MIXED LYMPHOCYTE CULTURE RESPONSES OF MICE*

GENETIC ANALYSIS OF THE RESPONSES TO *H-2D*^{d} SPECIFICITIES

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The mixed lymphocyte culture reaction (MLR)[1] through its association with the inheritance of the major histocompatibility antigens has received a great deal of interest in the field of transplantation biology (1). The recent demonstration that specific lymphoid cell types, i.e., θ-antigen-bearing thymus-derived lymphocytes (T cells) with allogeneic non-θ-antigen-bearing bone marrow-derived lymphocytes ("B" cells), interact to yield a proliferative response, broadens or extends the usefulness of this assay, namely to study the nature of cell-cell interactions (2). Any information, therefore, relating to the genetic control of responses in the MLR may aid in the delineation of the steps involved not only in the biological pathways of cell activation but also in T- and B-cell interaction.

The responses, in vitro, to the *H-2D* antigenic specificities of the mouse provided us with an ideal system in which to study the genetic control of responses in mixtures of allogeneic lymphocytes (3). The data presented in this paper will demonstrate that: (*a*) genetic material outside of the *H-2D* region is involved in determining the level of the response to *H-2D*^{d} antigens; (*b*) the control of the level of responsiveness was found to be a dominant trait; (*c*) multiple loci are involved in determining the level of MLR responses; (*d*) one of these loci is associated with the *H-2* region though responses to *H-2D*^{d} specificities were also observed, in the absence of differences between the cell donor strains at the *H-2K*, *H-2I*, and *H-2S* loci; and (*e*) responses are demonstrable even when genetic information determining MLR responsiveness is also present in the stimulating cell donor; i.e., unlike the *H-2*-associated specificities, differences

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[1] *Abbreviations used in this paper:* B cells, Non-θ-antigen-bearing bone marrow-derived lymphocytes; *H-2D*^{d}, major histocompatability antigenic determinants specified by the *H-2D* region of the *H-2*^{d} allele; Ir, immune response; *Ir-1A*, genotype symbol for the locus determining the *Ir-1* phenotype; *Ir-1B*, genotype symbol for the locus determining the *Ir-IgG* phenotype; MLR, mixed lymphocyte culture reaction; *n*, the number of independently segregating genetic loci; T cells, θ-antigen-bearing thymus-derived lymphocytes.

at these "response loci" are not required for the reaction to occur. This latter finding may indicate that the products of these "response genes" are not expressed on the cell surface.

Materials and Methods

Animals.—Inbred mice of the HTG/AoSfSn strain and the congenic resistant lines C57BL/10J, B10.D2/nSn, B10.A/Sn, and B10.A(5R)SgSn were purchased from the Jackson Laboratories, Bar Harbor, Maine. Breeding pairs of the HTI/Go and HTG/Ao strains and the B10.HTG congenic resistant line were received from Doctors J. Klein (University of Michigan Medical School), D. B. Amos (Duke University Medical School), and F. Lilly (Albert Einstein College of Medicine), respectively. The F_1 hybrid mice (C57BL/10 × HTG) and (B10.D2 × C57BL/10) and the backcross mice (C57BL/10 × HTG)F_1 × HTG were raised from matings in this laboratory.

H-2 Recombinants.—The $H\text{-}2^i$ allele of the B10.A(5R) line is a recombinant haplotype of the $H\text{-}2^a$ and $H\text{-}2^b$ alleles which arose during the production of the B10.A congenic resistant line. This mouse inherited the *H-2K* and *H-2I* loci of the $H\text{-}2^b$ allele and the *H-2S* (*Ss-Slp*) and *H-2D* loci of the $H\text{-}2^a$ allele (4, 5), (Fig. 1).

The $H\text{-}2^i$ allele of the HTI strain also resulted from a recombination between the $H\text{-}2^b$ allele of the C57BL/Go strain and the $H\text{-}2^a$ allele of the A/Go strain (6) (Fig. 1). This recombinant haplotype is comprised of the *H-2K*, *Ir-1A* (and presumably *Ir-1B*), and *H-2S* loci of the $H\text{-}2^b$ allele and the *H-2D* region of the $H\text{-}2^a$ allele (4, 7).

