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SHORT COMMUNICATION

Mixed Lymphocyte Culture Reactivity and H-2 Histocompatibility Loci Differences[1,2]

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The relationship between the major histocompatibility systems as defined serologically and by the mixed leukocyte culture (MLC) test is one which has received attention in both man (1) and mouse (2). In man, studies within families suggest that cells of siblings who inherit the same HL-A chromosomes from their parents and thus are serologically identical at HL-A do not stimulate in the MLC test. Cells of siblings who inherit different HL-A chromosomes do stimulate. This, combined with the finding that cells of all of several hundred unrelated pairs stimulate in the MLC test, suggests that a single genetic system controls reactivity in MLC, that this system is highly polymorphic and either the same as that coding for the HL-A antigens or very closely linked to the HL-A (1). Similarly, differences at the H-2 system in the mouse result in MLC activation (2).

The H-2 system in the mouse can be divided into two separate regions, H-2K and H-2D (3). Recently Rychlikova *et al.* (4) studied a series of mouse recombinant strains that differed only for the H-2D region or only for the H-2K region and other inbred strains that differed at both the H-2D and the H-2K regions. Their findings suggested that differences for H-2K alone or for both H-2K and H-2D resulted in MLC activation. Differences for the H-2D region alone, however, did not result in MLC activation in their study.

We have repeated these studies using a number of recombinant strains tested in

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all possible combinations of two in one-way mixed leukocyte culture tests (5). Our findings show unequivocally that whereas the H-2K region differences are associated with stronger MLC stimulation on the average than H-2D region differerences, some H-2D region differences by themselves do result in significant stimulation in MLC.

METHOD

The method used for culturing will be described in detail elsewhere (6); however, a brief description follows. Mouse spleen cells are cultured in RPMI-1640 (Gibco, Grand Island, NY), supplemented with penicillin, 100 units/ml; streptomycin, 100 μg/ml and heat-treated (56°C, 30 min) human plasma obtained from a frozen pool, 5 ml/100 ml (7). Responding cells and mitomycin C-treated stimulating cells (5) are cultured in 0.2-ml vol in Linbro microtiter plates (IS-FB-96-TC) at individual concentrations of 10^6 cells/well. After 72 hr of incubation in a humidified 5% CO_2, 95% air atmosphere, 2 μCi tritiated thymidine (sp act 1.9 Ci/mmole; Schwartz Bioresearch, Orangeburg, NY) is added to quadruplicate cultures. Sixteen hours later, samples are precipitated onto glass fiber filters and assayed for uptake of ^{3}H-TdR by liquid scintillation spectrophotometry (8).

Six of the 16 strains of mice used in these experiments are listed in Table 1; each combination has been tested a minimum of four times in MLC tests. All are congenic on a C57Bl/10 background; thus, any differences found between them can be ascribed to differences for the H-2 chromosomal region.

RESULTS

MLC test results on some of these strains are given in Table 2. These results were selected to demonstrate various points; they are representative of results obtained with other strain combinations as well as results obtained in other experiments. Six strains tested in all combinations of two are shown. Counts per minute (cpm) of tritiated thymidine incorporated ± standard deviation are given. In addition, the H-2 difference in each combination is given as either H-2K region alone, H-2D region alone, or H-2K and H-2D region differences. Lastly, for each combination it is indicated whether the stimulation in the allogeneic combination is statistically different from that in the isogeneic control culture using the same responding cell. This calculation is based on a *t* test performed on the log-

TABLE 1

LIST OF MOUSE STRAINS USED

Strain	Abbreviation	H-2 chromosome	Alleles at loci	
			H-2K	H-2D
C57B1/10Sn	B10	b	b	b
B10.A	A	a	k	d
B10.BR	BR	k	k	k
B10.A(3R)	3R	i-Sg	b	d
B10.A(4R)	4R	h-2Sg	k	b
B10.A(5R)	5R	i-2Sg	b	d

