H-2 EFFECTS ON CELL-CELL INTERACTIONS IN THE RESPONSE TO SINGLE NON-H-2 ALLOANTIGENS

II. H-2 D Region Control of H-7.1-Specific Stimulator Function in Mixed Lymphocyte Culture and Susceptibility to Lysis by H-7.1-Specific Cytotoxic Cells*

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In a preceding communication we have demonstrated that the relative immunogenicity of the murine H-7.1 alloantigen, as inferred from survival times of H-7.1-incompatible skin grafts, is determined by genes in the D region of the major histocompatibility (H-2) complex (1). Similar observations have been made previously for the male-specific (H-Y) antigen by other investigators (2, 3). The effects of these H-2-linked genes on the ability of non-H-2-incompatible cells to stimulate mixed lymphocyte culture (MLC) reactions or function as targets for cytotoxic effectors have not been investigated.

Extensive evidence has confirmed that the alloantigens which most strongly stimulate T-lymphocyte proliferation in primary MLC are coded for by genes in the H-2 complex (4) and more specifically by genes in the I region (5, 6). H-2K and H-2D alloantigens, most notably those generated by recent mutations, are stimulatory in MLC, although to a lesser degree than I region MLC determinants (6-9). However, the only individual non-H-2 alloantigens shown to be strongly stimulatory in primary MLC are antigens coded for by alleles at the Mls locus (10, 11). Marginally significant stimulation has been observed with single non-H-2 histocompatibility antigens (12) and Thy-1 antigen (13) in primary MLC. Additional attempts, including ours reported in this paper, have not resulted in significant stimulation by single non-H-2 antigens in primary MLC.

In this communication we report that nylon wool purified, splenic T lymphocytes obtained from donors primed with H-4- or H-7-incompatible spleen cells or skin grafts, proliferate in response to incompatible lymphocytes in secondary MLC. Further, we present results obtained in experiments intended to elucidate the role of H-2-linked genes in determining the relative ability of H-7.1-incompatible lymphocytes to stimulate proliferation in secondary MLC and

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‡ Abbreviations used in this paper: H, histocompatibility; MLC, mixed lymphocyte culture; SRBC, sheep erythrocytes.

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generation of H-7.1-specific cytotoxic effector cells. The results reported herein demonstrate that both the relative stimulation capacity of H-7.1-incompatible lymphocytes in MLC and susceptibility of H-7.1-incompatible lymphoblasts to lysis in cell-mediated lympholysis are controlled by a gene(s) in the H-2D region.

Materials and Methods

Mice. The mice employed in this study and their respective genotypes are listed in Table I of the preceding paper (1). In addition, B10.129 (21M) (referred to as B10.129-H-4a) mice were derived from breeding stock provided by Dr. George D. Snell, of The Jackson Laboratory, Bar Harbor, Maine. The B10.129-H-4a strain is congenic with B10, differs at only the H-4 locus (14) and in so doing, defines the H-4a: H-4b allelic combination, in turn the H-4.1 and H-4.2 alloantigens (15, 16).

