CROSS-REACTIVE CYTOTOXIC RESPONSES

H-2 Restricted are
More Specific Than Anti-H-2 Responses

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T-cell responses generated in vitro in mixed lymphocyte cultures are manifest first as a proliferative phase with a peak on day 3 or 4 followed by a cytotoxic phase which peaks on day 5. The majority of cells contributing to the proliferative phase are Ly-1-negative T cells responding to I region determinants and they help the Ly-2+ 3+ cytotoxic cells make their optimal response, which is directed against K/D coded antigens (1). When one-way mixed lymphocyte cultures (MLC)1 are set up between mice differing across the whole of the H-2 complex (i.e. KISD) the target cell specificity is such that the strongest cytotoxicity is seen against target cells of the same H-2 haplotype as the stimulating cell, but a certain degree of cross-killing is also seen on independent haplotypes (2, 3). In this paper we further document andquantitate the extent to which this type of cross-reactive kill is seen and compare it with the cross-reactive kill seen when cytotoxic responses are elicited between H-2b and H-2b mutant strains. The cross-reactivities elicited in both these ways is contrasted with the very specific H-2 restricted cytotoxicity obtained by priming H-2b female mice in vivo and boosting them in vitro in MLC with male spleen cells (4).

Materials and Methods

Mice were obtained either from the Division of Comparative Medicine, Clinical Research Centre, or The Jackson Laboratory, Bar Harbor, Maine.

MLC. MLC were set up in Falcon flasks (BioQuest, BBL & Falcon Products, Becton Dickinson & Co., Cockeysville, Md.) (25 cm²) using equal numbers of responder spleen cells, and 2,000 rads irradiated stimulator spleen cells, each at a concentration of 5 × 10⁶/ml in RPMI-1640 medium containing 10% fetal calf serum (FCS), 5 × 10⁻⁶ M 2 mercaptoethanol, 10 mM Hepes, glutamine, penicillin, and streptomycin. MLC flasks contained a total of 20 ml cell suspension, and were incubated upright in a humidified atmosphere with 10% CO₂, at 37°C for 5 days. They were then harvested and assayed for cytotoxicity. All anti-allogeneic and anti-H-2b mutant MLC were primary responses. All anti-H-Y MLC were secondary responses, after in vivo priming 2 wk–5 mo before in vitro boosting, with syngeneic male spleen cells.

Target cells were prepared 48 or 72 h before the assay by removing erythrocytes from freshly teased spleen cell suspensions using Gey’s hemolytic medium (5) and then incubating them in 4 μg/ml Concanavalin A in the RPMI medium described above, at a concentration of 4–5 × 10⁶/ml.

1 Abbreviations used in this paper: A/T, attacker:target; BSS, balanced salt solution; FCS, fetal calf serum; MHC, major histocompatibility complex; MLC, mixed lymphocyte cultures; MST, median survival time.

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The \( ^{51} \text{Cr} \) release assay was essentially as previously described (3). 1 \( \times 10^7-2 \times 10^7 \) target cells were labeled for 90 min at 37°C in 1 ml balanced salt solution (BSS) containing 5% FCS, with 100 \( \mu \text{Ci} \) \( ^{51} \text{Cr} \). They were then washed twice in a large volume (50 ml) BSS/5% FCS, counted, and resuspended in Eagle's minimal essential medium (Gibco 109G, Grand Island Biological Co., Grand Island, N.Y.) containing 10% FCS and 10 mM Hepes, at an appropriate concentration (usually between 1 \( \times 10^5 \) and 1 \( \times 10^6 \)/ml) chosen so that the attacking cells could be assayed at four different attacker:target (A:T) cell ratios, ranging from 20:1 down. 0.1-ml target cells were placed in wells in microtitre plates (most of the experiments reported here were done using flat-bottomed wells for the assay, but round-bottomed wells serve equally well). 0.1-ml attacking cells at four different, halving dilutions were then added to the target cells. There were three replicates for each A:T ratio. Maximum release was determined by adding 5% Triton to each of three wells containing target cells, and control, spontaneous release was determined by incubating target cells in each of three wells in medium alone. The plates were centrifuged immediately after addition of attackers and targets, at 500 rpm for 5 min and then incubated for 3 h at 37°C in a humidified atmosphere containing 10% CO\(_2\). The plates were then centrifuged again for 10 min at 1,000 rpm. An aliquot of the supernates (usually 100 \( \mu \text{l} \)) was then removed using an Eppendorf pipette, and the \( ^{51} \text{Cr} \) released into the supernate was determined using a gamma counter. The percent-specific release was determined according to the formula given in (5) and then regression analysis applied to the specific lysis values at each of the 4 A:T ratios. From the regression lines obtained, the value of specific lysis at a ratio of 10:1 or 4:1 was read. \( r^2 \) values of each of the regression lines were obtained, and results only accepted as positive where this value, indicating the goodness of fit of the four experimentally determined points to the line, lay between 0.9 and 1.0.