The HTG strain carries the $H\text{-}2^g$ allele which resulted from a recombination between the *H-2K* region of the $H\text{-}2^d$ allele of BALB/c strain and the *H-2D* region of the $H\text{-}2^b$ allele of the C57BL/Go strain (6). The precise location of the recombinant event giving rise to this $H\text{-}2^g$ haplotype presents an interesting and rather exceptional problem (Fig. 2). Phenotypically this strain appears to have inherited the $H\text{-}2K^d$, $H\text{-}2IA^b$, $H\text{-}2S^d$, and $H\text{-}2D^b$ regions (4, 7). The possible existence of multiple crossovers within *H-2*, however, has been questioned (8). The genotype of the *H-2I* region of the $H\text{-}2^g$ allele should become clarified through the deter-

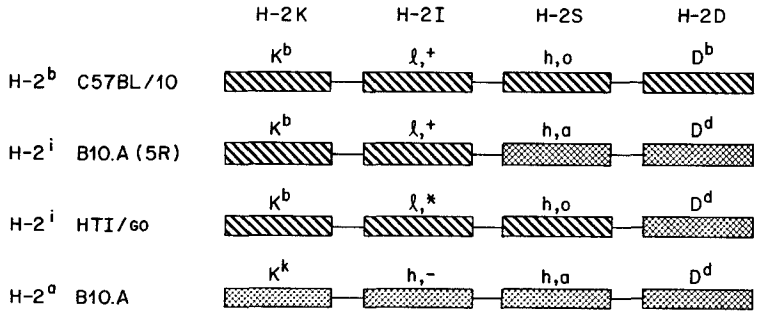

FIG. 1. A schematic diagram indicating the inheritance of the known regions and loci comprising the two recombinant $H\text{-}2^i$ alleles. (*) The phenotypes of both of the two known loci of the *H-2I* region, *Ir-1* (*Ir-1A*) and *Ir-IgG* (*Ir-1B*), are given with the exception of the *Ir-IgG* phenotype of the HTI/Go strain which has not yet been determined. The assignment of the *Ir-1* locus was based upon the response to the branched multichain synthetic polypeptide antigens ([H,G]-A–L) as determined by Grumet and McDevitt (7). Both the *Ss* and *Slp* phenotypes of the *H-2S* region are given.

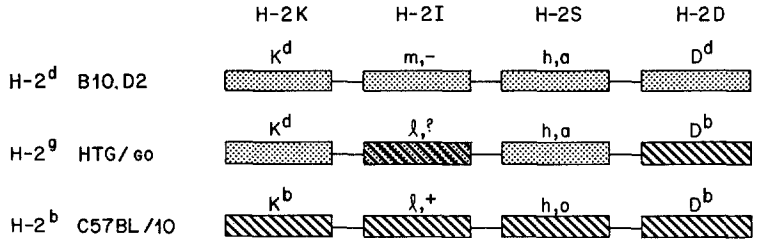

FIG. 2. A schematic map of the loci of the recombinant *H-2*^{g} allele. The *Ir-1B* or *Ir-IgG* phenotype of the HTG strain has not yet been determined.

mination of the *Ir-IgG* phenotype (*Ir-1B*) of the HTG strain and of the *Ir-1* responses (*Ir-1A* locus) of the B10.HTG congenic resistant line.

The Mixed Lymphoid Cell Culture Reaction Assay.—Mouse lymph node cell suspensions were cultured in RPMI 1640 medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 6% human serum and 0.5% normal (C57BL/10 × A/J)F_1 mouse serum (3). Human serum from one blood donor was utilized in all of the reported experiments. 1 ml each of responding and stimulating cells at a concentrtaion of 1.5×10^6 lymphoid cells/ml were mixed in 12 × 75 mm disposable glass tubes (Corning Glass Works, Corning, N. Y.), and were incubated at 37°C in a humidified atmosphere of 5% CO_2. Each test was cultured in triplicate.