MLC. MLC conditions were modified from those described by Peck and Bach (17). Protein-free Click's medium was prepared as described (18) with the exception that nucleic acid precursors were omitted, Heps added to a concentration of 30 mM, and penicillin-streptomycin replaced by gentamycin (Schering Corp. Kenilworth, N. Y.). Click's medium was supplemented with normal mouse serum (collected from donors syngeneic with responder cell donors) to a concentration of 0.5%. Stimulator cells were obtained from spleen cell suspensions after Tris-ammonium chloride treatment to lyse erythrocytes. The lymphocytes were treated with mitomycin C (Sigma Chemical Co., St. Louis, Mo.) at a concentration of 25 μg/2 × 10^7 cells for 30 min, washed, and adjusted to a concentration of 5 × 10^6 viable cells/ml. Spleens from responder mice were excised and reduced to a single cell suspension. Erythrocytes were lysed with Tris-ammonium chloride treatment and T lymphocytes were selected from the remaining lymphocyte population by passage over nylon wool columns according to the technique described previously (19). B-cell contamination of nylon wool T-cell suspensions was monitored by direct immunofluorescence testing with fluoresceinated, polyvalent goat anti-mouse immunoglobulin (Antibodies Inc., Davis, Calif.). B-cell contamination consistently varied between 5 and 10%; suspensions with greater than 10% B-cell contamination were discarded. Responder cell suspensions were adjusted to 5 × 10^6 viable cells/ml. Responder (5 × 10^5 cells) and stimulator (5 × 10^3 cells) cells were mixed in 0.20-ml quadruplicate cultures in wells of microtiter plates (Falcon Plastics, Oxnard, Calif.). Microtiter plates were placed in humidified Lucite boxes, gassed with 10% CO2, 7% O2, and 83% N2 (20), and incubated at 37°C for 3-5 days. 24 h before harvest, 2 μCi [3H]thymidine (2 Ci/mM) was added to each well in 50 μl unsupplemented Click's medium. Lymphocytes were collected on glass fiber filters and washed with normal saline and methanol with the aid of a semiautomatric cell harvester (Otto Hiller Co., Madison, Wis.). [3H]thymidine uptake was determined by using a scintillation counter (Beckman Instruments, Fullerton, Calif.). Mean uptake (± standard error) in quadruplicate wells was calculated. The significance of differences between means of allogeneic and syngeneic cultures was determined by Students' t test assuming equal variance (21). Antigen-specific uptake (allogeneic mean - syngeneic mean) was calculated when the allogeneic mean was significantly greater than the syngeneic mean at P < 0.001.

Cell-Mediated Lympholysis. The cell-mediated lympholysis assay was a short term 51Cr release assay performed as described (22). Effector cells were prepared by coculturing responder and stimulator cells at a 1:1 ratio for 5 days under conditions similar to those employed for the MLC assay; 5 ml of each suspension were mixed in 60-mm Petri dishes. Surviving lymphocytes were washed in RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 5% heat-inactivated fetal calf serum and the cell concentration adjusted to 2 × 10^6 viable cells/ml. Lymphoblast targets were prepared by incubating spleen cells (3 × 10^6 cells/ml) in RPMI-1640 supplemented with concanavalin A (2 μg/ml) (Calbiochem, La Jolla, Calif.) for 3 days. Blast cells were partially purified by differential centrifugation at 50 g and subsequently labeled with 51Cr. Labeled lymphoblasts were washed five times and adjusted to 1 × 10^6 cells/ml. One-half milliliter of effector and 51Cr-labeled target cell suspensions were mixed in 35-mm plastic dishes in an effector: target ratio of 20:1. The dishes were placed in a humidified Lucite box, gassed with 10% CO2, 7% O2, and 83% N2, rocked (seven cycles/minutes), and incubated at 37°C for 4 h. The cell suspensions were transferred to tubes and spun at 450 g for 10 min. One-half milliliter was
**H-2D Control of H-7.1 in Vitro Immunogenicity**

**Table I**

The Lack of Proliferative Response of B10 Normal Spleen Cells to H-4.2-Incompatible Stimulators in Primary MLC

| Stimulator cell | Relevant H locus incompatibility | Mean \(^{3}H\)uptake ± SE at 3 days | Mean \(^{3}H\)uptake ± SE at 4 days | Mean \(^{3}H\)uptake ± SE at 5 days |
|-----------------|----------------------------------|------------------------------------|------------------------------------|------------------------------------|
| B10.129-H-4 b   | H-4                              | 1,762 ± 359                        | 1,919 ± 511                        | 1,613 ± 361                        |
| B10.A(1R)       | H-2                              | 11,617 ± 1,666                     | 18,973 ± 1,761                     | 19,978 ± 1,297                     |
| B10             | None                             | 1,763 ± 199                        | 2,206 ± 517                        | 1,714 ± 219                        |
| -               | -                                | 1,107 ± 72                         | 1,377 ± 583                        | 1,104 ± 326                        |

removed from each sample for counting in a well-type gamma counter (Beckman Instruments, Fullerton, Calif.). Percent release was determined by the following formula:

\[
\text{Percent specific release} = \frac{\text{experimental cpm-spontaneous cpm (targets alone)}}{\text{total releasable cpm-spontaneous cpm (targets alone)}} \times 100
\]