Skin grafting was carried out according to the method of Billingham (6), using tail skin grafts placed on a graft bed prepared on the thorax of the recipient. Grafts were read daily after removing the plasters on day 8 after grafting and the end point scored when less than 10% viable skin was evident in the graft.

In vivo priming to the male-specific antigen, H-Y, was done by injecting female H-2\(^b\) mice with 10\(^7\) syngeneic male spleen cells 2 wk to several months before use in MLC (4).

**Results**

Tables I and II show the results of four experiments each where cytotoxicity generated in MLC between B10.S responders and B10 X-irradiated stimulator spleen cells (Table I) and between B10.G responders and B10 stimulators (Table II) was assayed against the specific target B10, and against spleen cells from a variety of different strains, representing 4 of H-2\(^b\) haplotype, three of H-2\(^d\) haplotype, and one each H-2\(^k\) and H-2\(^f\). Several points can be made from these data: first, there is a correlation between the level of kill against the specific target and that against the targets showing cross-reactive kill; where the one is high, so, comparatively, are the others, and conversely, when specific kill is relatively low, much less cross-reactive cytotoxicity is seen. Second, the level of cross-reactive kill against different strains of the same H-2 haplotype is comparable, within any one experiment, but there is an occasional exception, such as the failure to cross kill CBA targets in experiment 4, Table II, and the low kill by those same attackers against DBA/2 targets, in comparison with BALB/c and B10.D2 targets. Third, there is effectively no autokilling of targets syngeneic with the attackers. This third point is also made in Table III, where the cross-reactive killing by F\(_1\) cells is restricted, so that neither parental strain cells are killed.

That the cross-reactive killing as well as the specific killing is mediated by T cells is shown in Table IV, which is the result of assaying the same attacking cells shown...
specificity of anti-H-2 and H-2 restricted cytotoxicity

Table I

Specificity of H-2^a Anti-H-2^b Responses

| Exp. | H-2^a | H-2^b | Cytotoxicity* on target cells | H-2^a | H-2^b |
|------|-------|-------|-------------------------------|-------|-------|
|      |       |       | B10  | B10.S | B10.BR | C3H | CBA | AKR | B10.D2/| BALB/c | DBA/2 | B10.RIV | B10.M |
| 1    | 34.1 ± 0.5 | 1.5 ± 3.9 | 0.0 ± 1.4 | 13.7 ± 2.1 | ND | ND | 4.5 ± 0.9 | ND | ND | ND | ND |
| 2    | 17.8 ± 2.1 | 1.1 ± 0.5 | 8.7 ± 1.7 | ND | ND | ND | 5.2 ± 1.8 | ND | ND | ND | ND |
| 3    | 40.4 ± 1.1 | 1.9 ± 0.9 | 23.9 ± 2.1 | ND | 37.8 ± 1.9 | ND | 25.1 ± 0.2 | ND | ND | 17.0 ± 0.4 | 10.0 ± 1.5 |
| 4    | 38.9 ± 1.7 | ND | 16.5 ± 0.9 | 14.6 ± 0.9 | 5.6 ± 0.1 | 7.4 ± 0.9 | 15.2 ± 1.0 | 6.1 ± 0.4 | 6.4 ± 0.7 | ND | ND |
| Mean | 30.3 ± 4.8 | 1.2 ± 0.3 | 14.7 ± 2.5 | 14.2 ± 0.5 | 16.7 ± 1.1 | 7.4 ± 0.9 | 12.5 ± 0.9 | 6.1 ± 0.4 | 6.4 ± 0.7 | 17.0 ± 0.4 | 10.0 ± 1.3 |

* Cytotoxicity at A:T cell ratio 10:1, taken from a regression curve, see Materials and Methods.
† ND, Not done.

