen (6, 9). According to this idea, T stem cells expressing anti-self MHC receptors in the thymus would be selected and the anti-antigen (anti-X) receptor would be expressed independently. The thymic selection of T cell specificity could also be consistent with the altered-self hypothesis, which predicts that MHC-restricted T cells have one receptor that is specific for a complex of MHC (self)-plus-X antigens. In this model, exposure to self (thymic) H-2 drives the T cell repertoire to a state in which there is no demonstrable reactivity with pure self, but a high reactivity with self H-2-plus-X—i.e., modified-self or altered-self. The likelihood that the thymic selection of the H-2 restriction specificity is preferential rather than an absolute self-restriction is certainly consistent with the latter notion.

Jerne suggested in 1971 (before the discovery of H-2 restriction or thymic selection) that the T cell receptor repertoire was selected by the reactivity with self MHC antigens (10). An initial positive selection of T stem cells expressing anti-thymic MHC was followed by proliferation and mutation of these cells and by allowing only those mutants of these anti-self clones that lost strong reactivity to self to leave the thymus. According to this hypothesis one would certainly expect T cell receptors to be grossly influenced by self H-2 antigens and possibly that T cells would react better with antigens that could be considered slight variants of the selecting self H-2 antigens. Here we present data that support this notion. We show that F_1 (H-2^b/H-2^d) T cells from [F_1 → H-2^b] chimeras respond better to H-2^b mutant stimulator cells than do T cells from [F_1 → H-2^d] chimeras.

MATERIALS AND METHODS

Mice. C57BL/10 (B10, H-2^b), B10.D2 (H-2^d), C57BL/6 By (B6, H-2^b) mice, and two strains that carry *H-2K*^{b} mutations, B6.C-H-2^{bm1} (previously called H-2^{ba}) and B6.C-H-2^{bm4} (previously H-2^{bf}), were purchased from The Jackson Laboratory. BALB/cAn (H-2^d), BALB.HTG (HTG, H-2^g), B10.BR/SgSn (H-2^k), and B10.A(3R) (3R, H-2^{i3}) mice were maintained at our animal facility. The F_1 mice, (B10 × B10.D2) and (BALB/c × H-2^{bm4}), were also bred at Massachusetts Institute of Technology.

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Abbreviations: CTL, cytotoxic thymus-derived lymphocyte; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; TNP, 2,4,6-trinitrophenyl.

Radiation chimeras were made by using H-2^b/H-2^d hematopoietic stem cells to reconstitute lethally irradiated H-2 homozygous mice. Bone marrow was removed from the tibias and femurs of normal (B10 × B10.D2)F_1 mice and the cells were suspended in Hanks' balanced salt solution. To selectively kill T cells the bone marrow cells were treated with a mixture of hybridoma-derived anti-Thy-1 antibodies followed by rabbit serum as a source of complement. The monoclonal anti-Thy-1 antibodies were T24 (11) and 13-4 (12). Ten to 20 × 10^6 surviving bone marrow cells were injected into mice that had been irradiated [900–950 rad from a ^{137}Cs source (1 rad = 0.01 gray)] 2–4 hr previously. The irradiated host mice were B10, B10.D2, 3R, or HTG strains. At the time of sacrifice (at least 8 weeks after irradiation) pooled spleen and lymph node cells from the chimeras were typed with anti-H-2 serum plus complement: (C3H × DBA/2)F_1 anti-BALB.B sera (anti-H-2^b), absorbed with B10.D2 cells and (BALB.B × B10.BR)F_1 anti-B10.D2 (anti-H-2^d) sera were prepared in this laboratory. (A × B10)F_1 anti-B10.D2 (anti-K^d) and (A × B10.D2)F_1 anti-B10.A(5R) (anti-K^b) sera were generously provided by Kathy Wall and H. N. Eisen and were used to distinguish (B10 × B10.D2)F_1 cells from 3R and HTG cells, respectively. In all cases the cells from the chimeras were over 91% of donor F_1 type.

Mixed Lymphocyte Cultures (MLC). These cultures for the generation of CTL were set up as described (5). Briefly, 18–25 × 10^6 spleen and lymph node cells from unimmunized mice were cultured in 20 ml of RPMI 1640 medium with 25 × 10^6 stimulator cells that had received 1000 rads of irradiation. Cultures were incubated for 5 days at 37°C in an atmosphere of 5% CO_2 in air.