**Immunization Regimen.** Responder cells for the tests for H-2D dependence of stimulatory capacity of H-7.1-incompatible stimulators in MLC were obtained from (B10.C(H-2\(^b\))-H-7\(^b\) x B10-H-2\(^b\)-H-7\(^b\))F\(^1\) recipients of primary-quaternary H-7.1-incompatible skin grafts. These grafting procedures have been described in detail in the preceding communication (1). Spleens were excised 4 wk after quaternary grafting. T lymphocytes purified by passage through nylon wool columns served as both responders for MLC and precursors for cytotoxic effectors in CML.

**Results**

**Primary and Secondary MLC Responses to Single Non-H-2 Histocompatibility Antigens.** To determine if primary MLC responses could be detected in this test system, normal B10 responder spleen cells were mixed with H-4.2-incompatible stimulator cells from B10.129(H-2\(^b\))-H-4\(^b\) donors and the resulting proliferation measured by \(^{3}H\)thymidine incorporation. These results are presented in Table I. No significant proliferation was observed in primary MLC. Significant proliferation was observed when the same cells were stimulated by H-2-disparate stimulators. To amplify the frequency of H-4.2-specific responder lymphocytes in responder populations, responding cell donors were primed in vivo with either multiple sets of H-4.2-incompatible skin grafts or a single intraperitoneal injection of varying numbers (1-20 \(\times\) 10\(^6\)) of H-4.2-incompatible spleen cells. Spleens excised 1 wk after graft rejection or injection were the source of responder lymphocytes. The responder spleen cells were mixed with syngeneic and H-4.2-incompatible stimulators. The results of these assays are presented in Table II. Spleen cells obtained from donors primed with either H-4.2-incompatible skin grafts or lymphocytes responded with significant proliferation to H-4.2-incompatible stimulators, thus demonstrating the utility of priming with either skin grafts or spleen cells. To determine if the specific response could be improved by using purified responder T cells the response of splenic T lymphocytes obtained from B10 donors primed with a single injection of 10 \(\times\) 10\(^6\) H-4.2-incompatible B10.129(H-2\(^b\))-H-4\(^b\) spleen cells was tested 7 days postinjection. The results of this assay are included in Table II. Responder T cells were observed to proliferate in response to H-4.2-incompatible stimulators, suggesting that the responding cells in the spleen were T lymphocytes. These observations demonstrated that non-H-2 H antigens are
Table II

