SEROLOGICALLY DEFINED AND LYMPHOCYTE-DEFINED COMPONENTS OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN THE MOUSE*

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The mixed leukocyte culture (MLC)[1] test has been used as a measure of histocompatibility and as a model of the recognition phase of the homograft reaction. Studies in man (1), mouse (2), and rat (3) have suggested that activation or stimulation in MLC is dependent on differences at the major histocompatibility complex (MHC) although exceptions to this rule have been found (4). In the mouse the MHC includes two serologically defined (SD) loci, *H-2K* and *H-2D*, immune response (*Ir*) loci (5, 6), loci governing susceptibility or resistance to tumor viruses (7), and the *Ss-Slp* loci (8).

It was naturally assumed that since MLC activation was dependent on MHC differences, SD antigen differences were responsible for stimulation. Unusual and aberrant cases in human studies (9–12) suggested, however, that in addition to the SD loci, there may be MHC differences which are difficult to detect serologically using the usual methods of immunization and testing, but which can cause a lymphocyte proliferative response. We will refer to such differences as lymphocyte-defined (LD) differences (13).

We will present data in this paper which we have obtained in mouse MLC studies. We have for the most part made use of congenic strains of animals carrying recombinant MHC chromosomes (strains that are genetically identical except for the genes of the MHC). This allows us to test two animals differing for only some segment of the MHC. In a few cases we have studied animals differing for the MHC and for loci segregating independently of the MHC; these will be discussed in detail. Our results indicate that the strongest MLC activation is associated with *Ir* region differences; *H-2K* or *H-2D* differences

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[1] *Abbreviations used in this paper*: GvH, graft-*versus*-host; Ir, immune response; LD, lymphocyte defined; MHC, major histocompatibility complex; MLC, mixed leukocyte culture; SD, serologically defined.

without *Ir* region differences result in relatively weak or no MLC stimulation. Further, in some cases we have found mouse strain combinations that have only LD differences (with SD loci identity) whose cells stimulate in MLC. Brief reports dealing with some of these data have been published elsewhere (13, 14).

Materials and Methods

Mice.—For practical purposes it is useful to divide the MHC into four regions: *H-2K*, *Ir*, *Ss-Slp*, and *H-2D* (as shown in Fig. 1). The strains used are listed in Table I. With the exception of B10.A(1R) and B10.A(3R) mice which were kindly sent to us by Dr. Jack H. Stimpfling, McLaughlin Research Institute, Great Falls, Mont., all mice were from our own colonies at the

-----*H-2K*-----*Ir*------*Ss-Slp*------*H-2D*-----

FIG. 1. A map of the mouse MHC showing the two SD loci, *H-2K* and *H-2D*, with the *Ir* and *Ss-Slp* loci in between. The map is discussed in the text.

University of Michigan and University of Wisconsin. All strains, except AQR, C3H, and HTG, were congenic with strain C57BL/10Sn (=B10). They were derived by intercrossing two strains, one of which was B10, followed by repeated backcrossing to B10 with concomitant selection for the H-2 chromosome of the second strain. The eleven cogenic lines used in the present study underwent 11 or 12 backcrossings to B10 and several generations of intercrossing thereafter. Theoretically, after 11 generations of backcrossing, the genome of the congenic lines should be almost identical with the genome of the B10 strain except for the H-2 difference. In practice, one can expect that the lines still differ from B10 not only in the H-2 complex but also in whatever differences there might have been between B10 and the second strain in the neighboring segments on both sides of H-2.

Four of the strains used carried H-2 chromosomes of independent origin ($H\text{-}2^b$, $H\text{-}2^d$, $H\text{-}2^k$, and $H\text{-}2^q$), the rest of the strains carried recombinant H-2 chromosomes. The chromosomes of independent origin are those for which there is no evidence for exchange of genetic material with other chromosomes in their recent history. They differ from one another in their SD loci, in at least a portion of the *Ir* region, and, in some cases, also in the *Ss* region. Whether they also share some portions of the MHC is at present not known. For the purpose of the present study the independent MHC chromosomes will be considered as completely different from one another in all the regions. The difference is expressed in Table I by assignment of the H-2 chromosome symbols to the individual regions (e.g. all four regions of chromosome $H\text{-}2^b$ are designated with a capital letter "B," all four regions of chromosome $H\text{-}2^d$ with a capital letter "D," etc.).

The recombinant H-2 chromosomes were derived by intra-H-2 exchange of genetic material (crossing-over) between two chromosomes of an H-2 heterozygote. The origin of the individual recombinant chromosomes was described elsewhere (15). Since, at the present time, we know only a small fraction of the chromosome, the breakage point in the individual H-2 crossovers can be mapped with only a very limited degree of accuracy. Considering the great number of genes constituting the MHC, it is very unlikely that in any two of the approximately 35 known MHC recombinants the break occurred exactly in the same position. Those MHC recombinants which at the present time seem to be identical [e.g. B10.A(1R) and B10.A(2R)] will be most likely found different after new markers are discovered in the region of the break, as has already happened in at least one case [namely B10.A(2R) and B10.A(4R), see below].