Irradiation.—One-way stimulation was achieved and assessed as reported (3). One of the cell suspensions was submitted to 1,200 R of X irradiation delivered by a 280 kV Picker vanguard machine (Picker Corp., Cleveland, Ohio), utilizing a 1.53 mm copper half-layer filter, at the rate of 492 R/min at a focus skin distance of 25 cm. The efficiency of the X irradiation to block the cells from incorporating [^{3}H]thymidine was always assayed by culturing these cells with the nonspecific mitogen, phytohemagglutinin (PHA-P, Difco Laboratories, Detroit, Mich.).

Transformation Assay.—Between 72 and 96 h of culture 0.5 μCi of [^{3}H]thymidine/ml (sp act, 6.0 Ci/mmol; Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) was added and the cultures were incubated for an additional 18–19 h. The medium was then removed and the cells were washed once with phosphate-buffered isotonic saline (pH 7.10–7.20) containing 1% gelatin. The cells were then treated with 10% cold trichloroacetic acid, centrifuged, and washed once with 2% cold trichloroacetic acid. The resulting precipitate was dissolved in Nuclear Chicago solubilizer (Amersham/Searle Corp., Arlington Heights, Ill.), and transferred to toluene scintillation fluid. The results are expressed as the mean of repetitive experiments ± standard error. To facilitate the interpretation of the MLR data, the incorporation of less than 3,500 net cpm of [^{3}H]thymidine in the cell mixtures minus their control values was considered to be a minimal level of response.

Serology.—The backcross mice were all serotyped for their *H-2K* antigens, *H-2.31* and/or *H-2.33*. Anti-*H-2.31* serum which detected the *H-2*^{g} allele was produced in (C57BL/10 × A/J)F_1 hybrids against B10.D2 lymph node cells. The anti-*H-2.33* serum that was used to detect the *H-2*^{b} allele was produced in (B10.D2 × A/J)F_1 hybrid mice against B10.A(5R) skin grafts.[2] Each antiserum was negative, with cells bearing the antigens of the other allele. The two-stage cytotoxicity (trypan blue exclusion) test was performed utilizing splenic-nucleated cell suspensions and rabbit serum that had been absorbed with mouse cells (9).

[2] The anti-*H-2.33* serum was a generous gift from Dr. Ian McKenzie, Transplantation Unit, Massachusetts General Hospital, Boston, Mass.

RESULTS

Response to H-2D^d *Specificities in the Absence of Differences at the H-2K, H-2I and H-2S Regions.*—A high level of response was observed when cells from the C57BL/10 line were stimulated with X-irradiated cells from either the B10.A(5R) or HTI recombinants (Table I). The B10.A(5R) line differs from C57BL/10 at the *H-2S* and *H-2D* loci and depending upon the number of loci that may exist within the *Ir*-complex, these lines may also differ for some as yet undefined *H-2I* loci (5). The only known difference between the *H-2* complex of the HTI strain and that of the C57BL/10 is the *H-2D* region. "Non-*H-2*"-associated stimulation in this MLR combination was unlikely, since the B10.A(5R) line which is congenic with the C57BL/10 line did not readily respond to the HTI cells (Table I). Hence, in the C57BL/10 plus HTI cell combinations, positive stimulation was observed with cells from two strains in which there were no differences at their *H-2K*, *H-2I*, and *H-2S* loci.

TABLE I

The Mixed Lymphocyte Culture Response to H-2D^d *Antigenic Specificities*

Responder and stimulator cells	No. of exp.	Average cpm ± SE*	
		Control	MLR
C57BL/10 + B10.A(5R):1,200 R	12	2,822 ± 816	14,853 ± 4,211
C57BL/10 + HTI:1,200 R	6	2,940 ± 869	14,655 ± 4,544
B10.A(5R) + HTI:1,200 R	4	1,540 ± 608	2,791 ± 950

* The one-way cultures were assayed after an 18-h pulse with 0.5 μCi [^{3}H]thymidine/ml and are presented as the mean ± SE of the given number of replicate experiments. Triplicate cultures were set up in each individual experiment.