| Immunizing cells | Stimulator cells | Mean [3H] uptake (cpm ± SE) |
|------------------|------------------|-----------------------------|
|                  |                  | 3 days | 4 days | 5 days |
| Skin allografts  |                  |        |        |       |
| Three sets       | B10.129-H-4b     | 5,261 ± 1,023 (3,168) | 9,589 ± 2,479 (7,969) | 25,040 ± 1,550 (21,594) |
|                  | B10.A(1R)        | NT     | NT     | NT    |
|                  | B10              | 2,093 ± 444 | 1,820 ± 393 | 3,446 ± 566 |
|                  | -                | 1,380 ± 150 | 1,427 ± 375 | 2,165 ± 670 |
|                  |                  |        |        |       |
| Spleen cells     |                  |        |        |       |
| 1 x 10⁸          | B10.129-H-4b     | 2,834 ± 484 (1,277) | 1,576 ± 76 (988) | 1,821 ± 113 (1,126) |
|                  | B10.A(1R)        | 13,861 ± 837 (11,509) | 21,264 ± 2,879 (20,680) | 27,776 ± 2,872 (27,061) |
|                  | B10              | 1,357 ± 36 | 588 ± 205 | 695 ± 251 |
|                  | -                | 947 ± 97 | 862 ± 299 | 738 ± 231 |
|                  |                  |        |        |       |
| Spleen cells     |                  |        |        |       |
| 5 x 10⁸          | B10.129-H-4b     | 4,753 ± 215 (2,731) | 6,747 ± 1,226 (4,221) | 8,483 ± 2,017 (5,899) |
|                  | B10.A(1R)        | 15,124 ± 951 (13,109) | 23,394 ± 2,140 (20,868) | 24,240 ± 809 (21,656) |
|                  | B10              | 2,022 ± 317 | 2,526 ± 443 | 2,584 ± 900 |
|                  | -                | 1,462 ± 164 | 1,856 ± 419 | 2,592 ± 531 |
|                  |                  |        |        |       |
| Spleen cells     |                  |        |        |       |
| 10 x 10⁸         | B10.129-H-4b     | 5,058 ± 851 (2,962) | 10,463 ± 1,528 (7,869) | 9,238 ± 1,446 (5,846) |
|                  | B10.A(1R)        | 13,526 ± 2,402 (11,430) | 21,067 ± 2,524 (18,473) | 18,862 ± 2,928 (16,470) |
|                  | B10              | 2,096 ± 251 | 3,290 ± 221 | 3,392 ± 531 |
|                  | -                | 1,611 ± 145 | 1,689 ± 140 | 2,034 ± 195 |
|                  |                  |        |        |       |
| Spleen cells     |                  |        |        |       |
| 20 x 10⁸         | B10.129-H-4b     | 9,076 ± 1,086 (6,584) | 9,168 ± 1,044 (5,878) | 8,010 ± 1,401 (4,560) |
|                  | B10.A(1R)        | 14,289 ± 1,442 (11,797) | 20,061 ± 2,029 (16,771) | 17,445 ± 762 (13,960) |
|                  | B10              | 2,492 ± 230 | 3,290 ± 583 | 3,450 ± 892 |
|                  | -                | 1,705 ± 268 | 2,399 ± 291 | 2,538 ± 180 |
|                  |                  |        |        |       |
| Spleen cells     |                  |        |        |       |
| 10 x 10⁸         | B10.129-H-4b     | 1,925 ± 210 (1,021) | 3,004 ± 251 (3,507) | 4,903 ± 1,415 (4,344) |
|                  | B10.A(1R)        | NT | NT | NT |
|                  | B10              | 404 ± 75 | 407 ± 120 | 597 ± 190 |
|                  | -                | 284 ± 127 | 818 ± 202 | 157 ± 78 |

* A computed when mean uptake is significantly different from syngeneic combinations at P < 0.001.
† Not tested.
§ Response of nylon wool purified T cells.

stimulatory in MLC, although in vivo priming is required, presumably to increase the frequency of antigen-specific responder T cells to a level detectable by [3H]thymidine uptake.

H-2D Control of Stimulatory Capacity of H-7.1-Incompatible Stimulators. In a companion paper we presented evidence indicating that a gene(s) in the H-2D region regulates the relative rejectability of H-7.1-incompatible skin grafts (1). We have further investigated the possible role of this gene in regulating the ability of H-7.1-incompatible spleen cells to stimulate proliferation in MLC. Preimmunized (B10.C(H-2b)-H-7b x B10-H-2a-H-7b)F1 responder cells were mixed with H-7.1-incompatible, H-2-compatible stimulator cells from donors with different H-2 haplotypes. The results of these assays are presented in Table III. B10 stimulators cells were optimally stimulatory (Δ = 28,505 at 5 days) whereas B10.A stimulators were comparatively ineffective (Δ = 2,186 at 5 days). To ensure that proliferation of F1 responder cells was H-7.1-specific and not specific for Hh-1 determinants (23, 24) expressed on homozygous H-2b stimulators, two controls were performed. First, B10.C(H-2b)-H-7b and B10-H-2b-H-7b parental strain spleen cells were tested for stimulation and were found to be equally nonstimulatory. These cells are H-7 compatible but should express Hh-1 determinants identical to those of B10 and B10.A stimulators,
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TABLE