The different recombinant MHC chromosomes share portions of the H-2 complex with the chromosomes from which they were derived, and differ in others. Using a particular combina-

TABLE I

Strains Used in Mouse MHC Studies

Strain	*H-2*	*K*	*Ir-1*	*Ss-Slp*	*D*
C57BL/10	b	B	B	B	B
B10.Br	k	K	K	K	K
B10.D2	d	D	D	D	D
B10.A	a	K	K	D	D
B10.A(1R)	h-Sg	K	K	D	B
B10.A(2R)	h-2Sg	K	K	D	B
B10.A(3R)	i-Sg	B	B	D	D
B10.A(4R)	h-3Sg	K	K	B	B
B10.A(5R)	i-2Sg	B	B	D	D
B10.G	q	Q	Q	Q	Q
B10.AKM	m	K	K	K	Q
HTG	g	D	D	D	B
AQR	y-Klj	Q	K	D	D
B10.T(6R)	y-Sgi	Q	Q	Q	D
C3H	k	K	K	K	K

tion of two MHC chromosomes in an MLC test, a situation can be created in which the responding cell will differ from the stimulating cell not by the whole MHC, but by only a small portion of it. This principle is used in the present study.

The origin of the congenic lines used here was described by Stimpfling and Reichert (16). A few comments should, however, be added about some of the lines. Line B10.G was derived by Stimpfling from a noninbred stock obtained from Dr. Margaret C. Green. The stock carried two marker genes in linkage group IX, tufted (*tf*) and Brachyury (*T*). The H-2 chromosome derived from this stock was typed by Stimpfling and Reichert as a chromosome which "resembles but seems not to be identical with $H\text{-}2^m$." Our own analysis (Klein, J., unpublished data) of the B10.G line indicates that its H-2 type is serologically indistinguishable from the $H\text{-}2^q$ type of strains DBA/1, T138, and Green's *tf-T* stock. Thus for our purposes, the H-2 type of line B10.G will be considered identical with $H\text{-}2^q$.

Cells of lines B10.A(2R) and B10.A(4R) are serologically indistinguishable from one another. All efforts to date to produce antibodies by cross-immunization of these two lines have failed. Skin graft exchanges between the two lines are accepted for at least 40 days (although there might be some occasional rejections beyond this period of time). They also respond similarly to synthetic polypeptides, such as (T,G)-A- -L (17). This response is controlled by gene(s) in the *Ir* region. The two lines, however, are not completely identical. First, they differ in their response to gamma G_{2a} allotype (18), a trait which is also controlled by the *Ir* region, and second, they differ by the Slp antigen. Thus, although the two recombinants were derived from the same two MHC chromosomes ($H\text{-}2^a$ and $H\text{-}2^b$) by a recombinational event which occurred in the *Ir* region, the crossover apparently did not occur in exactly the same position.

Strain AQR was derived from a cross (T138 × B10.A)F_1 × B10 as an H-2 recombinant (19). After the first backcross, three additional backcrosses were made to B10 before the line was established by intercrossing. Thus, although the AQR line is not congenic with B10, it carries a considerable portion of the B10 genome.

Line B10.T(6R) was derived by Stimpfling and Reichert (16) as an H-2 recombinant between $H\text{-}2^a$ of B10.A and $H\text{-}2^q$ of Green's *tf-T* stock. The line still carries the markers outside the H-2 complex (*tf*,*T*) and apparently the whole chromosomal segment of the *tf-T* stock delineated by these two markers (over 15 map units long). The B10.T(6R) line is serologically indistinguishable from AQR, at least with respect to the known H-2 antigens. At-

tempts to obtain antibodies by cross-immunization of the two strains have so far been unsuccessful. However, skin grafts transplanted from B10.T(6R) donors onto (B10 × AQR)F_1 recipients are rejected within 3 wk after transplantation (Klein, J., unpublished data). Whether the rejection was due to residual minor histocompatibility differences [very unlikely since B10.T(6R) and B10 are thought to be congenic], to some difference within the MHC (the two lines differ in both the *Ir* and *Ss* regions), or an unknown histocompatibility locus outside of H-2 (possibly between T and H-2) is at present not known.

Strain HTG was derived as an H-2 recombinant from a cross (BALB/c × C57BL)F_1 × C57BL (20) and was maintained thereafter mostly by sib-sib matings. Thus it also contains a considerable portion of the B10 genome.

