Is the H-2K Locus of the Mouse Stronger Than the H-2D Locus?

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Recently, evidence has been gathered showing that the H-2 complex of the mouse consists of only two histocompatibility genes (regions), H–2K and H–2D (Klein & Shreffler 1972). The rest of the H–2 regions described in the literature (C,V,E,A) are probably serological artifacts. Rychlíková and co-workers (1971) reported that significant transformation of allogeneic lymphocytes in mixed cultures (MLC) occurred when congenic strain combinations differed at the H–2K locus and did not occur when the combinations differed at the H–2D locus. According to Démant (1970), a similar phenomenon can be observed in the graft-versus-host (GVH) reaction. Here again, the H–2K incompatibilities lead to a strong reaction while H–2D incompatibilities cause only insignificant or very mild splenomegaly. The authors interpret these results as evidence for the "superiority of the K-end incompatibilities over the D-end incompatibilities" (Rychlíková et al. 1971). In this communication, evidence is presented that: 1) skin graft rejection does occur across the H–2D barrier, and 2) H–2K, at least in the combinations studied, presents a stronger histocompatibility barrier than H–2D.

Four intra-H–2 crossovers (Table I), two proven (i.e. $H-2^{h-3Sg}, H-2^{i-2Sg}$) and two suspected (i.e. $H-2^a, H-2^m$), and four H–2 chromosomes from which the crossovers were derived ($H-2^b, H-2^d, H-2^k, H-2^q$), were arranged in eight different donor–recipient combinations (Table II). Four of these combinations differed at the H–2K locus and the other four at the H–2D locus. All the H–2 chromosomes were on the same genetic background of the C57BL/10Sn (=B10) strain, thus ex-

Table I

Origin of four H–2 crossovers

$$\frac{H\text{--}2^d}{H\text{--}2^k} = \frac{H\text{--}2K^d}{H\text{--}2K^k} \Big| \frac{H\text{--}2D^d}{H\text{--}2D^k} \longrightarrow H\text{--}2^a = H\text{--}2K^kH\text{--}2D^d \text{ (B10.A)*}$$

$$\frac{H\text{--}2^k}{H\text{--}2^q} = \frac{H\text{--}2K^k}{H\text{--}2K^q} \Big| \frac{H\text{--}2D^k}{H\text{--}2D^q} \longrightarrow H\text{--}2^m = H\text{--}2K^kH\text{--}2D^q \text{ (B10.AKM)*}$$

$$\frac{H\text{--}2^a}{H\text{--}2^b} = \frac{H\text{--}2K^k}{H\text{--}2K^b} \Big| \frac{H\text{--}2D^d}{H\text{--}2D^b} \longrightarrow H\text{--}2^{h\text{--}3\,Sg} = H\text{--}2K^kH\text{--}2D^d \text{ (B10.A[2R])}$$

$$\frac{H\text{--}2^a}{H\text{--}2^b} = \frac{H\text{--}2K^k}{H\text{--}2K^b} \Big| \frac{H\text{--}2D^d}{H\text{--}2D^b} \longrightarrow H\text{--}2^{i\text{--}2\,Sg} = H\text{--}2K^bH\text{--}2D^d \text{ (B10.A[5R])}$$

* Suspected but not proven H–2 crossovers.

Table II

Survival times of skin allografts transplanted in donor-recipient combinations which differ at the H–2K or the H–2D locus

Donor (H–2 chromosome)	Recipient (H–2 chromosome)	H–2 difference	MST ± S. D.*
B10.A (H–2^a)	B10.BR (H–2^k)	H–2D^d	17.0 ± 1.3
	B10.D2 (H–2^d)	H–2K^k	11.4 ± 0.8
B10.AKM (H–2^m)	B10.BR (H–2^k)	H–2D^q	14.0 ± 1.5
	B10.G (H–2^q)	H–2K^k	11.7 ± 0.7
B10.A (2R) (H–2$^{h\text{--}3\,Sg}$)	B10.BR (H–2^k)	H–2D^b	15.0 ± 1.3
	B10 (H–2^b)	H–2K^k	11.8 ± 0.9
B10.A. (5R) (H–2$^{i\text{--}2\,Sg}$)	B10 (H–2^b)	H–2D^d	15.3 ± 1.1
	B10.D2 (H–2^d)	H–2K^b	10.1 ± 0.8

* Mean survival time ± standard deviation.

cluding any non-H–2 incompatibilities. Both the donors and the recipients were 2- to 3-month-old males. The tail skin grafts, about 1 cm² in size, were obtained by simply cutting off the tail, stripping the skin and cutting it into five pieces. The grafts were placed on the left flank of the recipients. Otherwise, the same grafting technique described elsewhere (Klein &

Bailey 1971) was used. The bandages were removed seven days after transplantation, and the grafts were inspected daily until the end of the experiment. In each combination seven recipients were used. The results (Table II) indicate that in all combinations which differ at the H–2D locus, the skin grafts do not survive longer than an average of 17 days after grafting. This

mean survival time is shorter than MST of grafts exchanged across any of the known non-H–2 barriers. Thus, there is no question that the H–2D locus alone is a *histocompatibility* locus. In accordance with this conclusion, we have recently shown that the H–2D difference *can* cause a definite stimulation in MLC (Klein et al. 1972).

