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The H-2 Model for the Major Histocompatibility Systems

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Thirty-five years ago, Gorer (1936), working with inbred strains of mice, discovered four blood group antigens and showed (Gorer 1937) that one of them, antigen II, was also present in fixed tissues and played a decisive role in determining susceptibility or resistance to tumor transplants. Following the suggestion of Snell (1948) that antigens concerned in transplantation reactions should be called histocompatibility (H) antigens, the designation antigen II was changed to H-2. The gene controlling the H-2 antigen was shown to be linked with *Fused* (a gene for a tail anomaly) in the 9th linkage group (Gorer *et al.* 1948). It soon became apparent through histogenetical studies by Snell and his coworkers (for a review, see Snell 1953) that what looked at first like a simple biallelic locus was actually a multiallelic system with many different *H-2* alleles present in different inbred strains. At the same time, serological studies by Gorer, Amos, Hoecker, and others (for a review, see Gorer 1959) revealed that the H-2 antigen was not simple, but consisted of increasing numbers of antigenic components. In 1951, Snell showed that F_1 hybrids between inbred strains BALB/c ($H\text{-}2^d$) and CBA ($H\text{-}2^k$) were susceptible to an A strain tumor and suggested that the $H\text{-}2^a$ allele of strain A was actually composed of two components, *d* and *k*, and should be therefore written as $H\text{-}2^{dk}$. This was the first indication of a bipartite structure of the *H-2* locus. The bipartity was further supported by detection of crossing-over between the *d* and *k* components (Allen 1955), but was later obscured by discoveries of additional recombinants and an increasing serological complexity. The idea re-emerged in 1965 when it was discovered that the *Ss* (serum protein) locus was located within the complex chromosomal region controlling H-2 antigens (Shreffler 1965).

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In 1956 Snell and coworkers (Counce *et al.* 1956) reported that the *H-2* locus has a unique position among the 15 or more histocompatibility loci of the mouse in the sense that it can cause much more rapid rejection of incompatible tumor transplants than any of the non-*H-2* loci. The *H-2* locus was therefore referred to as a 'strong' locus and the non-*H-2* loci as 'weak' loci. We shall use the more neutral terms 'major' (*H-2*) and 'minor' (non-*H-2*) loci. Major histocompatibility systems similar to H-2 have since been discovered in man and in the rat, rabbit, chimpanzee, rhesus monkey, dog, pig, fowl, and other species (for a review, see Iványi 1970).

The first human leukocyte antigen was discovered in 1958 (Dausset 1958), twenty-two years after the discovery of the H-2 antigen. In the early sixties several investigators, stimulated by developments in the transplantation field, detected a number of other leukocyte antigens and antigenic groups. However, several years passed before it was realized that all of these antigens belonged to the same system and that this system was similar to the *H-2* complex of the mouse. The system was first called *Hu-1* (Dausset *et al.* 1966), then renamed *HL-A* (Human Leukocyte-A system). At that time definition of the H-2 system was already at a rather advanced stage and provided a direct or indirect, conscious or unconscious, stimulus for developments in the HL-A system. The more recent history of the HL-A system is almost a repetition of the history of the H-2 system. Beginning with the serological techniques developed for H-2 (the cytotoxic test of Gorer & O'Gorman (1956) and the leukoagglutination technique of Amos (1953)), and continuing through discoveries of the role in transplantation, cellular and sub-cellular distribution, development, genetic bipartity, intraregional recombination, chemical nature, and presently culminating in speculations about biological function, the HL-A investigations have strikingly paralleled in approach and in results what had previously been shown in studies of H-2. One area in which the HL-A studies have taken a lead is in population analyses, but progress is now also rapidly being made in this area with the H-2 system.

Substantial progress has been made in the understanding of the HL-A system in the past decade – enough that some workers have stated that HL-A is now the 'model' for all of the major histocompatibility systems. We do not intend to *debate* that issue here. Rather we would like to present, for the benefit of any who may believe that the 'former' model system might still have something to contribute, a review of recent studies and ideas bearing on the genetic complexity and serological polymorphism of the H-2 system, and then undertake some comparisons of the H-2 and HL-A systems with respect to these two points.