MLC Technique.—The MLC method we have used in these studies will be described in detail elsewhere. In brief: MLC tests were done using a micromethod recently described for human cultures (21). Mouse spleen cells were used as both responding and stimulating cells. In most experiments the cells were cultured using 1×10^6 responding cells and 1×10^6 mitomycin C-treated stimulating cells/0.2 ml culture well. In some combinations different cell concentrations were tested. The cells were cultured in tissue culture medium RPMI 1640 supplemented with pooled frozen human plasma, penicillin, and streptomycin. The human plasma, after thawing and heating at 56°C for 30 min, was used at a final concentration of 5% (v/v). The cells were cultured for 3 days after which time 2 μCi of tritiated thymidine (specific activity 1.9 C/mM) is added to each culture. The cultures are precipitated approximately 16 hr later after overnight labeling using an automatic multiple sample harvester (22).

Stimulation in allogeneic mixtures is expressed and evaluated in two ways. First, to test whether stimulation has occurred in a given allogeneic mixture, a *t* test is performed on the log converted counts per minute (cpm) of the allogeneic mixture compared with the log cpm in the isogeneic control mixture (the same responding cells with isogeneic or syngeneic mitomycin C-treated "stimulating" cells). The significance of stimulation is then expressed by a *P* value. Second, to compare the response of cells of one strain with a number of different stimulating cells, for instance differing from the responding cells by different regions of the MHC, results in each combination are expressed as a ratio of cpm in the allogeneic mixture divided by cpm in the isogeneic control. Whereas there are probably drawbacks to the use of the ratio method, it is one way to pool results from several experiments testing the same combinations.

RESULTS

In the studies presented in this paper, we have tested a total of 173 strain combinations in one-way mixed lymphocyte culture involving 15 different strains. Most experiments were set up by combinatorial protocol; one example of such an experiment is given in Table II.

TABLE II

MLC Responses in Selected Mouse Strains

Responder	Stimulator			
	B10	B10.Br	B10.D2	B10.G
B10	(3,865 ± 429)*	26,903 ± 2932	26,858 ± 1873	24,960 ± 5750
B10.Br	23,124 ± 170	(4,380 ± 715)	16,326 ± 3034	14,672 ± 4668
B10.D2	14,925 ± 2616	13,227 ± 628	(2,415 ± 467)	14,847 ± 1747
B10.G	21,619 ± 2185	18,715 ± 2493	25,730 ± 1667	(2,469 ± 474)

$P < 0.001$ in all allogeneic mixtures.

* Counts per minute (cpm) ± standard deviation.

In this particular experiment cells of four mouse strains that carry nonrecombinant MHC chromosomes are tested in all possible combinations. The control counts per minute plus or minus standard deviation are given in parentheses; cpm in the allogenic mixtures are compared with the control values using the same responding cells. The *P* value given in the table reflects the level of significance of stimulation as discussed above. In each allogenic one-way MLC there is highly significant stimulation ($P < 0.001$) between any two of these four strains. Results given in this paper are either representative experiments, such as the one given in Table II, or data pooled from several experiments involving that same strain combination. In the latter case, the ratio method will be used to express the results.

MHC Differences for the H-2D Region Only.—Five different strain combinations, identical for the *H-2K*, *Ir-1*, and *Ss-Slp* regions were tested for their ability to stimulate in one-way MLC. Stimulation in these combinations was uniformly low, and in some cases not significant. The results shown in Table III

TABLE III

MLC Response with only an H-2D Region Difference

Responder	Stimulator		
	B10.G	B10.T(6R)	B10.AKM
B10.G	(3,512 ± 983)*	10,434 ± 1300 $P < 0.005$‡	25,388 ± 3286 $P < 0.001$
B10.T(6R)	7,348 ± 720 $P < 0.025$	(3,440 ± 1409)	44,151 ± 1472 $P < 0.001$
B10.AKM	23,627 ± 4298 $P < 0.001$	39,535 ± 3982 $P < 0.001$	(3,727 ± 1068)

* Counts per minute (cpm) ± standard deviation.

‡ Probability that cpm in allogeneic mixture differs from the cpm in isogeneic control mixture using the same responding cell.

are from an experiment in which we noted one of the strongest stimulating combinations with only an *H-2D* region different. B10.AKM cells are included as "control" cells. The response of B10.G cells to B10.T(6R) mitomycin C-treated stimulating cells was significantly stronger than the response in the opposite direction in all four times of testing. On two occasions the response of B10.T(6R) cells to B10.G stimulating cells was not significant.

A total of 10 combinations involving 9 strains differing only for *H-2D* have been tested. The average ratio of stimulation was only 1.8 (varying from 0.8 to 5.4). In 11 of 24 allogenic mixtures the stimulation was insignificant by *t* test; in only 4 of 24 was the stimulation significant with $P < 0.001$.

One would thus have to conclude that *H-2D* region differences are either only weakly stimulatory or in some cases perhaps not at all stimulatory (14). It can not be ruled out that the failure to obtain stimulation in some cases represents an insensitivity of the MLC method being used.

