THE MAJOR HISTOCOMPATIBILITY COMPLEX DETERMINES SUSCEPTIBILITY TO CYTOTOXIC T CELLS DIRECTED AGAINST MINOR HISTOCOMPATIBILITY ANTIGENS*

BY MICHAEL JOHN BEVAN

(From The Salk Institute for Biological Studies, San Diego, California 92112)

The major histocompatibility complex (MHC)[1] of the mouse, *H-2*, is located on chromosome 17 (linkage group IX). The *H-2* gene complex is divided into four regions, *K, I, S,* and *D,* and the distance between the *K* and *D* regions is about 0.5 cm (1). The *H-2* gene complex was originally recognized as determining cell surface antigens which caused the rapid rejection of allografts and elicited the production of cytotoxic or agglutinating antibodies. Recently *H-2* has been shown to exert an influence on the responsiveness of thymus-derived (T) lymphocytes. The function of helper T cells is controlled by genes in the *I* region of *H-2*, whereas genes in the *K* and *D* regions control the responsiveness of cytotoxic T cells.

Immune response (Ir) genes which regulate the response of helper T cells to specific antigens are marker loci for the *I* region (2, 3). The *I* region also determines surface antigens which stimulate allogeneic T cells to divide in a mixed lymphocyte culture (MLC) or graft versus host (GVH) reaction (4, 5). A very large fraction, 1–10%, of T lymphocytes respond in MLC or GVH to allogeneic differences in the *I* region (6, 7). The classical, serologically-defined antigens are determined by genes in the *K* and *D* regions at either end of the *H-2* complex. Analyses with alloantisera have shown that the K and D antigens are present on most cells; they are glycoproteins of mol wt about 45,000 and are associated on the cell surface with β-2-microglobulin, a polypeptide that shows sequence homology with immunoglobulin (1, 8). In general, allelic differences in K or D antigens do not lead to a large T-cell proliferative response in MLC. They are, however, the target antigens for cytotoxic T cells derived from primary MLC or are physically and genetically linked to them (1, 9, 10). Work from this laboratory suggests that 1–4% of cytotoxic T-cell precursors are directed against H-2 antigens (11).

Recently, the lytic function of cytotoxic T cells directed against syngeneic cells was shown to be controlled by the *H-2* gene complex. Doherty and Zinkernagel (12, 13) and Blanden and Gardner et al. (14, 15) showed that cytotoxic T cells from an animal immune to lymphocytic choriomeningitis virus (LCM) or ectromelia virus could specifically lyse infected target cells only when the target shared the same *H-2* as the cytotoxic T cells. Shearer also showed that the *H-2* haplotype of the target was important in determining susceptibility to cytotoxic cells immunized with trinitrophenyl (TNP)-conjugated syngeneic cells (16). The regions of *H-2* important in restricting target cell lysis were mapped to the *K* and *D* regions (17, 18).

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; FCS, fetal calf serum; GVH, graft versus host; LCM, lymphocytic choriomeningitis; LPS, lipopolysaccharide; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; PHA, phytohemagglutinin; TNP, trinitrophenyl.

Here I report studies on the cytotoxic cells generated by immunization of one strain of mouse with cells from an allogeneic strain which carries the same *H-2* region. Thus, the immunization is to alloantigenic differences which do not map in *H-2*. The effector cytotoxic cells in a 4 h ^{51}Cr release assay are shown to be T cells and indistinguishable in many aspects from cytotoxic T cells directed against H-2 antigens. These cytotoxic T cells will lyse only those allogeneic target cells which carry the same *H-2* as the immunizing strain, targets from congenic strains differing at both *H-2K* and *H-2D* are not susceptible to lysis. Two lines of evidence are presented which argue against the possibility that the cytotoxic cells have to simultaneously recognize *H-2* structures and non-H-2 alloantigens on the target in order to cause lysis, i.e., the dual recognition or self recognition hypothesis. Consequently, I favor the interaction antigen hypothesis which states that H-2K and D coded products modify all of the non-H-2 coded alloantigenic surface structures which serve as target antigens for cytotoxic T cells. The non-H-2 products are probably coded for by minor histocompatibility genes.

Materials and Methods

Mice.   C57BL/6 (B6, *H-2^b*), B6.C/*H-2^d* (synonym, HW19), C57BL/10 (B10, *H-2^b*), B10.D2/nSn (*H-2^d*), DBA/2 (*H-2^d*), DBA/1 (*H-2^q*), and D1.C (*H-2^d*) mice were obtained from Jackson Laboratories, Bar Harbor, Maine. BALB/c (*H-2^d*), BALB.B (*H-2^b*), F$_1$(C57BL/6 × BALB/c) (*H-2$^{b/d}$*), and F$_1$ (BALB/c × BALB.B) (*H-2$^{d/b}$*) mice were bred at the Salk Institute. BALB.B mice were originally received from Dr. F. Lilly. All of the mice used as donors of responding and stimulating cells were female.

Mitogens.   Phytohemagglutinin-P (PHA) and lipopolysaccharide (Salmonella typhosa, 0901, LPS) were obtained from Difco Laboratories, Detroit, Mich., and Concanavalin A (Con A) was obtained from Calbiochem, San Diego, Calif.

Target Cells.   EL4, a B6 lymphoma, and P815, a DBA/2 mastocytoma, were maintained in tissue culture as described (19). Normal (nontumor) targets were spleen cells which had been cultured for 2–3 days with 10 μg/ml LPS (B-cell blasts) or 2 μg/ml Con A (T-cell blasts), as described previously (9, 19).