GENETIC ORGANIZATION OF THE H-2 COMPLEX*

The genetic complexity of the chromosomal region which controls the H-2 antigens is well known. This region is complex, not only in that recombination separates determinants for various H-2 antigens, but also in that a variety of different phenotypic manifestations of genetic differences in this region have been observed. Recombination within the *H-2* complex, separating the genetic sites determining different H-2 antigenic specificities, was first shown by Amos *et al.* (1955) and by Allen (1955), and confirmed by Pizarro *et al.* (1961) and by Stimpfling & Richardson (1965). It was later shown that a number of other, apparently unrelated traits were also controlled by the *H-2* complex. These included quantitative and qualitative variations in a specific serum protein, the Ss protein (Shreffler & Passmore 1971), the 'hybrid resistance' phenomenon (Cudkowicz 1968), differences in susceptibility or resistance to certain tumor viruses (Lilly 1966, 1968, Tennant & Snell 1968, Nandi 1967), and a number of differences in levels of antibody response to various synthetic antigens (McDevitt & Tyan 1968, Rathbun & Hildemann 1970, Vaz & Levine 1970).

Many questions of genetic interest can be raised about the organization of this *H-2* complex. (1) How many loci are included within the complex? (2) What do these loci do, i.e., what are their genetic functions and interrelationships? (3) How are these loci arranged on the chromosome? (4) How did this complex region evolve? We have approached these questions through studies in two areas – investigations of the 'genetic fine structure' of the *H-2* complex and investigations of the nature of the genetic control of the Ss serum protein variations.

By 'genetic fine structure' is implied the detailed genetic linkage map of the segment of chromosome which controls the various H-2 antigens and associated traits. At the present time, concepts of this genetic fine structure are in a state of flux. The first such genetic map was proposed by Gorer & Mi-

* In the past, the terms *H-2* locus or *H-2* region have been used to refer to the segment of chromosome which determines the H-2 antigenic specificities. However, as discussed below, we now feel that this segment of chromosome is, in fact, composed of a complex of a number of genes, some with functions unrelated to the H-2 antigens. Therefore, we will use the term *H-2* gene complex or simply *H-2 complex* to refer to that chromosomal segment which determines the specificities of the H-2 antigens and which includes the determinants for a number of other, apparently unrelated traits. We will use the term *region* to refer to subdivisions of the H-2 complex defined by recombination. (Terminology suggested by Snell & Dausset, personal communication. See also Lewis (1967).) We will use the term *H-2 chromosome* to refer to the distinctive combinations of genetic information carried in the *H-2* gene complexes of 9th chromosomes from different sources.

kulska (1959) as a result of their serological analyses of three H-2 types presumed to have arisen by crossing-over within the *H-2* complex. They subdivided the complex into four segments labeled *D*, *C*, *V*, *K*. Pizarro *et al.* (1961) showed that the complex could be divided into at least two segments, one the *D* region and the other the *K* region. Stimpfling & Richardson (1965) and Stimpfling (1965) suggested that five intra-*H-2* cross-overs which they had detected were compatible with a subdivision of the *H-2* complex into at least three regions, *D*, *C*, *K*. Our own finding that the Ss trait is determined by a segment within the *H-2* complex, as well as further serological analyses of a number of intra-*H-2* recombinants, led to *H-2* maps of gradually increasing complexity, with 6 subdivisions (Shreffler 1967), 7 subdivisions (Shreffler 1970), and 8 subdivisions (Shreffler & Klein 1970).