MHC Differences for the H-2K Region Only.—We have tested one combination which differs for only the *H-2K* region, B10.A *versus* AQR. This combination differs for minor loci segregating independently of the MHC as well as at the K end of the MHC. The results obtained in four separate experiments involving these two strain combinations, tested in both directions in each experiment, also suggest that there is little if any stimulation in this combination. One example of MLC tests in this combination is given in Table IV. This represents the strongest stimulation obtained in any of the experiments. In five of the eight cases, P values obtained were not significant. The average ratio of stimulation for AQR responding to B10.A was 1.5 (with a range of 0.8–2.2); for B10.A responding to AQR, 1.4 (with a range of 0.8–2.0).

MHC Differences with SD Loci Identity.—One set of three strains, B10.A(1R), B10.A(2R), and B10.A(4R), is of special interest since these strains were derived from different crossovers involving the same F_1 genotype. The genotypes of the $H\text{-}2^a/H\text{-}2^b$ heterozygote and three resultant recombinant chromosomes are given in Fig. 2. Since the 1R, 2R, and 4R strains were derived in this manner and are on a congenic background, they differ only for the portion of the MHC between the two SD loci as indicated. 1R and 2R are, with respect to the markers available to us, identical, although, the recombinational events may

TABLE IV

MLC Response with SD Loci Identity

Responders	Stimulator		
	AQR	B10.T(6R)	B10.A
AQR	3,567 ± 1190*	32,087 ± 1900 $P < 0.001$‡	7,869 ± 439 $0.01 < P < 0.025$
B10.T(6R)	44,181 ± 2071 $P < 0.001$	3,440 ± 1409	52,402 ± 4292 $P < 0.001$
B10.A	8,425 ± 722 $0.1 < P < 0.2$	25,823 ± 1583 $P < 0.001$	6,856 ± 1416

* Counts per minute (cpm) ± standard deviation.

‡ Probability that cpm in allogeneic mixture differs from the cpm in isogeneic control mixture using the same responding cell.

$H\text{-}2K^k$	$Ir\text{-}1^k$	$Ss\text{-}Slp^d$	$H\text{-}2D^d$	H-2 a/b heterozygote
$H\text{-}2K^b$	$Ir\text{-}1^b$	$Ss\text{-}Slp^b$	$H\text{-}2D^b$	
$H\text{-}2K^k$	$Ir\text{-}1^k$	$Ss\text{-}Slp^d$	$H\text{-}2D^b$	B10.A(1R) chromosome
$H\text{-}2K^k$	$Ir\text{-}1^k$	$Ss\text{-}Slp^d$	$H\text{-}2D^b$	B10.A(2R) chromosome
$H\text{-}2K^k$	$Ir\text{-}1^k$	$Ss\text{-}Slp^b$	$H\text{-}2D^b$	B10.A(4R) chromosome

FIG. 2. The strains B10.A(1R), B10.A(2R), and B10.A(3R) were all derived from an H-2 a/b heterozygote. The genotype of the heterozygote as well as the recombinant chromosomes are shown in this figure. 1R and 2R were derived by a recombinational event between *Ss-Slp* and *H-2D*; 4R by a recombinational event between *Ir*-1 and *Ss-Slp*. 2R and 4R differ for an *Ir* locus. (R. Lieberman. 1972. *Fed. Proc.* **31**:777.)

have taken place at different positions. Both 1R and 2R cells reproducibly and significantly stimulate the cells of the 4R strain, however there is either a very weak or no response of 1R or 2R cells to 4R stimulating cells. Since this unidirectional response is an unusual finding we have tested the cells of these strains at multiple concentrations of stimulating and responding cells assayed on several days to maximize response using this MLC method. Whereas we have obtained an occasional stimulation value where cells of 1R or 2R respond weakly but significantly to stimulating cells of 4R, for the most part MLC tests in this direction have failed to stimulate.

In this particular combination the Slp antigen is present in mice of the 1R and 2R genotype but absent from mice of the 4R genotype. Since the Slp antigen is present only in males given the presence of the *Slp*^{a} gene, we have tested whether there is a difference of response of males and females in this particular combination to further exclude the Slp antigen as reason for stimulation. Results of one such experiment which also illustrates the above discussion are shown in Table V. Cells of B10.D2 male and female of comparable age are included in the experiment as control cells. There is highly significant stimulation with cells of 4R responding to 1R and 2R; there appears to be no significant influence of sex on stimulation or response in this combination. These studies would suggest that stimulation is not due to the Slp antigen, but to LD loci of the MHC.

There are some examples in these experiments of weak but significant response of 1R to 4R and 2R to 4R, as well as mixtures in which 1R and 2R cells respond significantly to each other; neither response has been observed consistently.