Assay of Cytotoxic Activity. Surviving MLC cells were harvested after 5 days of culture and washed, and serial 1:3 dilutions of them were assayed for lysis of target cells (5). Target cells were spleen cells from various mice that had been cultured with the T cell mitogen concanavalin A for 2–3 days, then washed with α-methyl mannoside and labeled with sodium [^{51}Cr]chromate. Labeled target cells for the experiment in Fig. 3 were modified by suspension in 10 mM 2,4,6-trinitrobenzenesulfonate in phosphate-buffered saline for 10 min at room temperature, which attached 2,4,6-trinitrophenyl (TNP) groups. The assay was performed in a final volume of 1 ml of medium in 12 × 75 mm plastic tubes that were incubated upright for 4 hr at 37°C. Percent specific lysis was calculated as 100 × [(cpm released with CTL − cpm released alone)/(total cpm − cpm released alone)].

For the data in Table 2, 1 lytic unit was defined as the number of effector cells required to give 25% specific lysis of 4 × 10^4 target cells in 4 hr. The number of lytic units per culture (25 × 10^6 responders) was calculated (13).

RESULTS

Theory of the Experiments. The mutant K^b antigens differ in one or a few amino acid residues from the wild-type K^b antigen (14, 15), and are rather similar to wild type when analyzed by serology (15) or by third-party CTL responses (16, 17). The presence of K^b versus K^d on the thymic cells could influence the degree of reactivity of the mature CTL to K^b mutants; if the repertoire is selected to react with variants of self, then K^b-as-self should lead to greater reactivity than would K^d-as-self. In order to study this, (B10 × B10.D2)F_1 bone marrow cells were allowed to mature into CTLs in hosts of the various genotypes shown in Table 1. If the anti-K^b mutant repertoire of the F_1 CTL does derive largely from K^b-as-self, then reactivity to the mutants is expected to be high in B10 and 3R hosts and low in B10.D2 and HTG hosts (Table 1).

Table 1. Haplotypes of the mice used as chimera hosts*

Strain	H-2 haplotypes	Origin of *H-2* regions						
			I					
		K	*A*	*B*	*J*	*E*	*C*	*D*
B10	b	*b*	*b*	*b*	*b*	*b*	*b*	*b*
B10.D2	d	*d*	*d*	*d*	*d*	*d*	*d*	*d*
B10.A(3R)	i3	*b*	*b*	*b*	*b*	*k*	*d*	*d*
BALB.HTG	g	*d*	*d*	*d*	*d*	*d*	*d*	*b*

* Taken from ref. 18.

Response of Chimera Cells to H-2K^b Mutant Stimulator Cells. The data of representative experiments presented in Figs. 1 and 2 and Table 2 show that a high level of response to K^b mutants does correlate with the presence of K^b in the irradiated host. The CTL response to a third-party haplotype, H-2^k, does not vary in a reproducible fashion with cells from the various chimeras. In other experiments not shown here, responsiveness to H-2^s stimulator cells also did not vary signifi-