All of these genetic maps for the *H-2* complex were derived through standard multi-point genetic mapping procedures. Implicit in this approach were several assumptions: (1) that each of the recombinants analyzed resulted from a single, equal, cross-over event; (2) that the *H-2* complex in different chromosomes determining different *H-2* types is of the same length and has the same number of genetic subdivisions, as defined by recombination; and (3) that the genetic determinant of a given H-2 specificity is always located at the same linear position within the *H-2* complex of every chromosome which determines that specificity. For a long time, it was possible, on the basis of these assumptions, to construct a single, linear *H-2* map which was satisfyingly consistent for all of the intra-*H-2* recombinants analyzed. However, recently a number of difficulties, and in some instances clear inconsistencies, with the linear order in the *H-2* map have appeared. These difficulties have been enumerated elsewhere (Shreffler 1970, 1971, Shreffler *et al.* 1971); the problems arising with antigen H-2.3 will serve to illustrate them. In 1965, Stimpfling & Richardson noted that among 9 recombinants derived from the heterozygous combination, $H\text{-}2^{a}/H\text{-}2^{b}$, in which $H\text{-}2^{a}$ determines the presence of specificity H-2.3, but $H\text{-}2^{b}$ does not, all of the recombinants were positive for antigen H-2.3, even though equal numbers of 3-positive and 3-negative recombinants would be expected under standard genetic assumptions. In 1966, Shreffler *et al.* reported that a new H-2 recombinant, $H\text{-}2^{oh}$, derived from the heterozygous combination $H\text{-}2^{d}/H\text{-}2^{k}$, in which both parental alleles are 3-positive, had a very aberrant H-2.3 specificity and could almost be considered to lack this specificity. This was again inconsistent with genetic expectation. Finally a detailed analysis of *H-2* recombinants previously reported and classification for a new variant of the Ss system (the Slp allotype, Passmore & Shreffler 1970) led to the recognition of a clear inconsistency in map position for the determinant of H-2.3 in one recombinant, $H\text{-}2^{h\text{-}3Sg}$ (usually referred to as 4R) (Shreffler 1970). Similar kinds of inconsistencies have also

been noted for antigens H-2.1 and H-2.5 (Shreffler 1970) and for H-2.35 and H-2.36 (Démant *et al.* 1971b).

As a result of these inconsistencies, a suggestion first made in 1966 (Shreffler *et al.* 1966) that the *H-2*^{oh} difficulty might be accounted for if the determinants for the H-2.3 antigen were located in different positions on the two different parental *H-2* chromosomes has been elaborated and applied in a more general way (Shreffler 1970, 1971, Shreffler *et al.* 1971). This resulted in a new 'duplication model' for the genetic structure of the *H-2* complex which accounted for all of the inconsistencies in map position for antigens H-2.1, H-2.3, etc. This new model proposes that the genetic determinants for these specificities have been duplicated one or more times during evolution and that these determinants may therefore be located in different positions on different chromosomes and in some cases may be located in at least two different positions on the same chromosome. Figure 1 depicts a *postulated* organization for one *H-2* chromosome, *H-2*^{k}, and a generalized mechanism by which this organization might have evolved (Shreffler *et al.* 1971). It is emphasized that this Figure is intended to be highly diagrammatic. The several segments of the *H-2* complex which are depicted are postulated to be individual genes; however, these segments could also conceivably be mutational sites within a smaller number of genes or, at the opposite extreme, could be considered to be complexes of large numbers of genes. In this Figure and in previous discussions (Shreffler 1971), we have suggested that at least four regions of the *H-2* complex, two on either side of the *Ss-Slp* re-

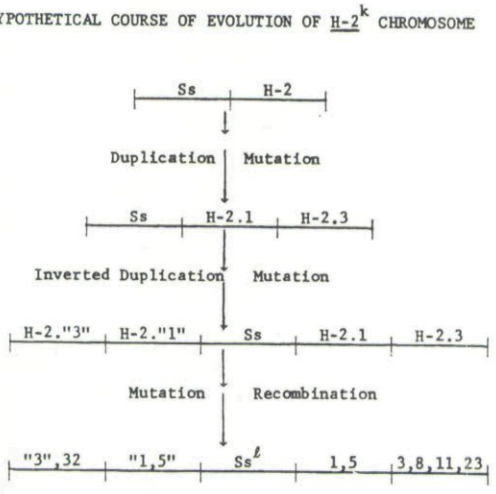

Figure 1. A hypothetical model for evolution of the *H-2* complex, relating specifically to the *H-2*^{k} chromosome (from Shreffler *et al.* 1971).

gion, can be identified as controlling distinct H-2 specificities and as separable by recombination. The evidence for four such regions is less firm than the evidence for a minimum of two regions, a *K* region and a *D* region on either side of *Ss-Slp*. Verification of additional subdivisions in the *K* and *D* regions must await further serological analyses.