Immunization.   There is no, or very little, cytotoxicity generated in a primary 4–5 day MLC when responder and stimulator cells share the same *H-2*. Immunizations were therefore performed by first priming the mice in vivo with allogeneic cells. 10^7 viable EL4 or P815 cells or 4–5 × 10^7 normal spleen and lymph node cells in balanced salt solution were injected intraperitoneally in a volume of 0.3–0.5 ml. 18–60 days later the spleens from these primed mice were removed and restimulated in vitro with cells from the original priming strain. The medium used for culture was RPMI 1640 supplemented with 50 μM 2-mercaptoethanol and 5% fetal calf serum (FCS) (20). Responding cells were cultured at 4 × 10^6 viable/ml and stimulated with 2 × 10^5/ml 5,000 rad (^{60}Co source) tumor cells or 4 × 10^6/ml 1,000 rad normal spleen cells. Cultures were fed with 200 μl of cocktail on day 1. They were harvested by pipetting after 4 or 5 days and washed and resuspended in assay medium (Dulbecco modified Eagle's medium containing 5% FCS).

^{51}Cr Labeling.   P815, EL4, or spleen cells which had been stimulated with LPS or Con A were washed and suspended in medium at a concentration of 2–4 × 10^6 in 0.2-ml medium containing 100–200 μCi [^{51}Cr]sodium chromate as described (19). After washing, the viable cells were counted in a hemocytometer and adjusted to 1 × 10^5 cells/ml. The mitogen-stimulated spleen cells contained over 60% blast cells, and I attempted to count only blasts, although the error involved may be up to 30%. 5 × 10^4 tumor cells incorporated 2–5 × 10^4 cpm and 5 × 10^4 blast cells, 1–3 × 10^4 cpm (counted at about 50% efficiency in a gamma counter).

Cytotoxic Assay.   0.5 ml of serial three- or fourfold dilutions of the cytotoxic cells (or control cells) were placed in 35-mm Petri dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.), and 5 × 10^4 ^{51}Cr-labeled target cells in 0.5 ml were added at the start of the assay. The dishes were placed in a box, gassed with 10% CO$_2$-air, and rocked at 7 cycles/min at 37°C for 4 h. The contents of the

dishes were transferred to tubes, centrifuged, and 0.5 ml of the supernate removed for counting. The figures are a plot of percent specific ^{51}Cr released vs. the logarithm of the aggressor: target ratio. The percent specific release was calculated as:

$$\frac{\text{experimental release cpm} - \text{spontaneous release cpm}}{\text{total cpm} - \text{spontaneous release cpm}} \times 100$$

Spontaneous release, by 5×10^4 targets alone, varied from 5–15% of the total for tumors and 10–38% for blast cells. The aggressor: target ratio is occasionally given as the ratio of responder cells originally cultured: target cells. This assumes 100% cell recovery. Cell recovery from cultures was always estimated and varied from 50–80%.

Treatment with Anti-Thy-1.2 Serum. Preparation of the anti-Thy-1.2 serum has been described fully elsewhere (19). Cells were incubated in serum in HEPES-buffered Eagle's medium containing 2 mg/ml bovine serum albumin for 15 min at 37°C, washed, and incubated in agarose-absorbed guniea pig serum (Grand Island Biological Co., Grand Island, N. Y.) at a final dilution of 1:10 for 45 min at 37°C. They were washed once more and resuspended in medium for the ^{51}Cr release assay.

Results

Primary In Vivo Cytotoxic Response of BALB/c to P815. BALB/c (H-2^d) mice were injected intraperitoneally with 10^7 viable DBA/2 mastocytes, P815 (H-2^d), and at intervals spleens were removed from injected and control mice and assayed for lysis of P815 in a 4 h ^{51}Cr release assay (Fig. 1). Spleen cells from unimmunized mice never caused lysis. Spleen cells from the injected mice were maximally cytotoxic at about day 11. The cytotoxicity of spleen cells had almost disappeared by day 20 after immunization. The curves for spleen cell cytoxicity are very flat, probably because the effector cells are infrequent, and the inert cells slow the rate of lysis (11). On day 9 and 10 the cells were also assayed against ^{51}Cr-EL4 (B6, H-2^b) and caused no lysis of this target, suggesting that lysis of P815 was specific.

During rejection of an H-2 different ascites tumor, a very active cytotoxic population could be obtained from the site of graft rejection (21). That the same is true in this situation is suggested in Fig. 1. On day 10, washings from the peritoneum of one mouse were passed through a cotton wool plug and mixed with the spleen cells. This mixed population was very active in the cytotoxic assay, probably due to the added peritoneal exudate cells. The kinetics of appearance and anatomical location of cytotoxic cells are correlated with rejection of the tumor graft suggesting they are active in the rejection process.

Anti-Thy-1 2 Sensitivity of Primary and Secondary Cytotoxic Cells. Spleen and peritoneal cells were obtained from BALB/c mice which had been injected 10 days earlier with P815. Aliquots were treated at a concentration of 10^7 viable cells/ml with a 1:10 dilution of anti-Thy-1.2 serum. The results in Table I show that under these conditions anti-Thy-1.2 serum plus complement completely abrogated measurable cytotoxicity, while normal mouse serum plus complement had no effect.