However, the results of skin grafting also show that in all the combinations tested, the grafts transplanted across the H–2D barrier survive, on the average, longer than those transplanted across the H–2K barrier. These results can be interpreted in three different ways. First, the difference between the H–2K and H–2D loci could be fortuitous. Some H–2 combinations could be more immunogenic than others. In the limited number of combinations tested, perhaps by chance, the weaker ones coincide with the H–2D incompatibilities. Testing additional combinations, as they become available, should either prove or disprove this possibility. Second, the H–2K locus for some reason may be stronger than the H–2D locus. (The H–2D product could be more deeply embedded in the membrane, or it could be slightly different chemically, etc.). This interpretation seems to be favored by the Prague group. Third, perhaps the H–2K and H–2D loci are of equal strength, and the observed difference in graft survival is attributable to a third locus which is closely linked to H–2 and located between the H–2K locus and the centromere. The products of this hypothetical third locus may not be detectable serologically, but play a significant role in cellular immunity. Such a locus has been postulated both for the mouse and man. In man, such a possibility was first considered in connection with the finding that leukocytes of some HL–A-identical siblings were mutually stimulatory in MLC tests (Amos & Bach 1969, Bach & Amos

1967). It was suggested that the stimulation could be due to a third locus which had no serologically detectable antigens associated with it. In the mouse, several circumstantial findings were interpreted by Amos as evidence for a third locus (Amos 1971, Amos & Yunis 1971). On the basis of the third locus hypothesis, the difference between the H–2K and H–2D incompatibilities could be explained by assuming that the third locus is stronger than either H–2K or H–2D. Alternatively a cumulative effect between the third locus and H–2K could result in a stronger incompatibility than the single H–2D difference. According to the third locus hypothesis, all the H–2K differences in Table II would also have to include third-locus differences. This is compatible with the origin of the four H–2 crossovers. The advantage of the third locus hypothesis is that it could explain not only the differential behavior of the H–2K and H–2D loci in the experiments of the Prague group and experiments reported in this communication, but also some inconsistencies associated with the H–2 complex. A striking example of this is the asymmetrical behavior of the parental variants selected from H–2 heterozygous tumors as described by George Klein's group in Stockholm. It has been shown by E. Klein (1961) that H–2^a/H–2^s heterozygous tumors when transplanted onto an H–2^d/H–2^s recipient can give rise to a variant which loses the H–2K^k allele but retains the H–2D^d allele of the original H–2^a (= H–2K^{k}H–2D^d) chromosome. However, transplantation onto an H–2^k/H–2^s recipient always leads to a concurrent loss of both H–2K^k and H–2D^d alleles, although the selection is directed against the H–2D^d allele only. A variant which retains the H–2K^k allele but loses the H–2D^d allele has never been found. The explanation offered for this asymmetry in variant formation is that the variant arises by mitotic

Tumor producing F_1 hybrid:

$$\frac{H-2^a}{H-2^s} = \frac{X^a H-2K^k H-2D^d}{X^s H-2K^s H-2D^s}$$

$\downarrow$

Tumor

Selective F_1 hybrids:

$$\frac{H-2^d}{H-2^s} = \frac{X^d H-2K^d H-2D^d}{X^s H-2K^s H-2D^s} \qquad\qquad \frac{H-2^k}{H-2^s} = \frac{X^k H-2K^k H-2D^k}{X^s H-2K^s H-2D^s}$$

Selection against X^a and $H-2K^k$ (X^a and $H-2K^k$ are lost), no selection against $H-2D^d$ ($H-2D^d$ is retained)

Selection against X^a and $H-2D^d$ (X^a and $H-2D^d$ are lost), $H-2K^k$ is also lost due to its central position

$H-2^a$ chromosome of the tumor variant:

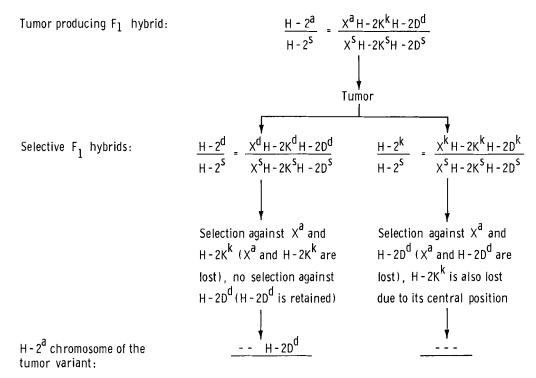$-\!-\ H-2D^d$ _____ $-\!-\!-$ _____

Figure 1. An explanation of the asymmetry in tumor variant formation on the basis of the third-locus hypothesis. (X = hypothetical third histocompatibility locus).

crossing-over within the H–2 complex. Such an explanation rests on the assumption that the centromere in the IXth linkage group of the mouse is proximal to the H–2D locus and distal to the H–2K locus. Recent evidence shows, however, that the order in the IXth linkage group is *centromere* .... *H–2K* .... *H–2D* .... (Lyon et al. 1968, Klein 1970, Miller et al. 1971 and J. Klein, unpublished results). This contradicts the gene order on which the mitotic crossing-over hypothesis was proposed. An explanation of the asymmetry of tumor variant formation on the basis of the hypothetical third locus (locus "X") is shown in Figure 1. A third histocompatibility locus at the centromeric side of H–2 would put the H–2K locus in the middle. An H–2ᵃ/H–2ˢ tumor which differs from an

H–2ᵏ/H–2ˢ recipient not only at the H–D2 locus but also at the third locus (X), apparently cannot lose the H–2Dᵈ allele without losing also the centrally located H–2K locus. This explanation requires the allele at the X-locus in H–2ᵏ to be distinct from the allele in H–2ᵃ, an assumption which is not quite in line with the cross-over hypothesis of the origin of H–2ᵃ from H–2ᵈ and H–2ᵏ. However, this discrepancy can be explained by an assumption of mutation at the X-locus of H–2ᵃ, or additional recombination between Xᵃ and H–2Kᵏ during the production of strains carrying the H–2ᵃ chromosome.

It should be stressed that at this moment it is not possible to decide between the alternative explanations offered for the observed difference in strength of the anti-

gens controlled by the H–2K and H–2D loci.

Acknowledgments

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