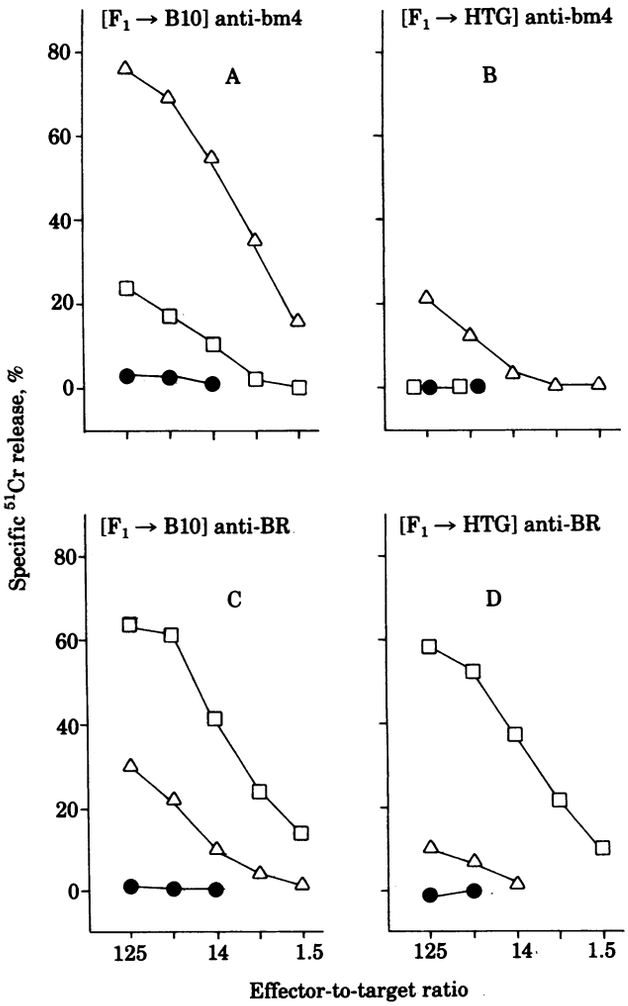

FIG. 1. Comparison of the CTL response of [F_1 → B10] and [F_1 → HTG] chimera cells to H-2^{bm4} and H-2^k stimulator cells. Spleen and lymph node cells from 61-day-old chimeras, [F_1 → B10] (*A* and *C*) and [F_1 → HTG] (*B* and *D*), were stimulated for 5 days in MLC with irradiated B6.C-H-2^{bm4} cells (*A* and *B*) or B10.BR cells (*C* and *D*). Cytotoxic activity was assayed on targets from B6.C-H-2^{bm4} (△), B10.BR (□) and B6 (●) mice. Spontaneous release of ^{51}Cr from the targets varied between 15% and 19%. All cytotoxic activity was sensitive to complement-mediated lysis by sera specific for F_1 cells.