In Table I, experiment 2, after treatment with anti-Thy 1.2 plus complement, there is complete abrogation of all cytotoxicity, against every target, after this treatment. Tables Va and b indicate that it is unlikely that the target antigens of the cross-reactive cytotoxicity are the public antigens identified serologically. Table Va lists the public specificities against which responses might be directed in an H-2^a anti-H-2^b response, and in an H-2^a anti-H-2^b response. Below this are listed the public H-2 specificities of the independent haplotypes k, q, s, r, b, and f. This information is taken from Klein (7). From the information in Table Va, the predictions of Table Vb can be made. The most important and easily tested predictions are the negative ones, i.e. that H-2^a and H-2^b targets should not be cross-killed by either q anti-b or s anti-b if serologically identified public specificities are the target antigens. However, it can be seen from Tables I and II that these predictions are not fulfilled, because both H-2^a and H-2^b targets are killed by both attackers. These results would appear to rule out the possibility that known serologically identified public antigens are targets for T-cell cytotoxicity, and may indicate that cytotoxic T cells have a different repertoire of reactivities from B cells, a point which has recently been made in an analysis of cytotoxic responses among H-2^b mutant strains (8). It could be argued that the cytotoxicity generated between two strains which differ across the whole MHC, including I as well as K/D differences, could be also directed against I region determinants, and that the cross-reactivities seen might be due to shared Ia specificities. That this is not the sole explanation of cross-reactive cytotoxicity is shown by experiments reported in Table VI, where cytotoxic responses were generated between H-2^b and H-2^b mutant strains. The H-2^a and H-2^b mice are H-2K^b mutants, and H-2^bo is an H-2D^b mutant (9, 10). The mutational events are thought to be restricted to the K or D end coded molecule, respectively, and certainly there is no evidence of any I region involvement (9, 10). Nevertheless, the high level of cytotoxicity shown against the specific target (first column) is also accompanied by cross-reactivity against target cells of H-2^k/d, H-2^d, H-2^a, H-2^b, H-2^k/q, and H-2^b haplotypes.

From the experiments reported above, both with anti-allogeneically induced cytotoxic responses, and with anti-K/D mutant cytotoxic responses, it would seem that if the MLC response is the in vitro correlate of graft rejection, then cross-priming for second-set graft rejection should be seen. There is already one report that this may be so (11), by judging histological evaluation of the second grafts early during the rejection process. We set up further experiments to investigate this point, with conventional macroscopic evaluation of second skin grafts to determine the median...
survival time (MST) of these grafts. Two groups of male B10.G mice were skin grafted for the first time either (1) with autologous male B10.G skin or (2) with male B10 skin. 35 days later, well after all the primary grafts in group 2 had been rejected, each group of mice was divided into two and regrafted, on the contralateral side, with either B10 male skin or B10.S male skin. The results in Fig. 1 show that autologous grafting of B10.G mice with B10.G skin leads to a primary rejection (MST 12.5 day) of subsequently grafted B10 or B10.S skin; see curves (a) and (b). Allogeneic priming of B10.G mice with B10 skin however elicits a more rapid rejection (MST 10.5 and 9.5 day) of subsequently grafted B10 or B10.S skin; compare curves (c) and (d) with curves (a) and (b) indicating presensitization by the first graft. Thus it would seem that B10 does cross-prime for a second set response to B10.S alloantigens on skin. Thus the in vivo data correlates with the in vitro findings of cross-reactivity at the level of the cytotoxic cell for anti-H-2 responses. This is of interest, since control of cytotoxicity and of graft rejection have recently been dissociated for responses to the male specific antigen, H-Y (12). It may be that such a disassociation for anti-H-2 responses is never seen because of the ubiquitous nature of both types of response to H-2 antigens.