The Dominant Effect of the C57BL/10 Response to H-2D^d *Specificities.*—HTG cells in a mixture with X-irradiated B10.D2 cells accords us with another MLR combination which also differs for the *H-2D*^{d} specificities. In contrast to the high responses observed, above, when the C57BL/10 was the responding cell donor, the HTG cells responded only minimally (Table II, *a*). If the locus (loci) that determines the expression of the *H-2D* antigens carried the only gene (genes) whose products were involved in determining these mixed lymphocyte culture reactions, then a higher level of response should have been observed in this latter combination. This point is further emphasized by the fact that the minimally responding HTG strain and the responder C57BL/10 strain express the same *H-2D*^{b} antigens (see Fig. 2). The positive response observed with the C57BL/10 line may then have been the result of the presence of other particular genetic factors which enabled its cells to respond to the *H-2D*^{d} antigenic specificities. To examine this possibility F_1 hybrids were raised from a mating of the C57BL/10 responder line and the minimally responding HTG line. Cells from these F_1 hybrids readily responded to the X-irradiated cells from the B10.D2 line (Table II, *b*). These results indicate that the C57BL/10 line has a dominant

TABLE II

The Mixed Lymphocyte Culture Response of Recombinant and F_1 Hybrid Mice to H-2D^d Antigenic Specificities

Responder and stimulating cells	No. of Exp.	Average cpm ± SE		Average MLR/Control ± SE
		Control	MLR	
(*a*) HTG* + B10.D2:1,200 R	7	1,940 ±798	3,678 ±1,063	2.8 ±0.6
(*b*) (C57BL/10 × HTG)F_1 + B10.D2:1,200 R	14	2,080 ±561	13,612 ±2,377	8.3 ±1.4
(*c*) (C57BL/10 × HTG)F_1 + (B10.D2 × C57BL/10)F_1: 1.200 R	2	982 ±266	10,009 ±964	10.7 ±1.9
(*d*) (C57BL/10 × HTG)F_1 + (C57BL/10 × HTG)-F_1:1,200 R	1	1,006	1,090	1.1
(*e*) (B10.D2 × C57BL/10)F_1 + (C57BL/10 × HTG)F_1:1,200 R	2	1,136 ±214	1,575 ±491	1.4 ±0.2

* The HTG/AoSfSn line was used for the studies presented in this table.

effect on the determination of responsiveness to the *H-2D^d* antigens. A high level of response was also observed between cells from responding and stimulating F_1 hybrid donors both derived from a mating with the C57BL/10 line, therefore, each group of cells had all of the genetic material of that line (Table II, *c*). This demonstrates that the dominant traits of the C57BL/10 line that determine the ability to respond to the *H-2D^d* antigens are functional even when the stimulating cells also carry the same genetic information.

The stimulus for this mixed lymphoid cell culture reaction was shown to be an effect of the *H-2D* region antigens. No response was detected with an F_1 hybrid MLR combination where the X-irradiated F_1 hybrid cells presented no foreign antigens to the responding cell population (Table II, *d* and *e*).

Genetic Analysis of the Contribution of the C57BL/10 Line to the Response to the H-2D^d Specificities.—As the HTG and C57BL/10 strains each express the *H-2.2* antigens determined at the *H-2D* loci, the mixed lymphocyte culture responsiveness to the *H-2D^d* specificities was unlikely to be attributable to this allele. These strains differ at the *H-2K*, *H-2S*, and possibly the *H-2I* regions of the *H-2* complex plus innumerable other loci since the original *H-2^g* allele-bearing recombinant mouse was backcrossed twice to the BALB/c strain (6). Therefore, to further analyze the genetic contribution of the C57BL/10 to the MLR response, backcross mice were produced by mating the (C57BL/10 × HTG)F_1 hybrids with the HTG parental strain. In the mixed lymphocyte culture assays performed with cells from these backcross mice, their lymph node cells were tested for their responses to the *H-2D^d* antigens of the B10.D2 line, to parental strain cells, and to cells bearing antigens of a different *H-2* allele. In all of these experiments, a positive control was included to demonstrate that each of the X-irradiated cell suspensions was capable of stimulation. The inheritance of the *H-2* alleles was also followed. Since the C57BL/10 and HTG lines have the

same *H-2D* antigens, the backcross cell donors were serotyped with sera specific for their respective *H-2K* "private" antigens.