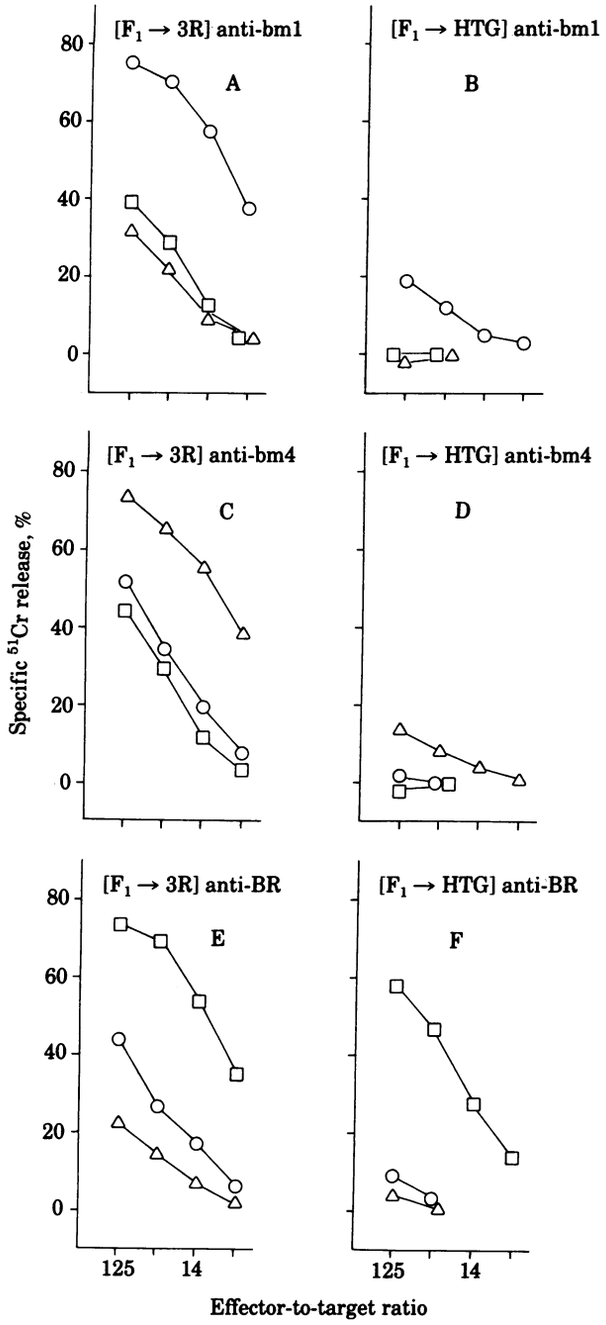

FIG. 2. Comparison of the CTL response of [F_1 → 3R] (*A*, *C*, and *E*) and [F_1 → HTG] (*B*, *D*, and *F*) chimera cells to H-2^{bm1}, H-2^{bm4}, and H-2^k stimulator cells. Spleen and lymph node cells from 53-day-old chimeras were stimulated for 5 days in MLC with irradiated B6.C-H-2^{bm1}, (*A* and *B*) B6.C-H^{bm4} (*C* and *D*), or B10.BR (*E* and *F*) spleen cells. CTL activity was assayed against targets from B6.C-H-2^{bm1} (○), B6.C-H-2^{bm4} (△), and B10.BR (□) mice. Spontaneous lysis of the targets varied between 16% and 18%. All cytotoxic activity was mediated by F_1 T cells.

cantly. For example, [F_1 → B10] and [F_1 → HTG] cells mounted a similar cytotoxic response to B10.BR (H-2^k) cells (Fig. 1 *C* and *D*); however, the response to H-2^{bm4} cells by the [F_1 → B10] (Fig. 1*A*) was much greater than the response of the [F_1 → HTG] cells (Fig. 1*B*). Similarly Fig. 2 shows that [F_1 → 3R] is a high responder to H-2^{bm1} and H-2^{bm4} cells compared to [F_1 → HTG]. These results are consistent with the notion that CTLs that restrict to K^b as a self H-2 antigen in the thymus are more likely to give rise to progeny that score as anti-K^b mutant reactive cells than are CTLs that see K^d, D^b, or D^d as self H-2 antigens.

Table 2. Cytotoxic response of chimera cells to (H-2^d × H-2^{bm4})F_1 stimulators

Cytotoxic cells	Lytic units on target cells		
	B6	B10.BR	H-2^{bm4}
[F_1 → B10] anti-(H-2^d × H-2^{bm4})	0	ND	18
[F_1 → B10] anti-BR	0	27	2.7
[F_1 → B10.D2] anti-(H-2^d ×H-2^{bm4})	0	ND	6.0
[F_1 → B10.D2] anti-BR	0	71	< 1.0

Spleen and lymph node cells from [F_1 → B10] and [F_1 → B10.D2] chimeras were stimulated for 5 days in MLC with irradiated (BALB/c × H-2^{bm4}) F_1 or B10.BR cells. Cytotoxic activity was assayed on concanavalin A-stimulated blasts and quantitated as described (13). ND, not determined.

As an alternative to this explanation, which is based only on CTL precursor frequency, one could propose that the high level of response to H-2^b mutant cells depends on learning I^b (*IA*, *IB*, or *IJ*) as self in the thymus. Helper T cells are restricted by the *I* region of the host thymus (7, 8, 19), and such helpers may determine the level of the CTL response to K^b-mutant stimulator cells. The mice that treat I^b as self ([F_1 → B10] and [F_1 → 3R]) could, according to this hypothesis, make a better helper cell response to K^{bm4} I^b D^b stimulator cells than mice that have an I^d thymus. There are at least two strong arguments against this explanation of our results:

(*i*) As is apparent in Fig. 1 *C* and *D*, Fig. 2 *E* and *F*, and Table 2, it is not necessary to stimulate the chimera cells with H-2^b mutant cells to detect the differences in CTL-mediated lysis of the mutants. Stimulation with totally allogeneic H-2^k (or H-2^s) cells gives some crossreactive lysis of H-2^{bm4} and H-2^{bm1} targets. The cross-reactivity of the high-responder chimeras is again at least 5-fold higher than the crossreactivity shown by the low-responder chimeras. Table 2 shows a clear example of this. The [F_1 → B10] anti-H-2^k response gave 10% crossreactive lysis on H-2^{bm4} targets, whereas the [F_1 → B10.D2] anti-H-2^k CTL crossreacted less than 2% on the mutant (Table 2). Because helper cells are not involved in the 4-hr cytotoxic assay, this argues against the helper cell explanation.