In Experiment 2, Table I, the anti-Thy-1.2 sensitivity of boosted cytotoxic cells derived from an H-2 identical and an H-2 different immunization were compared. The cytotoxic effector cells in a B6 (H-2^b) anti-DBA/2 (H-2^d) population are well established as being T cells. Secondary stimulated populations, BALB/c anti-DBA/2 and B6 anti-DBA/2, were treated at a concentration of 7×10^6 viable
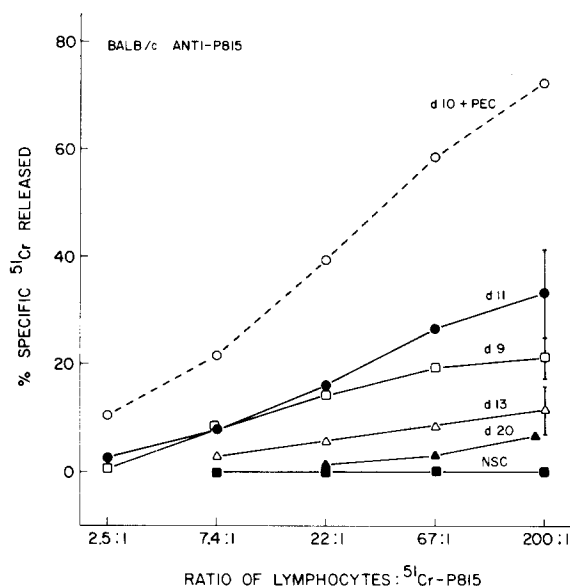
FIG. 1. Primary in vivo cytotoxic response of BALB/c mice (H-2^d) to P815 (H-2^d). On the indicated days after intraperitoneal injection of 10^7 P815 cells, spleens from two mice were removed and assayed for lysis of ^{51}Cr-P815. The vertical bars represent the range of lysis by the two spleen populations. On day 10 peritoneal exudate cells (PEC) and spleen cells were mixed and assayed for cytotoxicity (O---O).

cells/ml with serum at a final dilution of 1:17. The data in Table I show that treatment with normal AKR serum and complement did not diminish cytotoxicity. Treatment with AKR anti-C3H serum (anti-Thy-1.2) and complement drastically reduced cytotoxicity. The activity of the BALB/c anti-DBA/2 population was reduced 98%. The activity of the B6 anti-DBA/2 population was reduced 98.6%. Thus the two populations did not differ significantly in their sensitivity to anti-Thy-1.2 serum.

Nonspecific Lysis of Tumor Cells. In previous publications it was shown that cytotoxic T cells induced by Con A or H-2-different MLC could lyse tumor targets nonspecifically in the presence of PHA (19, 22). The results presented in Fig. 2 show that cytotoxic cells generated by an allogeneic, *H-2*-identical immunization can also be assayed in the same way. BALB/c (H-2^d) were immunized with DBA/2 cells (H-2^d) and BALB.B (H-2^b) with B6 cells (H-2^b). Both populations were assayed for lysis of P815 (DBA/2) and EL4 (B6) in the presence and absence of 10 μg/ml PHA. In the absence of PHA (specific assay) only the BALB/c anti-DBA/2 cells lysed P815, and only the BALB.B anti-B6 cells lysed EL4 efficiently. When the assay was performed in the presence of PHA (nonspecific assay) then both cytotoxic populations lysed both targets very efficiently (Fig. 2 a and b).

The data has shown that cytotoxic effector cells generated in *H-2*-identical and *H-2*-different immunizations have the following common properties: (*a*) both can be assayed in a short-term ^{51}Cr release assay, (*b*) anti-Thy-1.2 sensitivity, and (*c*) the ability to cause nonspecific lysis of tumor cells in the presence of PHA.

TABLE I

Sensitivity of Primary and Secondary BALB/c (H-2^d) anti-DBA/2 (H-2^d) Cytotoxic Cells
to Treatment with anti-Thy-1.2 Serum and Complement*

Exp.	Cytotoxic cells	Ratio	Percent specific cytotoxicity after:			Percent reduction of activity‡
			No treat-ment	Normal AKR serum plus c'	Anti-Thy-1.2 plus c'	
1	BALB/c anti-P815§	260:1	62.4	62.0	0	100
2	BALB/c anti-DBA/2§	50:1	85.5	85.4	15.3	98
	B6 anti-DBA/2§	50:1	88.4	89.0	11.5	98.6

* Cytotoxicity was assayed for 4 h on ^{51}Cr-P815 (DBA/2, H-2^d).
‡ Calculated from a complete titration curve as described previously (9).
§ In Experiment 1, cytotoxic cells were spleen and peritoneal exudate cells from BALB/c mice injected 10 days earlier with 10^7 P815. In Experiment 2, cytotoxic cells were spleen cells of BALB/c (H-2^d) or B6 (H-2^b) mice injected 2 mo previously with P815 and stimulated in vitro for 4 days with irradiated DBA/2 spleen cells.
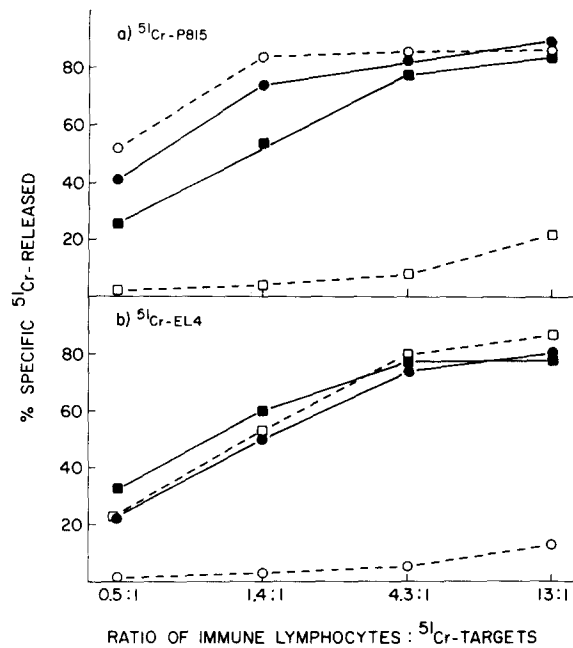

FIG. 2. Specific and nonspecific lysis of tumor cells mediated by cytotoxic cells generated by H-2 identical immunizations. Spleen cells from mice that had been injected 1 mo earlier with allogeneic H-2 identical tumor cells were removed and restimulated in culture with irradiated spleen cells from the original priming strain. BALB/c cells were primed with P815 and boosted with DBA/2 spleen cells (○, ●). BALB.B cells were primed with EL4 and boosted with B6 spleen cells (□, ■). Assayed in the absence (specific assay, open symbols) and the presence (nonspecific assay, closed symbols) of 10 μg/ml PHA against (a) ^{51}Cr-P815 and (b) ^{51}Cr-EL4.