TABLE 2

MIXED LYMPHOCYTE CULTURE STUDIES IN SIX DIFFERENT MOUSE STRAINS

	Stimulating cells					
	C57B1/10	BR	A	3R	4R	5R
Responding cells						
C57B1/10	(6911 ± 1336)[c]	K+D[a] <.001[b] 57217 ± 2384	K+D <.001 88123 ± 5041	D <.005 22298 ± 2146	K <.001 50139 ± 3024	D <.005 19922 ± 2913
BR	K+D <.001 77870 ± 4590	(16624 ± 3895)	D >0.4 18924 ± 2018	K+D <.001 70545 ± 5903	D >.05 23008 ± 2243	K+D <.005 45804 ± 2355
A	K+D <.001 129167 ± 4811	D <.005 26361 ± 4355	(10201 ± 1394)	K <.001 63841 ± 5782	D <.005 19712 ± 1146	K <.001 59434 ± 4288
3R	D <.02 18078 ± 2391	K+D <.001 75587 ± 4736	K <.001 47540 ± 3825	(7241 ± 2474)	K+D <.001 46368 ± 4895	>.50 6300 ± 1030[d]
4R	K <.001 92971 ± 8851	D <.001 44595 ± 3572	D <.005 33406 ± 7278	K+D <.001 75984 ± 12580	(9409 ± 727)	K+D <.001 71014 ± 4278
5R	D <.005 28373 ± 3054	K+D <.001 72529 ± 5812	K <.001 58738 ± 4833	>.10 11692 ± 2397[d]	K+D <.01 52703 ± 11748	(8652 ± 2307)

[a] Indicates whether difference is at H–2D, H–2K, or both loci.
[b] Indicates significance level of stimulation.
[c] Counts per minute ± SD
[d] Strains 3R and 5R are H–2 identical.

converted data. Strains 3R and 5R are identical for the H-2K and H-2D regions and the cells do not stimulate each other.

DISCUSSION

Two general statements can be made. First, cells of mice that differ for either the H-2K region alone or for the H-2D plus the H-2K region stimulate more in MLC than do cells of mice that differ only for the D region. All of these combinations can result in significant stimulation. Second, cells of mice that differ only for the H-2D region stimulate in some cases and do not stimulate in other cases. The stimulation in some of these instances is highly significant and is reproducible from experiment to experiment.

Several of the mouse strain combinations tested in MLC in this study have also had skin graft survival studies done. The results of those studies (9) showed that in four combinations in which there was a H-2K region difference skin grafts were rejected in 10.1–11.7 days, whereas when an H-2D region difference was present skin grafts were rejected in 14–17 days. As noted above we have found greater stimulation when H-2K region differences were involved than when H-2D region differences were involved. There is thus an overall correlation between the results of skin graft survival and MLC activation. Whereas we do not believe that MLC data for one given combination is optimally presented by studying ratios of cpm present in the allogeneic mixture divided by the cpm present in the control isogeneic mixture, it would seem useful here to summarize the MLC results from three experiments. All combinations in which skin grafting was done that differed only for the H-2D region had a ratio of MLC stimulation between 0.9 and 2.9 with an average ratio of 1.55; combinations differing for the H-2K region had stimulation ratios varying from 3.4 to 15.4 with a mean ratio of 6.77.

These results confirm the findings of Rychlikova *et al.* (4) that H-2K region differences do result in greater stimulation on the average than do H-2D region differences. However, they also indicate that certain H-2D region incompatibilities are associated with stimulation in MLC. Rychlikova *et al.* pointed out in their article that the lack of stimulation in MLC when there were only H-2D region differences may have been due to insensitivity of their MLC method. We would agree that this is a likely reason for the difference in our findings.

The observation that H-2D region differences alone do result in MLC activation is theoretically and practically important. In the original studies in which the HL-A–MLC correlation was described, one sibling pair was studied, the members of which had inherited the same HL-A chromosomes from their parents and thus had the same HL-A antigens. The cells of these two siblings, however, did stimulate in the MLC. We suggested at that time (1) that as one possible explanation of this finding there may be another locus linked to HL-A, differences at which could also cause stimulation in MLC. In one parent of this sibling pair a recombinational event would have taken place making the two siblings different at this other locus—thus the MLC activation. Recently, Yunis and Amos (10) have suggested that such a third locus, linked to HL-A in man, may, in fact, be the MLC locus. In anology with mice one could thus explain the stimulation associated with H-2K region differences by suggesting that if mice differ for the H-2K region, they will

also differ at this MLC locus (the MLC locus being on that side of H-2) and, therefore, there is a positive MLC. H-2D region differences would not result in stimulation since there is no difference for the MLC locus. The finding of clearly positive MLC tests in some H-2D region differences in this study is pertinent to this problem. We have used the term "region" when referring to H-2K or H-2D differences since it seems possible that genes linkd to but separate from the H-2K and H-2D loci can cause stimulation in MLC (11).

Very preliminary, since few combinations are involved, is the correlation between skin graft survival and MLC activation. This correlation needs to be strengthened by further studies.

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