The Figure implies that a number of different H-2 antigenic specificities may be determined by a single region or a single gene within the *H-2* complex. As we have discussed previously (Shreffler 1967), and as others have also pointed out, it is quite possible that multiple antigenic specificities might be determined by the same gene or even by the same mutational site within a gene, if one assumes that these different antigens reflect different populations of antibodies, all cross-reactive with a single antigenic combining site.

The duplication model has several interesting implications. First, it provides a basis for explanation of the varying degrees of serological cross-reactivity of anti-H-2.1 and anti-H-2.3 sera among various *H-2* alleles, which has been suggested by the work of Snell *et al.* (1971a) and Démant *et al.* (1971b). If the *K* and *D* regions of the *H-2* complex did in fact arise through duplication of a single ancestral gene, then it might be expected that the products of these two regions could have substantial structural similarities and that certain antisera might be found to react with the products of both regions. The model also provides a basis for understanding the inclusion, within a gene complex controlling cellular antigens, of the unrelated gene(s) controlling the Ss protein. *Ss* can be viewed as a linked, but unrelated, gene which was by chance included within the duplication (Figure 1). This could also apply to the immune response trait (see below).

Thus, at present, one in effect has a choice of two different genetic maps for the *H-2* complex. One is based on standard mapping principles. It is quite complex, with 7 or 8 regions, and is not entirely consistent with the data. The other, as described above, is somewhat unorthodox and speculative but is simpler and more consistent with available data. Further data will be required to determine which is correct. Regardless of these uncertainties, a number of points can be made about the genetic organization of the *H-2* complex which do seem to be rather well-established.

1. Despite the difficulties involved in precisely localizing specificities H-2.1, H-2.3, etc., a number of major genetic determinants or markers can be definitively mapped on the 9th chromosome. These are shown in Figure 2. Two regions controlling H-2 antigens can be considered as unequivocally established. These are designated *K* and *D*. We emphasize that this is a *minimal* number; the existence of at least these two discrete segments for control of H-2 antigens is beyond question. This does not necessarily imply that additional regions, e.g., *A*, *E*, *V*, etc., may not exist, only that they are less well

established. It would also seem now to be established beyond any doubt that the *K* and *D* regions are separated by the *Ss-Slp* determinant (Shreffler & Passmore 1971). (Note that the map positions of the *K* and *D* regions have been reversed from those shown on previous *H-2* maps. This results from new findings with respect to centromere position, which place the centromere of the 9th chromosome at the *K* end of the *H-2* complex (Lyon *et al.* 1968, Klein 1970). By accepted genetic practice, the genetic markers are ordered from left to right, beginning with the centromere.) Recent data on the Ir-1 trait (McDevitt *et al.*, to be published) rather strongly indicate that the genetic determinant for this trait is located between the *Ss-Slp* and *K* regions of the *H-2* complex. Two intra-*H-2* recombinants are consistent in positioning *Ir-1* to the right of the *K* region and three recombinants position it to the left of *Ss-Slp*. The position of the *Tla* locus was established some time ago (Boyse *et al.* 1965). The positions of the *tf* and *T-t* markers at the *K* end of the *H-2* complex were also previously established (Shreffler 1965, Stimpfling 1965).

2. The events giving rise to the exceptional H-2 types which we have referred to as 'recombinant' types seem now to be clearly established as classical genetic crossing-over. This has been accomplished through the use of genetic markers outside the *H-2* complex. One recombinant has been found among the progeny of a heterozygous parent carrying outside markers on both sides of the *H-2* complex. One of the parental chromosomes was marked on one side, at the *K* end, by the *T* (Brachyury) marker and at the *D* end by a translocation marker *T(2;9)138Ca*. The recombinant offspring from this cross showed the expected exchange of outside markers (Klein *et al.* 1970). Further progeny from this cross are currently being screened. In other studies, consistent exchanges have been observed between *D* region antigens and the *T* marker outside the *K* region (Shreffler 1965, Stimpfling 1965).