A broad range of reactivities was found in the MLR responses to the *H-2D*^{d} antigens among the 34 individual backcross mice examined (Table III). 6 of the 34 mice expressed a minimal level of response similar to that of the HTG parental strain and yielded stimulation ratios less than or equal to two (17.6%) (Table III). The proportion of backcross mice that reacted like the minimally responding HTG strain reflects the number of independently segregating genetic loci (n) that are involved in controlling the level of the MLR response and should be equal to $(\frac{1}{2})^n$. The data are consistent with an effect controlled by genes at two to four independently segregating loci ($P = 0.005$).[3]

One of these loci apparently is associated with the *H-2* complex as fewer of the *H-2* heterozygous backcross mice responded like the HTG minimally responding

TABLE III

Mixed Lymphocyte Culture Responses of (C57BL/10 × HTG/Ao)F$_1$ *× HTG/Ao Backcross Mice (34 Mice)*

Stimulation ratio	Percent of animals responding to stimulating cells (1,200 R)			
	B10.D2	HTG	C57BL/10	B10.A
≤2.0	17.6	70.6	14.7	2.9
≤4.0	61.8	88.2	35.3	11.8
≤6.0	76.5	100.0	47.1	23.6
≤8.0	85.3	100.0	55.9	32.4
≤10.0	91.2	100.0	61.8	50.0

parental strain than did the *H-2* homozygous backcross mice (Table IV). If the ability to respond to the *H-2D*^{d} antigens was completely controlled by the *H-2* complex, however, then only cells from the backcross mice that had inherited the *H-2*^{b} allele of the C57BL/10 line should be able to respond. On the other hand, if the dominant responsiveness traits of the C57BL/10 line were not all associated with the *H-2* complex then cells from at least some of the backcross mice that were homozygous for the *H-2*^{q} allele should also be capable of responding to the *H-2D*^{d} specificities, at a level similar to that of the F_1 hybrid. Indeed, relatively high levels of responses to cells bearing the *H-2D*^{d} antigens were observed in cultures with cells from some of the backcross mice that were homozygous for the *H-2*^{q} allele of the minimally responding HTG strain (Table IV). The fact that some of the backcross homozygous mice can respond to the *H-2D*^{d} antigens demonstrates that genetic information that segregates independently from the *H-2* complex is involved in determining the levels of the mixed lymphocyte culture responses to these antigens.

The ability to recognize or respond to the *H-2D*^{d} specificities apparently is

[3] $P = 0.013$ for less than four loci.

not associated with the sex of the backcross mice as cells from 20% of the male homozygous donors responded poorly (≤ 2.0) as compared with 23.1% of the female homozygous mice (≤ 2.0). Sex-associated factors, however, are involved in determining the levels of MLR responses to the *H-2D*^{d} antigens (Table V). Female lymph node cells yielded higher responses than did male cells. The stimulating cell donors in all cases were of the same sex as the responding cell donors.

TABLE IV

Relationship of the Inheritance of H-2 to the MLR Responses of (C57BL/10 × HTG/Ao)F$_1$ × HTG/Ao Backcross Mice

Stimulation ratio	Percent of animals responding							
	23 homozygous mice with stimulating cells (1,200 R)				11 heterozygous mice with stimulating cells (1,200 R)			
	B10.D2	HTG	C57BL/10	B10.A	B10.D2	HTG	C57BL/10	B10.A
≤ 2.0	21.7	82.6	4.3	4.3	9.0	44.5	36.4	0
≤ 4.0	73.9	95.7	10.0	13.0	45.5	72.8	81.8	9.0
≤ 6.0	82.6	100.0	21.7	26.1	63.6	100.0	100.0	18.2
≤ 8.0	87.0	100.0	34.8	39.1	81.8	100.0	100.0	18.2
≤ 10.0	91.3	100.0	43.5	60.9	90.9	100.0	100.0	27.3

TABLE V

Average MLR Stimulation Ratios of the (C57BL/10 × HTG/Ao)F$_1$ × HTG/Ao Backcross Mice, Sex and H-2 as Factors in the MLR Responses