In contrast, the very fine specificity of an H-2 restricted response of various types of responder mice to the male specific antigen, H-Y, is shown in Table VII. Responder status with respect to the generation of these cytotoxic responses is associated both with a dominant Ir gene(s) of the H-2\textsuperscript{b} haplotype, mapping in IA, and with complementary Ir genes, at least some mapping in IC, of a variety of haplotypes which themselves are nonresponders, but which give responder F\textsubscript{1} hybrids (13). One
TABLE IV

Effect of Anti-Thy 1.2 Plus Complement on Cross-Reactive Cytotoxicity Shown by H-2* and H-2b MLC Cells

| Cytotoxicity on target cells* | H-2b | H-2* | H-2a | H-2d | H-2b |
|------------------------------|------|------|------|------|------|
| Group                        |      |      |      |      |      |
| Untreated                    | B10  | B10 S| B10 G| B10 D2| B10 BR|
| Complement alone             | 17.8 ± 2.1 | 1.1 ± 0.5 | 7.4 ± 1.1 | 5.2 ± 1.8 | 8.7 ± 1.7 |
| Anti-Thy 1.2 plus complement | 22.8 ± 4.4 | 1.1 ± 0.3 | 6.3 ± 1.4 | 6.6 ± 1.5 | 8.6 ± 1.7 |

* See footnotes on Table I.

TABLE V

An Analysis of Public H-2 Antigens as Targets for Cross-Reactive Cytotoxicity

(a) The public H-2 antigens:

| Sensitization  | K end | D end |
|----------------|-------|-------|
| H-2* Anti-H-2b |      |       |
| H-2* Anti-H-2b | 35    | 36    | 39    |
|                | 27    | 29    | 35    | 39    |

(b) The predictions if public specificities are targets:

| H-2* Anti-H-2b | Positive | H-2* and H-2b if 33/36 are targets on |
|                |          | " H-2b if 39/46 are targets |
|                |          | " Negative H-2* and H-2b on |
|                |          | " Positive H-2* if 27/28/29 are targets on |
|                |          | " H-2* if 27/39 are targets |
|                |          | " H-2b if 27/29/35 are targets |
|                |          | " Negative H-2b and H-2* on |

very marked feature of anti-H-Y responses is their propensity to be associated, in terms of target cell specificity, with either K or D end antigens, and only rarely with both. In this respect the responses differ somewhat from the H-2 restricted cytotoxic responses to viruses (14) or haptens (15) which are usually associated with both K and
Table VI

Specificity of Cytotoxicity Generated in MLC between H-2<sup>b</sup> Mutant Strains, and between H-2<sup>b</sup> Mutant Strains and their Strain of Origin

| Sensitized cells | Target cells |
|------------------|--------------|
| H-2<sup>b</sup> Anti-H-2<sup>b</sup> | B10.B6 | B10.A | B10.M | AKM | B10.RIII |
| H-2<sup>b</sup> Anti-H-2<sup>b</sup> | 67.6 ± 3.5 | ND | ND | 14.0 ± 1.6 | ND | 7.9 ± 1.0 | 16.3 ± 1.6 | 17.2 ± 1.6 | 11.7 ± 1.2 |
| H-2<sup>b</sup> Anti-H-2<sup>b</sup> | 68.3 ± 1.8 | ND | ND | 12.2 ± 0.7 | ND | 8.0 ± 1.0 | 16.0 ± 0.9 | 14.8 ± 0.9 | 7.1 ± 0.1 |
| H-2<sup>b</sup> Anti-H-2<sup>b</sup> | 72.0 ± 0.7 | ND | ND | 26.5 ± 1.3 | ND | 7.9 ± 0.3 | 16.3 ± 0.3 | 35.5 ± 1.4 | 7.4 ± 0.9 |
| H-2<sup>b</sup> Anti-H-2<sup>b</sup> | 1.3 ± 0.6 | 50.8 ± 2.5 | 0.1 ± 0.3 | ND | 7.8 ± 1.1 | 4.8 ± 0.7 | 13.2 ± 1.2 | ND | ND |
| H-2<sup>b</sup> Anti-H-2<sup>b</sup> | 629 ± 3.9 | 564 ± 3.5 | 56.6 ± 4.0 | ND | 16.4 ± 1.1 | 8.3 ± 0.5 | 27.5 ± 2.1 | ND | ND |
| H-2<sup>b</sup> Anti-H-2<sup>b</sup> | 60.0 ± 2.3 | 38.0 ± 2.6 | 60.4 ± 4.8 | ND | 20.4 ± 0.7 | 8.3 ± 0.4 | 20.2 ± 2.2 | ND | ND |