The other strain combination of great interest is that of AQR-B10.T(6R). This combination differs not only for the MHC genotypes, as shown in Table I, but also for minor loci. Our studies indicate however that these minor loci differences either do not lead to MLC activation or are extremely weak in doing so. This is based on the finding already discussed above, wherein cells of AQR were tested for their response to stimulating cells of B10.A, which differ from AQR at *H-2K* as well as the same two minor loci by which AQR and B10.T(6R) differ (Klein, J., unpublished data). [B10.A and B10.T(6R) are genetically identical except for the MHC]. Since there was repeatedly very little stimulation in the AQR-B10.A combination, it must be concluded that, even if the *H-2K* difference does not lead to any MLC activation, stimulation due to minor loci differences is very weak.

Stimulation in the mixture of serologically identical AQR-B10.T(6R) cells is strong and highly significant. The results of one experiment are shown in Table IV. There is stimulation in both directions in one-way MLC tests and this stimulation is highly significant in both directions. We must therefore again conclude that stimulation is associated with LD differences of the MHC although in this particular case we cannot conclude whether MLC stimulation is due to LD differences located between the two SD loci and/or outside the SD loci.

TABLE V

MLC Responses With SD Loci Identity

Responders	Stimulators							
	B10.A(1R) male	B10.A(1R) female	B10.A(2R) male	B10.A(2R) female	B10.A(4R) male	B10.A(4R) female	B10.D2 male	B10.D2 female
B10.A(1R) Male	8,179 ± 1,497*	7,706 ± 1,845 $0.5 < P$	7,142 ± 516 $0.2 < P < 0.4$	5,018 ± 779 $P < 0.01$	4,789 ± 736 $P < 0.01$	3,987 ± 1,134 $P < 0.01$	47,791 ± 3,256 $P < 0.001$	29,860 ± 2,509 $P < 0.001$
B10.A(1R) Female	5,615 ± 1,437 0.5‡ $< P$	5,500 ± 1,110	4,074 ± 1,084 $0.1 < P$	5,058 ± 1,517 $0.5 < P$	6,309 ± 1,396 $0.4 < P$	5,081 ± 1,410 $0.5 < P$	49,128 ± 4,860 $P < 0.001$	27,691 ± 3,080 $P < 0.001$
B10.A(2R) Male	3,518 ± 443 $P < 0.001$	3,811 ± 835 $P < 0.005$	2,158 ± 102	2,340 ± 396 $0.4 < P$	3,275 ± 441 $P < 0.005$	2,812 ± 527 $0.05 < P$	44,333 ± 3,725 $P < 0.001$	24,895 ± 5,256 $P < 0.001$
B10.A(2R) Female	7,329 ± 2,116 $0.2 < P$	5,590 ± 1,045 $0.2 < P$	6,876 ± 1,090 $0.2 < P$	5,644 ± 1,816	6,315 ± 2,363 $0.5 < P$	5,947 ± 1,316 $0.5 < P$	31,628 ± 2,873 $P < 0.001$	25,462 ± 1,457 $P < 0.001$
B10.A(4R) Male	10,161 ± 508 $P < 0.001$	6,973 ± 1,735 $P < 0.001$	7,639 ± 458 $P < 0.001$	5,725 ± 875 $P < 0.001$	2,035 ± 528	1,778 ± 282 $0.5 < P$	68,372 ± 10,113 $P < 0.001$	43,930 ± 3,921 $P < 0.001$
B10.A(4R) Female	9,787 ± 1,736 $P < 0.001$	5,976 ± 1,412 $P < 0.001$	5,366 ± 793 $P < 0.001$	5,472 ± 201 $P < 0.001$	2,296 ± 99 $0.05 < P$	1,926 ± 304	45,005 ± 4,457 $P < 0.001$	22,952 ± 2,487 $P < 0.001$
B10.D2 Male	34,809 ± 4,646 $P < 0.001$	26,533 ± 3,775 $P < 0.001$	20,052 ± 2,887 $P < 0.001$	18,759 ± 2,952 $P < 0.001$	11,829 ± 1,491 $P < 0.001$	15,320 ± 4,227 $P < 0.005$	5,729 ± 858	5,035 ± 1,092 $0.2 < P$
B10.D2 Female	71,522 ± 8,287 $P < 0.001$	49,868 ± 8,010 $P < 0.001$	54,345 ± 9,524 $P < 0.001$	41,911 ± 11,244 $P < 0.001$	24,141 ± 1,821 $P < 0.001$	23,918 ± 5,667 $P < 0.001$	4,129 ± 466 $0.5 < P$	3,898 ± 472

* Counts per minute (cpm) ± standard deviation.

‡ Probability that cpm in allogeneic mixture differs from the cpm in isogeneic control mixture using the same responding cell.