The H-2 Haplotype of the Target Determines Its Susceptibility to Lysis. Cytotoxic populations immunized with *H-2*-identical cells were assayed for lysis of targets from many strains that differed genetically from the immunizing strain at *H-2* or other loci. It was necessary to use normal cells as targets since suitable tumor cell targets were not available from many strains. LPS (B-cell) blasts and Con A (T-cell) blasts are quite susceptible to lysis by cytotoxic T cells directed against H-2 antigens (19). They were found to be sensitive also to cytotoxic T cells induced by *H-2*-identical immunization.

Spleen cells from B10.D2 (*H-2^d*) mice which had been injected with P815 (DBA/2, *H-2^d*) were restimulated in culture with DBA/2 cells. After 4 days the cells recovered from the cultures were assayed for lysis of LPS blasts (Fig. 3 a) and Con A blasts (Fig. 3 b) from four strains of mice. B10.D2 targets, syngeneic with the cytotoxic cells, were not lysed. Targets from the immunizing strain DBA/2 were lysed very efficiently. DBA/1 (*H-2^q*) targets were not lysed, whereas D1.C targets (*H-2^d*, congenic with DBA/1) were lysed about as well as DBA/2

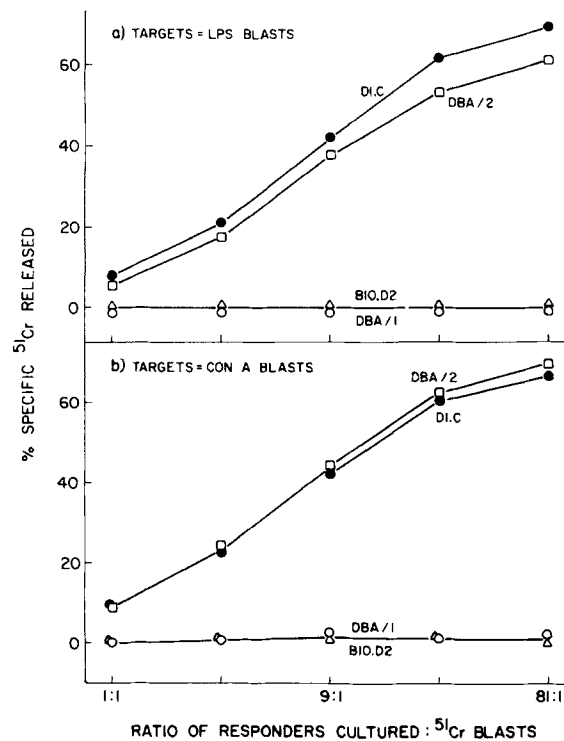RATIO OF RESPONDERS CULTURED : ^{51}Cr BLASTS

Fig. 3. The *H-2* haplotype of the target restricts susceptibility to lysis by B10.D2 (*H-2^d*) anti-DBA/2 (*H-2^d*) cytotoxic cells. Spleen cells from B10.D2 mice that had been injected 2 mo previously with P815 cells were restimulated for 4 days in culture with irradiated P815. Cultured cells were harvested and assayed for lysis of (a) LPS blast cells, and (b) Con A blast cells from four strains of mice. B10.D2 (*H-2^d*) targets (△—△); DBA/2 (*H-2^d*) targets (□—□); DBA/1 (*H-2^q*) targets (○—○); D1.C (*H-2^d*) targets (●—●). Spontaneous release by 5 × 10^4 targets cultured alone varied from 9.7 to 19.6%. Spleen cells from normal B10.D2 mice caused no lysis of any target in this experiment (not shown). Cell recovery from the 4-day cultures was 51%.

targets (Fig. 3 a and b). The difference in susceptibility of the congenic pair DBA/1 and D1.C is linked to H-2, even though the cytotoxic cells were not immunized to allelic differences which coded in the H-2 complex. The major finding of this report is that the H-2 complex restricts susceptibility of targets to lysis by allogeneic cytotoxic T cells immunized against non-H-2 allelic differences.

The data in Fig. 3 a and b showed that B- and T-cell blasts were about equally sensitive to lysis. The remaining experiments utilized B-cell blasts to avoid the cytotoxicity which T-cell mitogens induce.

BALB/c cells (H-2^d) were immunized with DBA/2 cells (H-2^d) and BALB.B (H-2^b) with B6 (H-2^b) cells. The two cytotoxic populations were assayed at the same time for lysis of ^{51}Cr-LPS blasts from six strains of mice. The results in Fig. 4 show that the restriction of target susceptibility imposed by the H-2 haplotype is not limited to H-2^d, but also applies to H-2^b. The BALB/c anti-DBA/2 population did not lyse syngeneic targets nor targets from the congenic partner BALB.B (H-2^b). Targets from the immunizing strain, DBA/2, were lysed efficiently. B6 (H-2^b) and B6.C/H-2^d are congenic and differ genetically at H-2; only targets from
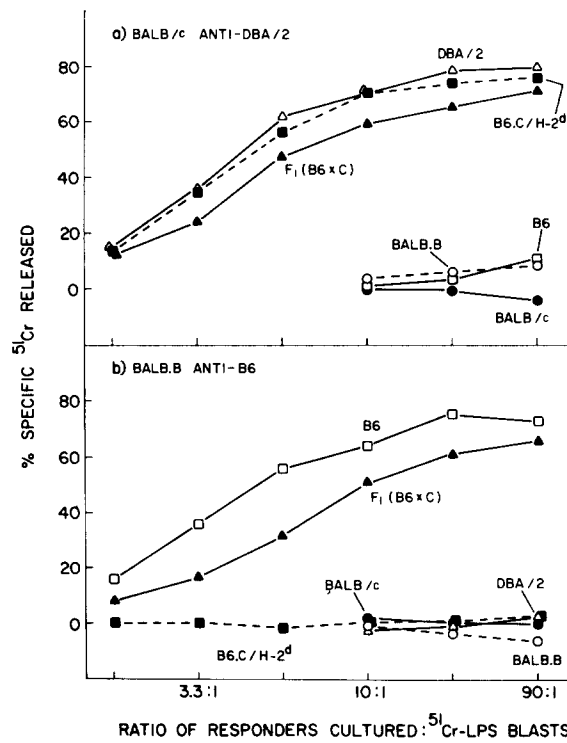