FIG. 1. Survival curves of second skin grafts of either B10 (groups a and c) or B10.S (groups b and d) placed on B10.G mice 25 days after a primary graft of syngeneic B10.G skin (a and b) or allogeneic B10 skin (c and d). All donors and recipients were male.

D antigens of any given haplotype. However, the most striking feature of these H-Y responses is the extraordinary specificity of the H-2 restriction, thus, H-2<sup>b</sup> (D<sup>b</sup>) associated anti-H-Y cytotoxic cells do not kill male cells of other haplotypes (exp. 1 and 2, Table VII), in marked contrast to the cross killing shown in Tables I and II, where anti-H-2<sup>b</sup> cytotoxic cells generated from H-2<sup>g</sup> or H-2<sup>a</sup> cells significantly cross killed a wide range of independent haplotypes. Likewise H-2<sup>a</sup> (D<sup>a</sup>) associated anti-H-Y killer cells did not cross kill other haplotypes nor do H-2<sup>b</sup> or H-2<sup>a</sup> associated killers cross kill (exp. 3–8, Table VII). This is true both when one is considering the anti-H-Y responses controlled by the dominant H-2<sup>b</sup> Ir gene(s) (exp. 1–3, 7, 8, Table VII) or the complementary Ir gene(s) of other haplotypes (exp. 4–6). Such data might argue for the associative H-2 antigen for H-2 restricted cytotoxicity being the private K or D end specificity, as defined serologically. However, this is unlikely in view of the failure of K and D end mutant strains, which share the same private specificity with the strain of origin (e.g. H-2<sup>ba</sup> and H-2<sup>bf</sup> are serologically difficult to distinguish from H-2<sup>b</sup> mice) to substitute for the strain of origin as virus infected or H-Y carrying target cells (16, 17). Such H-2 restricted data using mutant mouse strains as responders also argue for their being private specificities uniquely seen by T cells (17). The only H-Y responders for which the H-2 restriction is not limited to either end are those
### Table VII