Various MHC Differences.—Since so few strain combinations are available which differ for only one of the four regions of the MHC, we have given further examples of our MLC data from experiments in which mice carrying recombinant MHC chromosomes were tested (Table VI) so that the relative contributions of the different MHC regions (taken one, two or three at a time) can be compared; the MHC regions by which the stimulating cell differs from the responding cell are designated by the MHC chromosome carried by the stimulating cell. Each responding cell is shown with all of the different cells which have been used to stimulate it. For a responding cell-stimulating cell combination, the number of experiments in which that particular combination was included, the average ratio of stimulation and range of ratios obtained are given.

It is possible to evaluate the relative contribution of the MHC regions by comparing combinations that differ for different parts of the MHC. The data from experiments with 15 different responding cell strains are summarized in this fashion in Table VII. As judged by the ratio method, there is strong stimulation in all combinations as long as the *Ir* region difference is included. If the *Ir* region is the same in both responding and stimulating cells, the stimulation ratio obtained is much lower.

DISCUSSION

The mouse MHC map given in Fig. 1 needs discussion in view of recent findings regarding the genetics of this region. Calculations based on the frequency of crossing-over between *H-2K* and *H-2D* (approximately 0.4%) indicate that the chromosomal segment occupied by the MHC could encompass some 500 genes of 1000 nucleotide pairs each (23). According to the traditional interpretation of the H-2 system, many of these genes scattered throughout the whole chromosomal segment are involved in the control of the different H-2 antigens. This interpretation has led recently to a series of internal inconsistencies which could be overcome only by presupposition of such unlikely events as double or even triple crossing-over within a short chromosomal segment, distorted segregation, presence of two antigens controlled by two different genes on the same molecule, etc. In face of these difficulties it was suggested to abandon the traditional interpretation and replace it with a simple and virtually inconsistencies-free two-gene model of the H-2 system (24). According to the two-gene model, most, if not all, of the serologically detectable (SD) H-2 antigens are controlled by one of the two (and in some cases by both) H-2 loci, *H-2K* and *H-2D*, which are located at the opposite ends of the MHC. The chromosomal segment between the two SD loci can thus encompass a great number of genes. The products of these genes, however, do not seem to be serologically detectable (at least not so far). The loci of the middle segment of the H-2 complex have been shown to be involved in such functions as immune response to synthetic polypeptides (17) and gamma globulin allotypes (25), susceptibility or resistance to tumor viruses (7), production of serum proteins Ss and Slp (26), and as

TABLE VI

MLC Response in Various Mouse Strain Combinations

Responder	Stimulator	Major histocompatibility complex				No. Exp.	Average ratio of stimulation	Range
		K	*Ir-1*	*Ss-Slp*	*D*			
B10.Br	C57BL/10	B	B	B	B	3	5.2	4.7– 5.7
	B10.D2	D	D	D	D	3	3.9	3.7– 4.2
	B10.A			D	D	3	1.2	1.1– 1.5
	B10.A(1R)			D	B	3	1.3	1.1– 1.6
	B10.A(2R)			D	B	3	1.2	1.1– 1.3
	B10.A(3R)	B	B	D	D	3	5.0	4.2– 6.0
	B10.A(4R)			B	B	3	1.6	1.4– 1.7
	B10.A(5R)	B	B	D	D	2	4.2	2.7– 5.7
	B10.G	Q	Q	Q	Q	1	3.3	
	B10.AKM				Q	1	0.9	
	HTG	D	D	D	B	1	5.5	
	AQR	Q		D	D	1	2.3	
B10.A	C57BL/10	B	B	B	B	4	7.0	3.6–12.7
	B10.Br			K	K	3	1.9	1.2– 2.6
	B10.D2	D	D			4	5.0	3.8– 8.3
	B10.A(1R)				B	3	1.5	1.0– 1.4
	B10.A(2R)				B	3	1.7	1.5– 1.9
	B10.A(3R)	B	B			3	5.4	4.4– 6.1
	B10.A(4R)			B	B	4	1.7	0.7– 2.2
	B10.A(5R)	B	B			3	5.4	4.4– 6.1
	B10.G	Q	Q	Q	Q	4	3.0	1.7– 4.5
	B10.AKM			K	Q	4	1.4	1.0– 1.7
	HTG	D	D		B	1	5.8	
	AQR	Q				4	1.4	0.8– 2.0
B10.A(2R)	C57BL/10	B	B	B		4	5.9	5.4– 6.2
	B10.Br			K	K	3	1.8	1.7– 1.9
	B10.D2	D	D		D	4	9.3	4.4–20.5
	B10.A				D	3	1.3	1.0– 1.7
	B10.A(1R)					4	1.3	0.8– 1.6
	B10.A(3R)	B	B		D	3	8.2	4.2–10.6
	B10.A(4R)			B		5	1.3	0.9– 1.7
	B10.A(5R)	B	B		D	3	6.5	5.0– 8.0
	B10.G	Q	Q	Q	Q	1	5.9	
	B10.AKM			K	Q	1	1.9	
	HTG	D	D			1	8.6	
	AQR	Q			D	1	3.1	
B10.AKM	C57BL/10	B	B	B	B	2	6.6	5.9– 7.3
	B10.Br				K	1	2.1	
	B10.D2	D	D	D	D	2	6.9	5.9– 7.8
	B10.A			D	D	3	1.7	1.5– 1.9
	B10.A(1R)			D	B	2	1.8	1.7– 1.9
	B10.A(2R)			D	B	1	2.0	
	B10.A(3R)	B	B	D	D	1	8.5	
	B10.A(4R)			B	B	2	1.5	0.9– 2.1
	B10.G	Q	Q	Q		4	3.4	1.6– 6.3
	HTG	D	D	D	B	1	7.1	
	AQR	Q		D	D	4	2.4	1.5– 3.7
	B10.T(6R)	Q	Q	Q	D	3	6.9	1.2–10.6