FIG. 4. The H-2 haplotype of the target restricts the susceptibility to lysis mediated by BALB/c anti-DBA/2, and BALB.B anti-B6 cytotoxic cells and detection of hybrid-specific antigens. The cytotoxic cells were generated as described for Fig. 2. (a) BALB/c anti-DBA/2, and (b) BALB.B anti-B6 cytotoxic cells assayed against ^{51}Cr-labeled LPS blast targets from: DBA/2, H-2^d (△—△); B6, H-2^b (□—□); BALB/c, H-2^d (●—●); BALB.B, H-2^b (O - - - O); B6.C/H-2^d (■- - -■); and F$_1$(B6 × BALB/c), H-$2^{b/d}$ (▲—▲). Spontaneous release by 5 × 10⁴ targets varied from 26.9 to 36.3%.

B6.C/H-2^d were lysed efficiently, however. An explanation for the cross-reaction between DBA/2 and B6.C/H-2^d detected by the BALB/c cytotoxic population is given in the discussion. This BALB/c anti-DBA/2 cytotoxic population also showed F_1 specific lysis, that is, B6 (H-2^b) and BALB/c targets were not lysed while targets from the hybrid between these two strains, F_1(B6 × BALB/c), were lysed efficiently (Fig. 4 a).

The H-2^b cytotoxic population, BALB.B anti-B6, did not lyse targets from DBA/2 (H-2^d), BALB/c (H-2^d), or BALB.B (H-2^b) (Fig. 4 b). Targets from the immunizing strain, B6 (H-2^b), were lysed very well, whereas targets from the congenic strain, B6.C/H-2^d, were not lysed. Targets from F_1(B6 × BALB/c) were lysed efficiently, showing that susceptibility is dominant in an F_1 (Fig. 4 b).

The remaining experiments attempted to discriminate between two hypotheses that can explain these results: (a) The dual recognition hypothesis states that the cytotoxic population has two types of receptors to separately recognize H-2 and non-H-2 components on the target surface. (b) The interaction antigen hypothesis states that the antigens recognized are the result of interaction between the products of H-2 and non-H-2 genes.

Discrimination between the Hypotheses: F_1 Cytotoxic Effector Cells. This experiment asks whether an F_1 immunized against a strain that carries one of the parental H-2 haplotypes will be able to lyse cells from a strain congenic with the immunizing strain, but carrying the H-2 haplotype of the other parent of the F_1. F_1(BALB/c × BALB.B) (H-$2^{d/b}$) mice were immunized with either B10.D2 cells (H-2^d) or with B10 cells (H-2^b). B10.D2 and B10 are congenic and presumed to be genetically identical except for a region of the chromosome bearing H-2. Both immunized F_1 populations were assayed for lysis of F_1(BALB/c × BALB.B), B10.D2 and B10 targets (Fig. 5). Neither population lysed syngeneic targets. The F_1 cells immunized with B10.D2 lysed B10.D2, but not B10 targets (Fig. 5 a). The F_1 cells immunized with B10 lysed B10, but not B10.D2 targets (Fig. 5 b).

This result is consistent with the interaction antigen hypothesis. It is not consistent with the dual recognition hypothesis in a form which states that an F_1 cytotoxic T cell has recognition structures for both parental H-2 haplotypes which it possesses.