(*ii*) We used (H-2^d × H-2^{bm4})F_1 mice as donors of stimulator cells in MLC. The difference in the CTL response to K^{bm4} was still evident in comparing [F_1 → B10] and [F_1 → B10.D2] chimeras (Table 2). In another experiment [F_1 → HTG] cells were also low responders to these F_1 stimulator cells (data not shown). Because these stimulator cells express both I^d and I^b, the variation in *I*-region restriction of the chimera helper cells cannot be responsible for the differences in the magnitude of the reaction. We therefore feel that the most probable explanation is that T cells that become restricted to K^b are more likely to react strongly against K^b mutant antigens than are CTLs that are restricted to K^d.

Anti-Mutant Crossreactivity on TNP-Modified Self. CTL from normal (B10 × B10.D2)F_1 mice make a large alloreactive response to H-2^{bm4} (and H-2^{bm1}) stimulator cells. We predicted that most of the (H-2^b/H-2^d)F_1 anti-K^{bm4} CTL clones would be restricted to H-2K^b. Therefore, such alloreactive anti-mutant CTL and control (H-2^b/H-2^d)F_1 anti-H-2^k CTL were tested for crossreactive lysis of TNP-modified and unmodified B10, B10.D2, and 3R targets (Fig. 3). F_1 anti-H-2^k CTL crossreacted to about the 10% level on all three TNP-modified targets. The F_1 anti-K^{bm4} CTL response crossreacted more on TNP-B10 (K^bD^b) and TNP-3R (K^bD^d) targets than on TNP-B10.D2 (K^dD^d) targets. Thus the bulk of the TNP-specific crossreaction in the

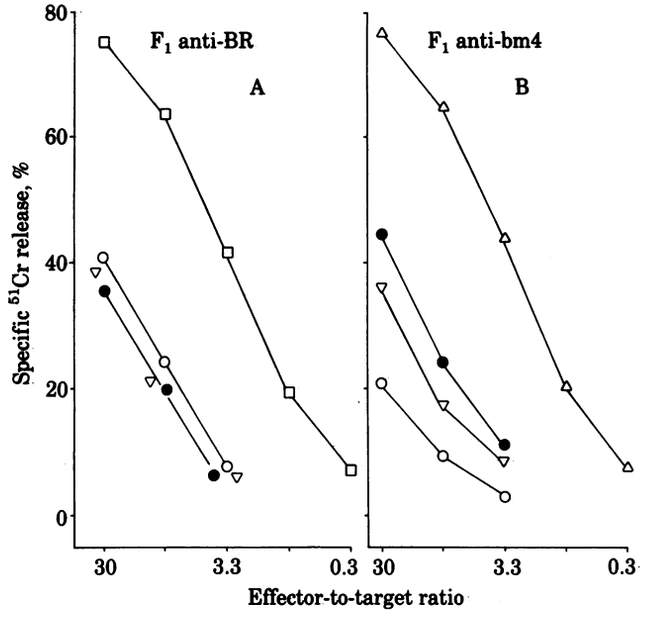

FIG. 3. Crossreactive lysis of TNP-modified self target cells by CTL from normal H-2^b/H-2^d mice generated against H-2^k (*A*) or H-2^{bm4} (*B*) stimulator cells. Spleen and lymph node cells from normal (B10 × B10.D2)F_1 mice were stimulated in MLC for 5 days with irradiated B10.BR or B6.C-H-2^{bm4} cells. CTL activity was assayed against B10.BR (□), B6.C-H-2^{bm4} (△), TNP-B10 (●), TNP-B10.D2 (○), and TNP-3R (▽) target cells. Spontaneous lysis of the targets varied between 15% and 32%. Lysis of unmodified target cells was less than 10% at the highest effector-to-target ratio.

anti-K^{bm4} response appears to be restricted to K^b. The results in Fig. 3 suggest that there is a specific deficit in H-2^d-restricted activity (as measured on TNP-modified B10.D2) in the F_1 anti-mutant response.

DISCUSSION

All CTL responses in the mouse, whether to virus, hapten, or any non-H-2 antigen, are restricted by the syngeneic *K* or *D* regions of *H-2* expressed by the immunizing cells (3). CTL responses to allogeneic (or xenogeneic) MHC antigens are special in that they are huge responses and the CTLs appear to be specific for the foreign *K* or *D* allele only and do not need to see self H-2 on the targets (3, 20). One of the most remarkable examples of alloreactivity is the response of H-2^b mice (wild type) to cells that carry mutant H-2K^b antigens. Mutant and wild-type molecules are very similar in structure, yet the two strains show a mutual high T cell reactivity (16, 17, 21–24).