Proliferative Response of (B10.C-H-7b × B10-H-2b)F1 Lympohocytes Primed with Multiple Graftings of H-7.1-Incompatible Grafts

| Stimulator cell donor | Origins of H-2 subregions | Mean [H] uptake (cpm ± SE [h*]) |
|-----------------------|---------------------------|---------------------------------|
|                       | K  | A  | B  | J  | E  | C  | S  | D  | Tla | H-7 |
|                       | 3 days | 4 days | 5 days | 3 days | 4 days | 5 days |
| C57BL/10              | b  | b  | b  | b  | b  | b  | b  | a  | 8,644 ± 740 (7,282) | 18,756 ± 3,625 (17,219) | 30,123 ± 5,593 (28,506) |
| B10.A                 | k  | k  | k  | k  | k  | d  | d  | a  | 2,507 ± 425 (886)  | 2,796 ± 533 (1,228)  | 3,852 ± 1,180 (2,196)  |
| (B10.A × B10.C-H-7 b) | b  | b  | b  | b  | b  | b  | b  | a  | 7,312 ± 983 (5,650) | 13,815 ± 1,282 (12,378) | 20,708 ± 3,368 (19,090) |
| B10(1R)               | k  | k  | k  | k  | k  | d  | d  | d  | 7,547 ± 1,144 (5,888) | 15,732 ± 1,231 (14,396) | 18,521 ± 2,633 (16,905) |
| B10(4R)               | k  | k  | b  | b  | b  | b  | b  | a  | 7,312 ± 983 (5,650) | 13,815 ± 1,282 (12,378) | 20,708 ± 3,368 (19,090) |
| B10(5R)               | b  | b  | b  | k  | k  | d  | d  | d  | 1,893 ± 355 (722)  | 3,725 ± 847 (3,109)  | 5,932 ± 1,511 (5,310)  |
| B6-T/a a              | b  | b  | b  | b  | b  | b  | b  | b  | 1,662 ± 241 (977)  | 4,241 ± 460 (3,680)  | 7,799 ± 948 (6,716)   |
|                       | 993 ± 194 | S37 ± 22 | 383 ± 140 | 593 ± 241 | 4,241 ± 460 | 7,799 ± 948 |

* a computed when mean is significantly different from the mean for the combination of (B10.C-H-7 b × B10-H-2b)F1 + B10.C-H-7 b at p < 0.001.

respectively. Second, (B10 × B10.A)F1, spleen cells which should not express Hh-1 determinants but do express the relevant H-7.1 antigen effectively stimulated proliferation in MLC. These results confirmed that the differential proliferative response of (B10.C-H-2b-H-7 b × B10-H-2b-H-7 b)F1 responders to B10 and B10.A stimulators was H-7.1-specific and not Hh-1-specific. The relatively high stimulation capacity of B10 stimulators was inherited as a dominant trait. The map position of the gene controlling the relative stimulation capacity of H-7.1-incompatible stimulators was determined through the use of stimulator cells from mice with H-2 haplotypes derived from intra-H-2 recombinations between H-2 b and H-2 D. Spleen cells obtained from B10.A(1R) and B10.A(4R) donors stimulated proliferation to a level comparable to that induced by B10 stimulators (Table III). However, B10.A(5R) spleen cells were similar in stimulatory capacity to B10.A spleen cells. The haplotype origins of the H-2 regions of these H-2 recombinants (Table III) suggests that the gene controlling the efficacy of H-7.1-incompatible stimulators maps in the region telomeric to the H-2D-Tla a recombination site.