**Specificity of Anti-H-Y Cytotoxic Responses**

| Exp. | Responder | Antigens in vivo and in vitro | Target cells (haplotype) | Specific cytotoxicity* | Associative antigen |
|------|-----------|-----------------------------|--------------------------|-----------------------|-------------------|
| 1    | B10?      | B10? (bbbbb)                | B10? (bbbbb)             | 18.5 ± 1.6            | D^p               |
|      |           | bbbbbbb                     | B10A(2R)? (kkkdb)        | 15.8 ± 1.8            |                   |
|      |           |                             | B10A(2R)? (kkkdb)        | −1.6 ± 1.2            |                   |
|      |           |                             | B10A(5R)? (bbbd)         | 0.78 ± 0.3            |                   |
|      |           |                             | BALB/cf (dddddd)         | 2.3 ± 0.8             |                   |
| 2    | (B10 × BALB/c)F_12 | B10? (bbbbb)                | B10? (bbbbb)             | 19.9 ± 3.4            | D^p               |
|      |           | bbbbbbb                     | B10? (bbbbb)             | 1.7 ± 0.9             |                   |
|      |           |                             | CBA? (kkkkk)             | −3.9 ± 1.1            |                   |
|      |           |                             | BALB/cf (dddddd)         | 0.1 ± 0.7             |                   |
|      |           |                             | Ad (kkkddd)              | 0.9 ± 0.8             |                   |
|      |           |                             | B10A(2R)? (kkkdb)        | 26.7 ± 0.3            |                   |
|      |           |                             | B10A(5R)? (bbbd)         | 0.4 ± 0.5             |                   |
| 3    | (B10 × B10S)F_12 | B10S? (bbbbb)                | B10? (bbbbb)             | 29.2 ± 0.7            | D^p               |
|      |           | bbbbbb/assass               | B10? (bbbbb)             | 4.2 ± 1.1             |                   |
|      |           |                             | B10A(2R)? (kkkdb)        | 26.9 ± 1.7            |                   |
|      |           |                             | B10A(5R)? (bbbd)         | 2.2 ± 0.7             |                   |
| 4    | (CBA × BALB/c)F_12 | CBA? (kkkkk)                | CBA? (kkkkk)             | 48.0 ± 2.9            | K^4 D^4           |
|      |           | kkkkkk/ddddddd              | CBA? (kkkkk)             | −2.8 ± 2.0            |                   |
|      |           |                             | A^d (kkkddd)             | 39.5 ± 2.5            |                   |
|      |           |                             | B10? (bbbbb)             | 0.9 ± 0.1             |                   |
|      |           |                             | B10A(4R)? (kkkbb)        | 23.8 ± 4.3            |                   |
|      |           |                             | B10A(2R)? (kkkdb)        | 20.9 ± 1.4            |                   |
|      |           |                             | C3H.OH? (ddddkk)         | 40.8 ± 1.3            |                   |
|      |           |                             | BALB/cf (dddddd)         | 2.0 ± 3.1             |                   |
| 5    | (CBA × B10.G)F_12 | CBA? (kkkkk)                | CBA? (kkkkk)             | 16.0 ± 0.6            | K^4 D^4           |
|      |           | kkkkkk/qqqqqq               | CBA? (kkkkk)             | 3.6 ± 0.3             |                   |
|      |           |                             | B10.G? (qqqqqq)          | −1.0 ± 0.7            |                   |
|      |           |                             | AQK? (qkkddd)            | −3.2 ± 2.4            |                   |
|      |           |                             | C3H.OH? (ddddkk)         | 12.5 ± 1.4            |                   |
|      |           |                             | A^d (kkkddd)             | 13.7 ± 0.4            |                   |
| 6    | (CBA × B10.G)F_12 | B10.G? (qqqqqq)             | B10.G? (qqqqqq)          | 13.1 ± 1.1            | D^4               |
|      |           | kkkkkk/qqqqqq               | B10.G? (qqqqqq)          | 3.6 ± 0.1             |                   |
|      |           |                             | CBA? (kkkkk)             | 1.0 ± 0.2             |                   |
|      |           |                             | AQK? (qkkddd)            | 0.5 ± 1.1             |                   |
|      |           |                             | C3H.OH? (ddddkk)         | 2.2 ± 1.1             |                   |
|      |           |                             | A^d (kkkddd)             | 1.5 ± 0.5             |                   |
| 7    | (B10.A × B10.A(5R))F_12 | B10.A? (kkkddd)             | B10.A? (kkkddd)         | 40.0 ± 1.9            | K^4 D^4           |
|      |           | kkkddd/bbbddd               | B10.A? (kkkddd)         | −3.1 ± 2.0            |                   |
|      |           |                             | C3H.OH? (ddddkk)         | 20.9 ± 1.3            |                   |
|      |           |                             | B10.BR? (kkkddd)         | 51.2 ± 2.8            |                   |
|      |           |                             | BALB/cf (dddddd)         | −1.6 ± 0.2            |                   |
| 8    | (B10.A × HTD)F_12 | B10.A? (kkkddd)             | B10.A? (kkkddd)         | 40.7 ± 2.5            | K^4 D^4           |
|      |           | kkkddd/bbbddd               | B10.A? (kkkddd)         | 2.8 ± 1.4             |                   |
|      |           |                             | C3H.OH? (ddddkk)         | 47.8 ± 3.9            |                   |
|      |           |                             | B10.BR? (kkkddd)         | 25.5 ± 1.7            |                   |
|      |           |                             | B10.DZ? (dddddd)         | 1.9 ± 1.2             |                   |
|      |           |                             | B10? (bbbbb)             | 3.9 ± 1.1             |                   |