The availability of a new stock carrying a single metacentric chromosome, one arm of which bears the ninth linkage group, should now permit cytogenetic verification of crossing-over. This stock was established by crossing mice of the species *Mus poschiavinus* with inbred *Mus musculus*, then successively backcrossing to the inbred *Mus musculus* stock while selecting for

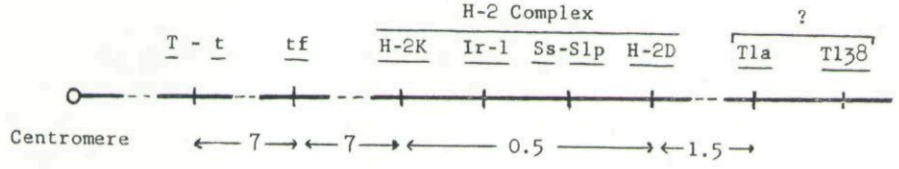

Figure 2. A simplified map of linkage group IX of the mouse.

Mus poschiavinus H-2 type. The *Mus poschiavinus* species carries 7 pairs of metacentric chromosomes, apparently derived from previously telocentric chromosomes through Robertsonian fusion. It appears that the 9th chromosome was included in such a fusion, and the continued backcrossing and selection has led to isolation of a stock carrying a single metacentric chromosome bearing the *H-2* complex (Klein, to be published). This stock promises to be useful for a variety of cytogenetic studies of *H-2*.

3. The frequency of recombination within the *H-2* complex is on the order of 0.5 per cent (Shreffler 1970). The frequency may vary between about 0.1 per cent and about 1 per cent for various heterozygous combinations, but this is the order of magnitude with which the event occurs, at least in inbred mouse strains. If one assumes that recombination frequency is proportional to physical length of DNA, the *H-2* complex *could* encompass several hundred genes; however, such a figure must be viewed with reservation (Shreffler & Klein 1970).

4. Rather a large number of 'private' H-2 specificities have been clearly positioned in either the *K* region or the *D* region of the *H-2* complex (see Table I below). As discussed below, the present data both for inbred mouse strains and wild populations suggest that a rough separation of H-2 specificities into two classes, the 'private' and 'public', is possible. As a further generality, the private specificities appear to be relatively sharply defined, identified by high titered antisera, restricted to a very few *H-2* chromosomes, and always restricted either to the *D* or to the *K* region. On the other hand, the public specificities tend to be less sharply defined, are more likely to show variable degrees of reactivity among different *H-2* chromosomes, are more widely distributed among different *H-2* chromosomes, and in some instances it appears that a given specificity may be determined sometimes by the *D* region, sometimes by the *K* region, or sometimes by both regions (Shreffler *et al.* 1971).

The next major question regarding the organization of the *H-2* complex concerns how many genetic loci occur within the complex, and what the functions of these loci may be. A number of aspects of these questions have already been discussed (Shreffler & Klein 1970). Of particular importance here are the relationships of the *Ss-Slp* and *Ir-1* determinants to the H-2 antigens. The principal features of the Ss-Slp system were recently reviewed (Shreffler & Passmore 1971). They are briefly summarized below.

The Ss-Slp trait involves two types of variation in a single serum protein, the Ss protein. The *Ss* variation is quantitative, detected on immunodiffusion by rabbit antiserum against the mouse Ss protein. Differences of about 20-fold in the level of this protein are observed between animals homozygous for an Ss^h allele, which determines a high level of the protein, and an Ss^l al-

lele, which determines a low level of the protein; the heterozygous type is intermediate. This quantitative variation involves no structural variation which can be detected by the rabbit antiserum. The Slp variation is allotypic, detected on immunodiffusion by specific alloimmune sera. The presence of Slp antigen in the serum is controlled by a dominant autosomal gene, Slp^a; its allele, Slp^o, determines absence of the antigen. The expression of the Slp^a gene is limited to normal males (hence Slp = sex-limited protein) and is under the control of male hormone (Passmore & Shreffler 1971). The Slp antigen can be induced in Slp^a/- females by administration of testosterone. Slp antigenic sites occur upon the same molecules which react with rabbit anti-Ss, indicating that Slp reflects structural variation in these Ss molecules. However, in the serum of the Slp-positive animal, not all Ss molecules carry Slp antigenic sites. Therefore, such sera have two populations of Ss molecules, those with and those without Slp antigenic sites. Whether this reflects separate synthesis of the two populations of molecules or synthesis of a single population, some fraction of which is structurally modified, has not yet been established.