Discrimination between the Hypotheses: the Cold Target Competition Experiment. With regard to the structures recognized by cytotoxic T cells, the dual recognition hypotheses predicts that cells from a pair of congenic strains which differ at H-2 have the same non-H-2 alloantigens on their surface. The interaction antigen hypothesis predicts the opposite. If the dual recognition hypothesis were correct then cells from B6.C/H-2^d might be able to be bound by the receptors on BALB.B (H-2^b) anti-B6 (H-2^b) cytotoxic cells which combine with B6 non-H-2 antigens. Thus unlabeled B6.C/H-2^d cells might inhibit ^{51}Cr release from labeled B6 targets (9, 23). In the experiment shown in Fig. 6, 3 × 10^4 [^{51}Cr]B6 LPS blasts were titred against BALB.B anti-B6 cytotoxic cells either alone or in the presence of a 30-fold excess (9 × 10^5) of unlabeled LPS blasts from BALB.B, B6.C/H-2^d, or B6 mice. BALB.B blasts, syngeneic with the cytotoxic cells, inhibited ^{51}Cr release 2.7-fold compared to release with no unlabeled blasts present; B6.C/H-2^d blasts inhibited 3.9-fold (i.e., 1.4-fold relative to the BALB.B
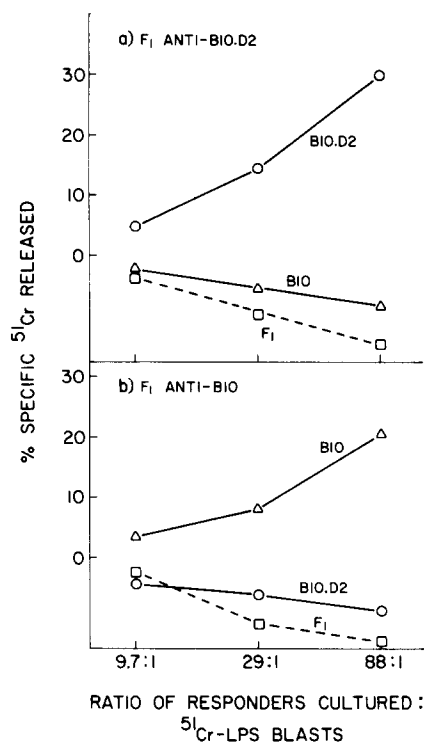
Fig. 5. Evidence against the requirement for self *H-2* recognition in lysis. F_1(BALB/c ×
BALB.B) *(H-2^{d/b})* mice were injected intraperitoneally with 42 × 10⁶ spleen and lymph node
cells from either B10.D2 *(H-2^d)* or B10 *(H-2^b)* mice. 18 days later spleen cells from the
injected mice were restimulated in vitro with irradiated spleen cells from mice of the
original priming strain. After 4 days the cultured cells were harvested and assayed for lysis
of ⁵¹Cr-LPS blasts from F_1(BALB/c × BALB.B) (□—□), B10.D2 (○—○), and B10 (△—△). *(a)*
Cells immunized against B10.D2; *(b)* cells immunized against B10. Spontaneous release of
⁵¹Cr varied from 20.3 to 25.3%.

inhibition); B6 blasts inhibited 23-fold (i.e., 8.5-fold relative to the BALB.B
inhibition). The difference in inhibition caused by B6.C/*H-2^d* and BALB.B blasts
is well within the limits of errors in counting the blasts. In a separate experi-
ment there was no difference in inhibition caused by blasts syngeneic with the
cytotoxic cells and blasts congenic with the ⁵¹Cr-labeled susceptible targets (24).

This result argues that the non-H-2 antigens on B6 which are recognized by
BALB.B anti-B6 cytotoxic cells are not present on B6.C/H-2^d in a way in which
they can be bound efficiently by the cytotoxic cells.

The Regions of H-2 Important in Restricting Target Susceptibility. In the T-
cell lysis of virus-infected or TNP-modified cells, evidence was presented that *K*
and/or *D* region compatibility between killer and target cells was required for
lysis. *H-2I* or *S* region compatibility was neither required nor sufficient (17, 18).
Table II presents evidence that the same compatibility requirements must be
met to achieve target lysis by cytotoxic cells directed against minor alloanti-
gens. BALB.B *(H-2^b)* cytotoxic T cells were generated by immunization with B6
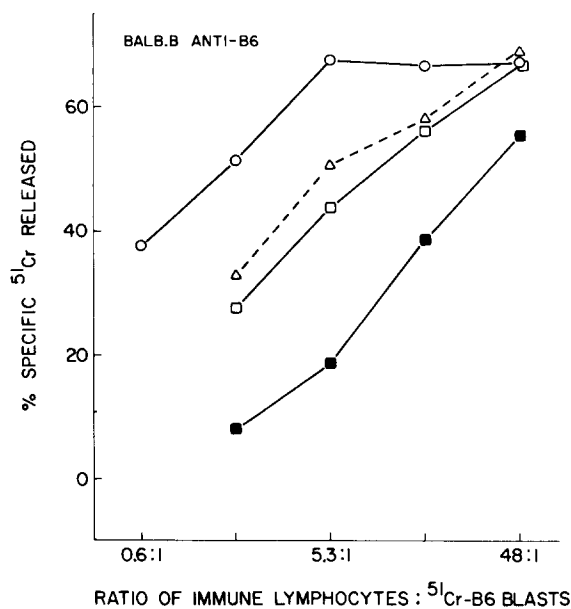cells *(H-2^b)*. These cytotoxic cells lysed B10 *(H-2^b)* LPS blasts very efficiently (B6

RATIO OF IMMUNE LYMPHOCYTES : ^{51}Cr-B6 BLASTS

Fig. 6.   B6.C/H-2^d cells fail to specifically inhibit the lysis of ^{51}Cr-B6 cells by BALB.B anti-B6 cytotoxic cells in a cold target competition experiment. BALB.B cells (H-2^b) were immunized against B6 cells (H-2^b) as in Fig. 2. They were assayed for lysis of 3 × 10^4 ^{51}Cr-B6 LPS blasts either alone (O—O) or in the presence of 9 × 10^5 unlabeled LPS blasts from BALB.B (△———△), B6.C/H-2^d (□—□), or B6 (■—■).

TABLE II

The Regions of the H-2 Complex Important in Restricting Target Susceptibility

Cytotoxic cells*	Target cells‡	H-2 genotype of target cells:						Specific lysis§
		K	IA	IB	IC	S	D	
								%
BALB.B anti-B6	B10	b	b	b	b	b	b	85.1
BALB.B anti-B6	B10.BR	k	k	k	k	k	k	-3.7
BALB.B anti-B6	B10.D2	d	d	d	d	d	d	1.5
BALB.B anti-B6	B10.A(2R)	k	k	k	d	d	b	75.7
BALB.B anti-B6	B10.A(5R)	b	b	b	d	d	d	30.9

* Spleen cells of BALB.B (H-2^b) mice primed 2 mo earlier with 10^7 EL4 (B6, H-2^b) were stimulated in vitro for 4 days with irradiated B6 spleen cells.