The high proliferation induced by B6-Tla a spleen cells further maps the gene to the D region as defined by the H-2D-Tla a recombination site on the centromeric side and the site of the recombination separating H-2 a and Tla a in the production of B6-Tla a on the telomeric side. It should be noted that B6-Tla a differs from B10 at a minimum of one non-H-2 H locus independent of H-2 (25) and at H-31 and H-32 linked to H-2 (26). The Tla a allele derived from the H-2 a haplotype is shared by B10-H-2b-H-7 b and B6-Tla a, therefore complementing for the H-31 and H-32 alleles of B6-Tla a which map in the H-2D-Tla a interval (27). The inability to detect proliferation in primary MLC to single non-H-2 H antigens makes a primary response to non-H-2 H antigens of B6-Tla a not shared with B10 highly unlikely. The single exception to the D region map position of the operative regulatory gene is the stimulatory capacity of B10.A(18R) spleen cells. B10.A(18R) spleen cells were intermediate stimulators (Δ = 11,953 cpm at 5 days) despite the fact that they are H-2D a; this observation suggests that the control of stimulating capacity of H-7.1-incompatible stimula-
| Boosting stimulator cells | Mean percent lysis of $^{35}$Cr lymphoblast targets |
|--------------------------|--------------------------------------------------|
|                          | B10 | B10.A | (B10.A × B10.A)F | B10.A(1R) | B10.A(5R) | B6-Tla* | B10.A-H-7* |
| B10                      | 39  | 0     | 45               | 70        | 9         | 45      | 0         |
| B10.A                    | 0   | 0     | 23               | 41        | 5         | 26      | 0         |
| (B10.A × B10.A)F         | 45  | 0     | 52               | 67        | 10        | 41      | 0         |
| B10.A(1R)                | 45  | 0     | 55               | 61        | 7         | 44      | 0         |
| B10.A(5R)                | 0   | 0     | 0                | 3         | 8         | 8       | 0         |
| B10.C-H-7               | 0   | 0     | 0                | 0         | 6         | 7       | 0         |

* Specific release < spontaneous release.

H-2 Determination of Capacity of H-7.1-Incompatible Stimulators to Boost for Restricted Lysis of H-7.1-Incompatible Targets

Boosting stimulator cells are under multigenic control by genes in the S-D interval. The 18R recombinant could then be assumed to carry one of the high stimulation genes derived from the H-2^b haplotype which would map to the centromeric side of the H-2^18 recombination site, and to carry a low stimulation gene derived from the H-2^c haplotype mapping in the D region as bounded on the centromeric side by the H-2^18 recombination site.

H-2D Control of Stimulation of Generation of H-7.1-Specific Cytotoxic Effector Cells. We determined the effects of H-2D genotype on the effectiveness of H-7.1-incompatible spleen cells in stimulating generation of H-7.1-specific effectors and susceptibility of H-7.1-incompatible lymphoblast targets to lysis. Prenmunized (B10.C(H-2^b)-H-7 b × B10-H-2 a H-7 a)F1 responder nylon wool T cells were stimulated with H-7.1-incompatible stimulators from donors with different H-2 haplotypes in macro MLC and the surviving cells tested for their ability to lyse H-7.1-incompatible, $^{35}$Cr-labeled lymphoblast targets. The results of these CML assays are presented in Table IV. The important observations may be summarized as follows: (a) H-7.1-incompatible stimulators possessing H-2D^b more effectively stimulated generation of H-7.1-specific effectors than H-2D^a homozygous stimulators and (b) H-7.1-incompatible, lymphoblast targets possessing the H-2D region of H-2^b were sensitive to detectable H-7.1-specific lysis whereas targets homozygous for the H-2D region of H-2^a were not. It is particularly important to emphasize that coexpression of H-7^a and H-2D^b by lymphoblast targets is essential for susceptibility to H-7.1-specific cytotoxic activity. The reciprocal H-2 restriction (involving F1 effector lysis exclusively of targets sharing the H-2 haplotype of the boosting cells) of the type reported for non-H-2 H antigens was not observed with H-7.1-incompatible lymphoblast targets. Targets must express H-2D^b and share a segment of the H-2 complex with the MLC stimulators and express H-7^a. Of particular interest was the observation that effectors boosted by B10.A stimulators lysed B6-Tla^a targets. Although B6-Tla^a and B10.A differ at both H-2K and H-2D, they share the genes in the region telomeric to the recombination splitting H-2D^a and Tla^b in the selection of B6-Tla^a. This result would suggest that the identity between MLC stimulator and CML target was not required at H-2D but rather at a locus telomeric to H-2D. H-2 identity between stimulator and
target without target expression of H-2D\(^d\) is simply not sufficient for detectable lysis as demonstrated by the inability of B10.A stimulators to boost for detectable lysis of B10.A lymphoblast targets (Table IV). It is interesting to note that while B10.A stimulators cannot stimulate the production of cytotoxic cells which can kill B10.A targets, they do stimulate the production of effectors which can kill cells which express H-2D\(^b\) and share a portion of the H-2 complex with H-2\(^a\). Thus (B10 × B10.A)F\(_1\), B10.A(1R), and B6-Tla\(^a\) targets are lysed by effectors stimulated by B10.A cells whereas B10 and B10.A targets are not. The inability of B10.A(5R) to stimulate generation of H-7.1-specific cytotoxic effectors suggests that such stimulation by H-2D\(^d\) stimulators may require simultaneous expression in the stimulator of an H-2\(^a\) allele at a locus centromeric to the H-2\(^{5}\) recombination site and H-2D\(^d\).