* Cytotoxicity at A:T cell ratio 4:1.
associated with H-2k and exemplified by responses to H-2k male cells by complementary F₁ hybrid females with one H-2k parent. In most cases the associative antigens are at both ends (exp. 4, 5, 7, and 8, Table VII). An insight into a possible underlying reason for these both-end associated responses comes from experiments, done with F₁ female mice having one B10.A parent (Table VII, exp. 7 and 8). When such hybrids are stimulated with B10.A male cells, they kill male targets bearing Kk antigens, fail to kill targets bearing Dd antigens, or K or D end antigens of other independent haplotypes, but, surprisingly, they do kill male targets bearing Dk antigens, with which they have not been ostensibly associated during sensitization. This suggests that there is reduplication of the Kk associative private antigen, as seen by T cells, at Dk.

Discussion

The very clear fine specificity of H-2 restricted cytotoxicity may argue for the differentiation of idiotypically homogeneous clones of T cells during the response to antigens which can only be seen by T cells in association with self H-2. In contrast, an anti-H-2 cytotoxic response may be extremely heterogeneous, consisting of clones of cells with many different specificities, even though a majority may be directed against haplotype-specific private antigens recognized by T cells. Experiments where target cell specificity of putatively individual clones of cytotoxic T cells have been examined also suggest that cross-reactivity lies at the level of different clones having different specificities, rather than any one clone having several different target specificities (18). Our use of H-2b mutant strains to elicit cytotoxicity with H-2b mice provides evidence that the cytotoxic cross-reactivities seen are due to antigens on K or D end coded molecules, rather than Ia antigens. However, the complication of cytotoxic responses to public Ia antigens being generated whenever responses are elicited across the whole MHC cannot be excluded, especially because it is known that cytotoxic cells can be generated between strains just differing at various parts of the I region (19, 20).

If indeed H-2 restricted responses are idiotypically homogeneous while anti-H-2 responses are heterogeneous, then what is the biological significance of these differences? It has been suggested that the raison d'être of H-2K and D antigens is to provide appropriate associative antigens for H-2 restricted responses, which may be of vital biological importance to recovery from virus infections (14). The duplication of genes during the phylogeny of the major histocompatibility complex (MHC), to provide at least two sets of antigens, K and D, as well as the extraordinary polymorphism of H-2K and D antigens, argues for a specialized function of each allelic product, and perhaps this specialization (specificity) provides the fine discriminatory properties observed for the MHC.

In contrast, anti-H-2 responses, both in vivo and in vitro, can hardly have any evolutionary or survival value, because it is unlikely that they have been elicited before the 20th century, unless they play a role in fetomaternal relationships as has been proposed (21). Thus anti-H-2 responses may be an accident, their magnitude a mere reflection of underlying and important anti-altered self responses, and therefore the question of their specificity is not important, nor are they very specific.

Summary

Cross-reactive T-cell cytotoxicity is seen when cytotoxic responses are generated in mixed lymphocyte cultures either between mouse strains which differ at the major
histocompatibility complex, H-2, or between H-2\textsuperscript{a} mutant strains and the strain from which they were derived. This cross-reactivity can be measured with \[^{51}Cr\] labeled target cells from a number of different H-2 haplotypes, and the pattern of cross-reaction indicates that the target antigens are unlikely to be any of the serologically defined public specificities. In contrast, the specificity of H-2 restricted cytotoxic responses, such as that to the male-specific antigen, H-Y, is exquisite, and male cells from strains of mice carrying H-2 haplotypes other than the responder have never been found to act as appropriate targets. The contrast between the specificity of anti-H-2 and H-2 restricted responses may argue for a greater idiotypic homogeneity of the cells making H-2 restricted responses, and the greater specificity of these responses may be necessary for their biological function.

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