‡ Spleen cells stimulated for 2 days with LPS and labeled with ^{51}Cr.

§ Attacker: target ratio 63:1. The titrations indicated that lysis of B10.A (2R) was 7–10 times stronger than lysis of B10.A (5R).

and B10 mice are almost identical), but caused no lysis of B10.BR (H-2^k) or B10.D2 (H-2^d) targets which differ from B10 throughout the whole H-2 complex. Targets from B10.A(2R) mice (H-2^{h2}), which share only the H-$2D^b$ region with B10, were lysed very efficiently. B10.A(5R) targets (H-2^{i5}), which share the H-$2K^b$, IA^b, and IB^b regions with B10, were also lysed though less efficiently than B10.A(2R) targets. Thus, for the cytotoxic BALB.B anti-B6 cells, H-2 compatibil-

ity to the right of the S region or to the left of the IC region was sufficient to allow lysis.

Discussion

Characterization of the Cytotoxic Effector Cells. Cytotoxic effector cells induced by immunization with allogeneic cells which carry the same H-2 can be assayed for target cell lysis in a 4 h ^{51}Cr release assay. Tumor cells, B-cell blasts, and T-cell blasts are sensitive targets. Thus, in effector function, they are similar to cytotoxic T cells directed against H-2 alloantigens. The cytotoxic effector cells in a BALB/c (H-2^d) anti-DBA/2 (H-2^d) population are sensitive to treatment with anti-Thy-1.2 serum and complement, and the degree of sensitivity is indistinguishable from that of the effector cells in a B6 (H-2^b) anti-DBA/2 (H-2^d) population (Table I). Furthermore, cytotoxic cells generated by allogeneic, H-2-identical immunization can cause efficient lysis of even syngeneic tumor targets when agglutinated with PHA (Fig. 2). In this latter property also, they resemble cytotoxic effector T cells directed against H-2 alloantigens (19). Thus, it is concluded that the effectors in H-2-identical and H-2-different cell-mediated lysis are the same, both are T cells.

The frequency of cytotoxic T-cell precursors reactive to H-2 coded alloantigens is probably much higher than of those reactive to non-H-2 coded allelic differences. Whereas H-2 reactive cytotoxic T cells can be easily demonstrated after a primary, 4–5 day MLC, this is not true for cytotoxic cells reactive to non-H-2 differences (25). Furthermore, after a polyclonal induction with Con A in the absence of antigen, cytotoxic T cells with specificity for foreign (nonself) H-2 can be detected, whereas cytotoxic activity against non-H-2 allelic differences cannot (11). Thus, Con A induced BALB.B (H-2^b) and B10.BR (H-2^k) cells lyse P815 (H-2^d) specifically, but Con A induced BALB/c (H-2^d) and B10.D2 (H-2^d) do not. This difference in the antigenic and mitogenic induction is most plausibly explained by assuming that H-2-reactive cytotoxic cells exists at a much higher frequency than non-H-2-reactive cells.

The Role of H-2 in Determining Target Susceptibility. Cytotoxic T cells of one strain of mouse immunized against cells of another strain, which carries the same H-2, will lyse targets of the immunizing strain but not targets from a strain congenic with the immunizing strain which differs at H-2. Schematically the results may be summarized as follows: cytotoxic T cells of strain A (H-2^d) immune to cells of strain B(H-2^d) will lyse B(H-2^d) targets but not B(H-2^k) targets. The cytotoxic population cross-reacts strongly on other strains that carry H-2^d, i.e., C(H-2^d) targets are lysed but C(H-2^b) targets are not. A(H-2^d) anti-B(H-2^d) cytotoxic cells do not lyse A(H-2^d) nor B(H-2^b) targets, but cells from the hybrid between these strains, F_1(A(H-2^d) × B(H-2^b)), are lysed very well. For example, BALB/c (H-2^d) anti-DBA/2(H-2^d) cytotoxic cells do not lyse targets from B6 (H-2^b) or BALB/c. However, B6.C/H-2^d targets and F_1 (B6 × BALB/c) targets are lysed very well (Fig. 4). B6.C/H-2^d is congenic with B6 and derived H-2^d from BALB/c. The susceptibility of these two targets rules out the explanation that the BALB/c anti-DBA/2 cytotoxic cells recognize an alloantigen closely linked to H-2 on chromosome 17.

The non-H-2 genetic loci which specify the alloantigenic differences measured

in the ^{51}Cr release assay are probably minor histocompatibility loci. Cytotoxic T cells are found at the site of rejection of a tumor graft when donor and recipient share the same *H-2* (Fig. 1), and they are presumably active in the rejection. By definition then the genes specifying the antigens are minor histocompatibility genes (26). The large degree of cross-reaction detected by the cytotoxic cells could also be explained if they were immunized against minor histocompatibility differences, since strains differ at a large number of minor histocompatibility loci, but the number of alleles at each locus is small (27). Recently, I have generated cytotoxic cells within the *H-3* congenic pair, B10 and B10.LP, and the *H-7* pair, B10 and B10.C(47N). Cytotoxicity in this restricted system shows the same *H-2* compatibility requirements described here. This strongly suggests that the minor histocompatibility loci, 35 of which have been isolated in congenic lines (27), are the same as those coding for allelic differences which immunize cytotoxic T cells.

To achieve an efficient, antigen-specific cooperative interaction between T and B cells (28) or T cells and macrophages (29), there is a requirement for the cells to share the same *I* region (or its homolog in other species). In these cooperative systems the *K* and *D* regions appear irrelevant. In the killing assays, described for viruses, TNP, and allogeneic cells the compatibility requirement is just the reverse (17, 18, Table II). This correlates very well with our knowledge of H-2-different MLC reactions, i.e. an excellent mitogenic response (by helper T cells?) is stimulated by *I*-region differences, whereas most or all killer cells derived from MLC are directed at *K*- and *D*-region differences (1, 4, 9, 25). If a cooperative interaction between T helpers and T killers is required to induce a cytotoxic response, then it is likely that this interaction will require *I*-region compatibility.