Responding cell donors	Average stimulation ratio ± SE, with stimulating cells (1,200 R)			
	B10.D2	HTG	B10.A	C57BL/10
23 homozygous mice	4.2 ± 0.84	1.4 ± 0.25	21.5 ± 5.70	21.9 ± 5.59*
10 ♂♂	2.6 ± 0.38	1.3 ± 0.20	20.2 ± 7.78	23.2 ± 10.39*
13 ♀♀	5.3 ± 1.38	1.4 ± 0.42	22.5 ± 8.39	21.0 ± 6.52
11 heterozygous mice	5.9 ± 1.10	2.8 ± 0.45	17.5 ± 2.85	2.7 ± 0.52
7 ♂♂	4.8 ± 1.10	2.9 ± 0.63	18.4 ± 3.89	2.8 ± 0.83
4 ♀♀	7.7 ± 2.30	2.6 ± 0.63	16.0 ± 4.51	2.4 ± 0.16

* One mouse with a stimulation ratio of 291.1 was omitted from these averages. If included the average for the homozygous mice would be 33.6 ± 12.86 and for the males, 50.0 ± 28.35.

That genetic factors distinct from the *H-2* complex play a role in determining the level of response to the *H-2D*^{d} antigens was further demonstrated through studies with the B10.HTG congenic resistant mice. This line genetically should be comprised of all of the genes of the C57BL/10 strain with the exception of its *H-2* region which was derived from the HTG strain. The B10.HTG cells exhibited a relatively high level of response to the B10.D2 cells (Table VI). The B10 genetic material must therefore be functional in determining the higher

TABLE VI

Comparison of the Responses of the HTG and B10.HTG Lines to the H-2D^d *Antigenic Specificities*

Stimulating cells (1,200 rads)		Average cpm of [^{3}H]thymidine incorporation ± SE in responding cells			
		None	HTG/Ao	B10.HTG	C57BL/10
Exp. 1	None		231 ± 44	924 ± 189	1,952 ± 481
	B10.D2	61 ± 15	842 ± 281	7,409 ± 391	19,531 ± 2,717
	HTG	42 ± 5	—	1,047 ± 233	14,288 ± 876
	C57BL/10	54 ± 9	50,332 ± 3,720	52,701 ± 1,348	—
Exp. 2	None		1,280 ± 663	735 ± 69	1,848 ± 302
	B10.D2	98 ± 59	1,982 ± 37	7,826 ± 1,515	9,908 ± 1,429
	HTG	29 ± 1	—	629 ± 165	6,370 ± 788
	C57BL/10	26 ± 5	24,897 ± 2,198	21,868 ± 2,921	
Exp. 3	None		3,203 ± 1,236	1,334 ± 324	
	B10.D2	58 ± 16	5,165 ± 270	9,455 ± 1,935	
	HTG	86 ± 12	2,215 ± 125	752 ± 89	
	C57BL/10	60 ± 9	50,674 ± 476	47,527 ± 2,144	

responses of the B10.HTG as contrasted with those of the HTG cells (Table VI). As the B10.HTG and B10.D2 lines are congenic resistant partners, the only antigenic differences in these cell mixtures are those of the *H-2D* region (and possibly the *H-2I*, see Materials and Methods). Although the HTG cells only respond minimally to the B10.D2 cells, both the HTG and B10.HTG cells readily responded to cells from the C57BL/10 line indicating that the responses to antigenic differences associated with the *K* end of *H-2* may be quite distinct from those determining the level of responses to the *D* end.

DISCUSSION

Through these studies it has become apparent that different genetic loci may be involved in determining the level of the responses to the respective *H-2I* and *H-2D* region antigenic specificities. Genetic information that does not appear to be associated with the *H-2* complex plays a role in the responses to the *H-2D* region antigens. The genetic control of responses to *H-2K* antigens in the absence of *H-2I* differences has not yet been studied. It has been demonstrated, however, that the high levels of responses observed to *K*-end differences are clearly related to associated differences at the closely linked *H-2I* or *H-2S* region (10). Differences within the known *Ir* complex or the *H-2S* region, however, are not required to obtain a response to the *H-2D*^{d} antigenic specificities. This is exemplified by the relatively high levels of responses that were observed with the C57BL/10 line to the *H-2D*^{d} antigens of the B10.A(5R) and HTI recombinant lines. The responses that were observed to the HTI cells also demonstrate an association of the stimulation with the *H-2D*^{d} region itself

as the recombinational event giving rise to this *H-2*^{i} allele occurred between the *H-2S* region of the *H-2*^{b} allele and the *H-2D* region of the *H-2*^{d} allele (see Fig. 1). "Non-*H-2*"-associated stimulation in the MLR combinations with cells from the HTI strain was ruled out by the minimal response exhibited by cells from the B10.A(5R) line to the X-irradiated HTI cells.