**Discussion**

In this communication we have shown that stimulator cells differing at single non-H-2 histocompatibility (H) loci (H-7 and H-4) stimulate significant proliferation of responder T lymphocytes in secondary mixed lymphocyte culture. In addition, the stimulatory capacity of H-7.1-incompatible stimulators and susceptibility of targets to lysis are regulated by a gene(s) in the H-2D region. It would appear that in vivo priming is required to expand the number of T cells specific for single non-H-2 H antigens enabling detection of proliferation in MLC. This supposition would predict that the frequency of T lymphocytes responsive to alloantigens in MLC range in frequency from those T cells in relatively high frequency, responsive to I region determinants and Mls antigens, to those T cells responsive to single non-H-2 histocompatibility alloantigens normally present in relatively low frequency. The relative levels of proliferation in MLC across H-2 and HLA barriers were believed to correlate inversely with the probability of graft rejection across the same barriers, until a lack of correlation was shown, initially for HLA(28) and subsequently for H-2 (5). On the contrary, the results in this communication and preliminary data on Ir gene control of the response to single non-H-2 H antigens demonstrate that relative levels of proliferation in secondary MLC inversely correlate with relative survival times of skin allografts transplanted across the same non-H-2 H barriers. The survival time of H-4.2-incompatible skin grafts is dependent on recipient H-2 genotype (29); H-2\(^b\) and H-2\(^d\) recipients are fast responders whereas H-2\(^a\) recipients are slow responders. Splenic T cells obtained from primed H-2\(^b\) and H-2\(^d\) donors proliferate in response to H-4.2-incompatible stimulators in secondary MLC whereas H-2\(^a\) T cells do not (P. J. Wettstein, unpublished observations).

The actual stimulator cell in mitomycin C-treated, H-7.1-incompatible spleen cell suspensions has not been identified. It has been inferred that non-H-2 H antigens are expressed on macrophages, based on the observations that: (a) non-H-2-incompatible hyperimmune spleen and peritoneal exudate cells (which transfer anti-SRBC immunity) are inactivated by recipients in vivo (30–33) and (b) the cells actually required for transfer of immunity are macrophages (34). We are presently investigating the identity of the actual cell stimulating non-H-2 H antigen-specific proliferation in secondary MLC. The stimulation of
non-H-2 H antigen-specific proliferation may be analogous to stimulation of guinea pig T cells by antigen-pulsed macrophages (30). If this situation were the case, an extensive study of this in vitro response may yield important information regarding the genetic control of macrophage:T cell and target:effector interactions.

The antigenic specificity of H-2D control of relative immunogenicity of non-H-2 H antigens is presently unknown. We are examining the effects of H-2D region genes on the immunogenicity of H-4, H-3, and H-Y alloantigens. It has been suggested that H-Y immunogenicity is controlled by a gene(s) in the H-2K end (c) although the data do not strongly support the proposed mapping. However, H-2D control of susceptibility of H-Y-positive lymphoblast targets to H-Y-specific lysis would account for the difference in susceptibility of B10.A and B10.A(2R) male targets (35) previously suspected to be the result of H-2D-linked restriction. If, as we suspect, the H-2 control of relative immunogenicity is not limited to the H antigens studied to date, this phenomenon will be important for the study of the response to non-H-2 H antigens in general. H-2 restricted in vitro cytotoxicity specific for multiple non-H-2 H antigens (36) could be partially accounted for by differential control of immunogenicity of subsets of non-H-2 H antigens by different H-2 haplotypes.