We have recently demonstrated that alloreactive CTLs from [F_1 → parent] chimeras have a self-preference as measured by their crossreactivity on TNP-modified parent 1 and parent 2 targets (25). This result could be explained in a two-receptor framework by supposing that the thymic selection worked only on the anti-self receptor, whereas the anti-X-receptor repertoires of [F_1 → parent 1] and [F_1 → parent 2] chimeras would be the same or very similar (9, 10, 26). The experiments we have presented here go further in that they show that the receptor used to respond to foreign antigen (in this case a foreign MHC antigen) is influenced by self MHC antigens. Also, because high responsiveness to the H-2K^b mutants is dependent on "seeing" the wild-type H-2K^b molecule as self and does not come about with D^b, K^d, or D^d antigens as self, these results suggest that self MHC antigens select a T cell repertoire that reacts preferentially with antigens that are "close to self."

One could explain the host-determined differences in response to K^b mutants either by *I* region-controlled differences in specific T-helper cells or by a specific suppressor mechanism operating in [F_1 → B10.D2] and [F_1 → HTG] chimeras. The *I*-region-restricted helper cell explanation is made unlikely by the data in Table 2 showing that F_1 (H-2^d × H-2^{bm4}) stimulators (which express both I^d and I^b) give the same result and because anti-H-2^k stimulated CTL, assayed for crossreactivity on mutant targets, also reveal high and low levels of activity (Figs. 1 and 2 and Table 2). This latter result also argues strongly against any explanation based on specific suppression of K^b-associated responses in the low-responder chimeras.

According to the altered-self hypothesis (which we prefer), the one receptor on CTL is therefore selected in the thymus to be reactive with complexes of self-K or D-plus-foreign antigen. Receptors expressed by T stem cells and selected by reactivity to self K^b, for example, are likely to be reactive eventually with K^b-plus-X or with a K^b mutant allele, whereas receptors selected by self D^b are more likely to respond to D^b-plus-X. But the results presented here are also consistent with a version of the two-receptor hypothesis suggested by Janeway *et al.* (27). This theory predicts that the receptor used in reactions to foreign MHC is the anti-self receptor; the anti-self receptors are heterogeneous and are all selected in the thymus to react weakly with self, while a fraction of them will react strongly with any one foreign haplotype. Because many other experiments have suggested that the ability to react with many conventional antigens is controlled by self MHC (reviewed in refs. 1 and 3) and our experiments suggest that the anti-MHC receptor is similarly influenced, then the simplest explanation is given by a one-receptor model.

The results presented here do speak against the suggestion of Jerne and colleagues (10, 28) that alloreactive cells are a separate subset from self-plus-X reactive cells and are not influenced by thymic H-2 antigens.

Another possibility for the self (host)-determined high reactivity to K^b mutants, which has been suggested to us by R. E. Langman and M. Cohn (personal communication) is that the high response is due to the mutant determinant being seen in conjunction with a self wild-type K^b determinant. That is, the alloreactive response to K^b-mutant cells may actually be a K^b-restricted response if a self-restricting determinant is shared by wild-type and mutant molecules.

It should be noted that the property of being alloreactive to a mutant H-2 difference is not absolutely dependent on the thymus bearing the wild-type molecule. For example, [F_1 → B10.D2] and [F_1 → HTG] chimera cells do mount a primary *in vitro* CTL response to stimulator cells bearing an *H-2K*^{b} mutation (Figs. 1 and 2 and Table 2); the response of these mice is lower than that of chimeras that do express K^b in the thymus. This degree of slackness is to be expected from the generation of diversity driven by a self antigen and fits well with the finding that responsiveness to conventional antigens is not absolutely restricted by self MHC antigens (19, 29). After all, every type of chimera we use makes a strong response to independent haplotypes such as H-2^k or H-2^s. These large responses may represent a summation of responses to the large number of differences between self and allo H-2. It is only when the antigenic difference is small that one can detect high or low responsiveness mapping to the H-2 complex.

Finally, it should be noted that Egorov *et al.* (30) have noted the *K*-region control of graft-versus-host reactivity to foreign *K*-region antigens. These results are apparently similar to those we report here.

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