The ability to respond to the *H-2D*^{d} specificities appears to be controlled by two to four independently segregating genetic loci. The data from our backcross mice demonstrate that genetic information both outside of *H-2* and associated with *H-2* is involved in determining mixed lymphocyte responses. The responses of the B10.HTG cells as compared with those of the HTG strain also demonstrate that the *H-2* complex does not by itself completely control the ability to respond specifically to the *H-2D*^{d} specificities. It may well be that the *H-2* complex exerts an amplification effect (quantitative) when it is associated with other genes that may be involved in the initial events of a mixed lymphocyte reaction. This could be accomplished by committing a previously nonreacting subpopulation of cells, such as helper cells, into the reaction.

Sex-associated factors also affect the levels of the MLR responses to the *H-2D*^{d} antigens. Sex differences in responsiveness to skin allografts have frequently been observed between donors and recipients that have restricted non-*H-2* or *H-2* histocompatibility differences (11, 12). These findings do not necessarily imply that a sex-linked *Ir* gene directs these responses as androgens apparently have a substantial effect on many aspects of the immune system (13).

Our data also demonstrate that the genetic loci that code for the *H-2D*^{d} antigens are quite distinct from the genetic information determining the response to the stimulus provided by those antigens. Separate response and stimulation loci have previously been suggested in the human HL-A system (14, 15). The stimulating locus (loci) of the mouse cells in our studies is situated within the *H-2D* region. The response by the C57BL/10 strain to this stimulus, however, is not determined by the *H-2D* antigens themselves. This was demonstrated through the low level of response exhibited by cells obtained from the recombinant HTG strain which originally inherited its *H-2D* region from the *H-2*^{b} allele.

The role that *Ir* and *MLR-D* genes may actually play in the response to histocompatibility antigens in vivo could be related to the type of immunological response that is manifested; cell-mediated immunity, antibody production, or tolerance. Their function could then be related to the selection of the different types of cells that must interact to formulate the respective effector products. It is also possible that several alternate pathways can lead to one particular type of immunological response to a histocompatibility antigen and that the selection of a pathway is dependent upon the nature of the presentation of that antigen. For example, the minimal level of responses to some of the *H-2D* antigens, as detected in the MLR, are not associated with a corresponding delay

in the rejection of skin grafts in the same immunogenic combination as compared with the rate of their rejection by an MLR-responding F_1 hybrid (3). Apparently the limited stimulation of lymphoid cell division, as observed in vitro after 3–5 days of culture, is not reflected in a corresponding limitation on the effector processes in vivo.

Congenic resistant partners are frequently utilized in the MLR assays to rule out the possible contribution of genes other than those of the *H-2* complex. The data presented here, however, demonstrate that the genetic products, the actions of which result in a high level of response, are functional even though the stimulating cells also have the capacity to synthesize those same products. This is not a situation without precedent. In the strains which carry an *Ir* gene that determines the rejection of male skin by the females of that strain, for example, the males also must carry that *Ir* gene. That the capacity to mount a response to one's own antigens does exist is also supported by the occurrence of autoimmune diseases. In any genetic system, one can only study the contributions of genes that are segregating. Therefore, caution should be used in the interpretation of results from studies utilizing mice that are congenic resistant partners.

SUMMARY

Cellular responses in vitro to *H-2D* region histocompatibility antigens were demonstrated to be under the genetic control of two or three ($P = 0.013$) independently segregating loci. The *H-2* region itself accounts for one of these loci, however, its activity appears to be dependent upon an association with other non-*H-2*-associated genetic information. The ability to stimulate a response and to respond to that stimulus are two separate genetic functions in certain MLR combinations. The stimuli in our studies were products of the *H-2D* region and cell donors must differ at that region in order for a response to occur. The control of the level of responses was determined by other genetic material. Differences at these "response loci" were not necessary for the induction of a proliferative response in the mixed lymphocyte cultures.

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