If the experiments, in which responders are stimulated by a complex array of H antigens, represented a general case for single H antigens, one would have expected reciprocal restriction of H-7.1-specific effectors. In this communication we have demonstrated that splenic T cells removed from animals primed to H-7.1 and boosted in vitro can produce H-2-restricted cytotoxic cells when stimulated by H-7.1-incompatible stimulators possessing H-2D\(^d\). The resistance of H-2D\(^d\) homozygous, H-7.1-incompatible targets to lysis places important limitations on the restriction in vitro since H-2D\(^d\) homozygous stimulators can not boost for cytotoxic effectors capable of lysing H-2D\(^d\) targets. These generated effectors can lyse H-2D\(^h\)-bearing targets if they share part of the H-2\(^h\) haplotype, suggesting classical restriction is operative in vitro but in a manner considerably more complex than predicted by any current restriction theory, either altered self or dual recognition. The unexpected observation that H-7.1-specific effector cells generated by boosting with B10.A stimulators lysed B6-Tla\(^a\) targets suggests that the previously observed requirement for sharing K or D regions between killer and target is not absolute and can also be satisfied by products of a locus telomeric to H-2D in the Tla region. Further experiments are required to confirm this observation and to map the locus responsible for restriction of H-7.1-specific effectors relative to the other genes in the D-Tla interval. The discrepancy between in vivo and in vitro results indicates that rechallenge with non-H-2-incompatible stimulators in vitro results in either a differentiation of previously unrestricted cytotoxic T cells into T cells with restricted cytotoxic activity or selective amplification of a subset of cytotoxic T cells preferentially reactive with targets expressing a particular H-2 haplotype. Selective depletion of proliferating T cells in MLC through the use of high levels of [\(\text{H}\)]thymidine or treatment with bromodeoxyuridine and light and restimulation of the remaining cells should allow us to discriminate between these two alternatives.
Summary

The relative immunogenicity of the H-7.1 alloantigen has been shown in a previous communication to be regulated by a gene in the D region of the mouse major histocompatibility (H-2) complex. The level of relative immunogenicity was inferred from survival times of H-7.1-incompatible skin grafts donated by donors with different H-2 haplotype origins of H-2D region genes. In this communication we report the results of an extension of these previous investigations into the possible role of H-2D region genes in controlling the capacity of H-7.1-incompatible lymphocytes to stimulate H-7.1-specific mixed lymphocyte culture proliferation and generation of cytotoxic effector cells. The results reported herein demonstrate that the H-2D genotype of H-7.1-incompatible stimulator cells determines the relative H-7.1-specific capacity of those lymphocytes to stimulate H-7.1-specific proliferation of in vivo primed responder T cells in secondary mixed lymphocyte culture. H-2Dd-bearing, H-7.1-incompatible stimulators were significantly more effective in stimulating H-7.1-specific proliferation than H-2Dd-bearing stimulators. As expected, H-2Db, H-7.1-incompatible stimulators were also more effective than H-2Da stimulators in generating H-7.1-specific cytotoxic effector cells. Further, the susceptibility of ³¹Cr-labeled, H-7.1-incompatible lymphoblast targets to H-7.1-specific lysis was similarly regulated by an H-2D gene. Reciprocal H-2 restriction (F₁ cells are capable of killing only the cells bearing the immunizing cell parental H-2 haplotype) observed by other investigators for cytolysis of non-H-2-incompatible targets was not observed. H-2Dd-bearing, H-7.1-incompatible stimulators stimulated generation of cytotoxic effectors capable of detectably lysing H-2Db but not H-2Da-bearing, H-7.1-incompatible targets. The impact of these observations on the proposed models for H-2 restriction of non-H-2 histocompatibility antigen-specific cytolysis is discussed.

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