There are two hypotheses that are consistent with the results of the cytotoxic assay summarized schematically above. The dual recognition hypothesis argues that the cytotoxic cell has two types of receptors, one clonally restricted with specificity for a minor histocompatibility antigen on the target and another receptor that interacts with an *H-2K* or *D*-coded structure on the target. The target has to present both structures to be susceptible to lysis. The interaction antigen hypothesis argues that there is only one type of receptor on the cytotoxic T cell, and this receptor recognizes a determinant that is the result of interaction between the products of *H-2* and non-*H-2*-coded genes. For example, *H-7* is a minor histocompatibility gene, and B6 (*H-2^b*) and B6.C/*H-2^d* both carry the *H-7^a* allele (27). The interaction antigen hypothesis predicts that the H-7 determinant which BALB.B (*H-2^b*, *H-7^b*) cytotoxic T cells recognize on B6 cells does not exist on B6.C/*H-2^d* cells. The experiment in Fig. 5 showed that F$_1$ (BALB/c × BALB.B) (*H-2$^{d/b}$*) cytotoxic cells immune to, and capable of lysing B10 (*H-2^b*) cells, did not lyse B10.D2 (*H-2^d*) cells, and vice versa, F$_1$ cytotoxic cells immune to B10.D2 did not lyse B10 cells. Zinkernagel and Doherty (30, 31) and Shearer et al. (18) have reported similar results with F$_1$ cytotoxic cells immunized with LCM-infected or TNP-modified parental cells. These results are consistent with the interaction antigen hypothesis. They limit the dual recognition hypothesis to saying that an F$_1$ cytotoxic T cell does not have H-2 recognition sites for both the H-2 haplotypes it possesses, i.e., the receptors for H-2 are clonally re-

stricted. The cold target competition experiment (Fig. 6) showed that an excess of B6.C/H-2^d cells did not inhibit lysis of B6 (H-2^b) cells mediated by BALB.B (H-2^b) anti-B6 cytotoxic cells any more than did an excess of BALB.B cells (18, 24, 31). Again this result is entirely consistent with the interaction antigen hypothesis. It argues strongly, but not definitively, against the dual recognition hypothesis. It could be for instance, that the affinity of interaction of the cytotoxic cells with non-H-2 antigens is weak in the absence of the second interaction with H-2 structures.

At the moment the data favor the interaction antigen hypothesis to explain the H-2 restriction of target lysis by allogeneic T cells directed against non-H-2 coded differences. The nature of the interaction between the genes and whether it occurs in the nucleus, cytoplasm, or at the cell surface is not known. It is unlikely that the products of over 35 minor histocompatibility loci modify the K and D antigens (15, 18, 30). Therefore, I have suggested that K and D region products (possibly glycosyl transferases) in fact modify products coded elsewhere or introduced by viruses. Admittedly it is difficult to apply this notion to the I region control of cooperative interactions involving T cells specific for protein antigens (28, 29).

The results reported here and the recent work on the response to viral infection (12, 13, 15, 32) and to TNP-modified syngeneic cells (16, 18) are suggestive that cytotoxic T cells exist only to recognize and eliminate cells that show variations of structures coded for in the major histocompatibility complex. The regions of H-2 important in restricting target susceptibility to cytotoxic T cells generated during LCM or ectromelia infection, or by immunization with TNP-modified syngeneic cells, were mapped to K and D (17, 18). Experiments in the allogeneic system described here have located activity in the D region and in the K plus IA plus IB region (Table II). The associations described between disease susceptibility and MHC phenotype have used the serologically-defined antigens as indicators of the MHC (33–35), i.e., products of the K and D regions in mice and their equivalents in other species. Therefore, these regions may be polymorphic so that for any virulent infectious agent there is a high probability that it will be modified by at least one of the alleles in a way which is immunogenic to cytotoxic T cells (36).

Summary

Cytotoxic cells were generated by immunizing one strain of mouse with cells from an allogeneic strain which carries the same H-2 region. The effector cells assayed in a 4 h ^{51}Cr release assay were shown to be T cells and indistinguishable, except in specificity, from cytotoxic T cells directed at H-2 alloantigens. Although the genetic differences between responder and stimulator cells responsible for the immunization did not code in H-2, the H-2 complex did restrict susceptibility of target cells. For example, BALB.B cytotoxic cells (H-2^b) immunized against and capable of lysing C57BL/6 cells (H-2^b) would not lyse B6.C/H-2^d target cells. C57BL/6 and B6.C/H-2^d are congenic and differ in the H-2 region. Two hypotheses are considered to explain the H-2 restriction of susceptibility to cytotoxic T cells generated by an H-2 identical alloimmunization. (a) The dual (self) recognition hypothesis states that the cytotoxic cell has two recognition

units, one for *H-2*-coded structures and another clonally restricted receptor for the minor alloantigen. (*b*) The interaction antigen hypothesis states that all the surface alloantigenic determinants recognized by cytotoxic T cells are the result of interaction between *H-2*- and non-*H-2*-coded gene products. Two lines of evidence, one with F_1 effector cells and the other a cold target competition experiment, are presented which argue strongly in favor of the interaction antigen hypothesis. The regions of *H-2* required to be histocompatible were mapped to the *D* region and to the left of *IC*, probably the *K* region. These results, and recent work on the response to virus-infected and TNP-modified syngeneic cells, suggest that cytotoxic cells are restricted in specificity to preferentially recognizing alterations in structures that are coded in the major histocompatibility complex.

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