TABLE VII

Summary of MLC Responses

MHC regions which are different	Ratio of stimulation	
	Range*	Average
K, Ir-1, Ss-Slp, D	1.2–33.6	7.2
K, Ir-1, Ss-Slp	1.4–15.7	6.1
K, Ir-1, D	3.3–20.5	7.0
K, Ss-Slp, D	1.5– 8.6	3.3
Ir-1, Ss-Slp, D	2.7–15.1	8.3
K, Ir-1	3.2–18.3	6.6
K, Ss-Slp	No experiments	
Ir-1, Ss-Slp	2.7–12.8	5.8
K, D	3.0– 3.8	3.4
Ir-1, D	No experiments	
Ss-Slp, D	0.7– 4.7	2.0
K	0.8– 2.2	1.4
Ir-1	No experiments	
D	0.8– 5.4	1.8
Ss-Slp	0.6– 4.9	2.0
None	0.6– 1.9	1.2

* These numbers represent the lowest and highest ratios of stimulation noted.

demonstrated here, control of MLC response (13). We have divided the MHC into four regions, *H-2K*, *Ir*, *Ss*, and *H-2D*, as shown in Fig. 1. However this does not imply that all the immune response genes have to be limited to the *Ir* region, or genes for serum proteins to *Ss* region, etc. It is quite possible that *Ir* genes asociated with the *H-2D* locus rather than *H-2K* locus will also be found.

The evidence for the two-gene model was discussed by Klein and Shreffler (15). All the considerations in the present paper are based on the two-gene model. Obviously, the interpretation of the results presented here would change considerably if the traditional and not the two-gene model were true.

The initial correlation suggesting that the MLC reaction was activated by differences of the MHC assumed in large measure that activation was due to differences for the SD antigens. The present studies indicate that MHC differences which lead to MLC activation are at least in some cases genetically separable from the SD loci. Further, an over-all evaluation of the data indicates that the SD differences themselves are only weakly stimulatory or not stimulatory at all in the MLC. For instance, some of the *H-2D* region differences do not stimulate. Other *H-2D* region differences stimulate weakly. These findings could be explained either by assuming that the SD antigens of *H-2D* vary in their strength as reflected in MLC and that the cases of nonstimulation noted by us simply reflect an insensitivity of the MLC technique, or that in those *H-2D* differences which result in stimulation, the recombinational event producing the *H-2D* different strains occurred such that LD differences were included. The single case of an isolated *H-2K* region difference which we have

studied includes minor loci incompatibilities as well as the MHC SD difference and as such the low level of stimulation is difficult to analyze. Whatever the case, it is clear that this *H-2K* difference is not a strong one with respect to MLC.

The strongest stimulation is seen when there are differences for the *Ir* region. This is true when all the data is summarized as seen in Table VII, as well as in individual experiments involving as many as thirteen strains. Most revealing are those combinations that differ only for the genetic region between the two SD loci, such as 1R, 2R, and 4R. The 2R and 4R strains, which differ for the immune response locus described by Lieberman, stimulate repeatedly and significantly in one direction, 4R responding to 2R, and much more weakly or not at all in the other direction. This demonstrates unequivocably that MLC activation can be caused by genetic differences other than the SD loci and fits in well with the over-all pattern of stimulation associated with the *Ir* region. The studies with AQR-B10.T(6R) are also in accord with this finding although here there may be genetic differences outside the SD loci as well as between them. It is not clear with which of these differences the MLC activation is associated. The occasional instances of significant stimulation in the 1R-2R combination may indicate that these strains differ for an LD locus.

Rychlikova et al. (27) claimed that the MLC stimulation is an exclusive property of the *H-2*K end of the MHC. In view of our results, this claim now has to be modified in the following way: First, MLC stimulation is not limited to the K end; D end differences, at least in some combinations, do stimulate. Second, although the K end differences usually show much stronger stimulation than the D end differences, the stimulation is not primarily due to the *H-2K* locus itself, but to the *Ir* region. Since the *Ir* region is closely linked to the *H-2K* locus, a false impression that the *H-2K* locus was stronger than the *H-2D* locus was obtained from the results of these authors.

It is of course of great interest to ask what the LD locus product is. Since the LD locus (loci) map with the *Ir* loci it is possible to suggest that the *Ir* and LD loci are identical. Benacerraf and McDevitt (5) have suggested that the *Ir* locus product may be a T cell receptor; we would extend this suggestion to say that the *Ir* (LD) locus product is a T cell receptor and that this same cell surface component can act as a stimulatory molecule (in the stimulating cells) to which responding lymphocytes can react. Any cell that has one or more *Ir* products that a second cell does not have might stimulate that second cell in MLC. It seems quite likely that there are *Ir* loci in this region other than those currently described; the product of each of these could thus function as a stimulatory molecule. While it appears extremely difficult (and perhaps impossible) to obtain cytotoxic or agglutinating antibodies against these differences, it may be that another form of antibody, such as a blocking antibody, is produced (28). Studies are currently underway to investigate this possibility.

However, the LD product may be different from the *Ir* product; several possi-

bilities exist. The LD product might be recognized by thymus-derived (T) lymphocytes which are involved in cell-mediated immunity (graft rejection) and probably to a large extent in the MLC reaction, but cannot stimulate those bone marrow (B) lymphocytes which produce classical antibody. A close physical association in the membrane between the LD and SD loci products would also explain certain correlations found in studies between the SD antigens and MLC response as discussed below. As such, the LD locus product may function as a "carrier" while the SD product is the "hapten." Alternatively it is known that there are loci in the MHC which affect susceptibility to viral infection; perhaps in a case such as the 2R-4R combination, the 2R cells carry viral antigens which are recognized by the 4R cells.

Our studies in the mouse show the following picture for the relationship between the LD loci, MLC activation, and graft survival. In one combination, AQR-B10.T(6R), skin grafts are rejected in 14–18 days, in all probability due to the MHC incompatibility (see Materials and Methods section). These findings are similar to the studies of Bailey et al. in which MHC difference(s) with SD identity can lead to graft rejection (29) and reciprocal stimulation in MLC.[2] On the other hand, in the 2R-4R combination, which causes clear MLC activation in at least one direction, there is no rapid skin graft rejection, but there is a graft-*versus*-host (GvH) reaction as assayed by splenomegaly. Cells of B10.A(4R) animals cause a GvH reaction in B10.A(2R) recipients; there is no GvH reaction when B10.A(2R) cells are injected into B10.A(4R) recipients.[3] Whether this is due to a greater sensitivity of the MLC method compared with skin grafting, to the finding of only one-way stimulation which is reproducibly demonstrable in the MLC test (which is a very unusual observation in normal combinations), to the fact that in some cases it is possible to get MLC activation in vitro without having a histocompatibility difference which can lead to skin graft rejection, or to differences between skin and lymphoid cells as target cells, remains the subject of further investigation.

While it is impossible to conclude from these studies whether the SD antigens can themselves result in MLC activation, it does appear that loci spread over a large portion of the MHC region between the two SD loci and perhaps outside the SD loci can determine products which are stimulatory in MLC. Again several interpretations of this phenomenon are possible. Two are perhaps of special interest. First, it may be that there are several evolutionarily similar LD loci in the MHC, all with a similar phenotypic product. These products, quite likely cell surface components, could function as histocompatibility antigens. Second, it may be that the LD loci determine molecularly and perhaps functionally different surface membrane components which can be recognized as foreign, thereby stimulating in MLC.

The findings presented in this paper force us to reevaluate the genetic and

[2] Widmer, M. B., F. H. Bach, and D. W. Bailey. Manuscript in preparation.

[3] Livnat, S., J. Klein, and F. H. Bach. Manuscript in preparation.

immunological complexity of the major histocompatibility complex. The possibility that the MLC test can be used to detect the *Ir* loci products or alternatively define yet other loci of this region present exciting possibilities for further research.

SUMMARY

The mixed leukocyte culture (MLC) test is an in vitro model of the recognition phase of the homograft response. For the most part, activation in MLC is dependent on differences of the major histocompatibility complex (MHC). Our present studies in the mouse suggest that activation is primarily associated with differences of genetic regions of the MHC other than those which control the serologically defined (H-2) antigens. These differences do not lead to cytotoxic or agglutinating antibody formation despite extensive immunization; we have called these differences lymphocyte-defined (LD) differences. The strongest stimulation in MLC is associated with differences of the *Ir* region. It is possible that the *Ir* product is the T cell receptor and that it is this same molecule which can act as the stimulatory agent in MLC. Other possibilities are discussed.

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