Genetically, the Ss quantitative variation and the Slp structural variation are determined by a single locus or by loci so closely linked that they have not been separated by recombination. It has been unequivocally established, through analyses of 16 intra-*H-2* recombinants, that the *Ss-Slp* genetic determinant maps within the *H-2* gene complex between the *K* region and the *D* region. The Ss and Slp differences appear to be functionally unrelated to H-2 alloantigenic differences. This is most clearly demonstrated by the long-term survival of skin grafts from mice carrying the $H\text{-}2^{oh}$ allele to mice of a congenic background carrying the $H\text{-}2^{ol}$ allele, which differs from $H\text{-}2^{oh}$ only in that it carries the Ss^l determinant rather than the Ss^h determinant (Shreffler, unpublished). The Ss difference thus confers no demonstrable histocompatibility difference. Stimpfling & Reichert (1970) have shown long-term survival of skin grafts exchanged among strains B10.A (1R), B10.A (2R), and B10.A (4R), which apparently differ only in that the 4R strain is Slp^o whereas the other two are Slp^a. This would indicate that Slp differences likewise confer no histoincompatibility. Furthermore, among *H-2* chromosomes differing only with respect to Ss type, there is no apparent difference in level of expression of H-2 antigens, indicating that the quantitative Ss differences do not play any regulatory role with regard to H-2 antigens. It has also been shown that Ss protein and H-2 antigen preparations do not cross-react serologically to any detectable degree (Ferraro & Nathenson, personal communication). These findings, plus a number of other pieces of evidence previously summarized (Shreffler & Passmore 1971), indicate that the *H-2* and *Ss-Slp* genetic determinants are probably entirely unrelated functionally.

TABLE

The H-2 antigens and associated

	H-2 chromosome symbol	*H-2K* region symbol	*H-2K* region: Private 20	21	19	25	17	16	23	15	31	33	11	*H-2K* region: Public 36	35	34	8	5	3	1	*Ss* region: Ss	Slp
H-2 chromosomes of independent origin	b	b	–	–	–	–	–	–	–	–	–	33	–	36	35	–	–	–	–	–	h	o
	d	d	–	–	–	–	–	–	–	–	31	–	–	–	–	34	8	–	–	–	h	a
	f	f	–	–	–	–	–	–	–	–	–	–	–	–	–	.	8	–	–	–	h	o
	j	j	–	–	–	–	–	–	–	15	–	–	–	–	–	.	–	–	–	–	h	a
	k	k	–	–	–	25	–	–	23	–	–	–	11	–	–	.	8	5	3	1	*l*	o
	p	p	–	–	–	–	–	16	–	–	–	–	–	–	–	.	8	–	–	–	h	a
	q	q	–	–	–	–	17	–	–	–	–	–	11	–	–	34	–	5	–	–	h	o
	r	r	–	–	–	25	–	–	–	–	–	–	11	–	–	–	8	5	–	–	h	o
	s	s	–	–	19	–	–	–	–	–	–	–	–	–	–	.	–	5	–	–	h	a
	v	v	–	21	–	–	–	–	–	–	–	–	–	–	–	.	–	5	–	–	h	a
	u	u	20	–	–	–	–	–	–	–	–	–	–	–	–	.	8?	5	–	–	h	o
H-2 chromosomes derived by recombination	a	k	–	–	–	25	–	–	23	–	–	–	11	–	–	–	8	5	3	1	h, *l*	a, r
	g	d	–	–	–	–	–	–	–	–	31	–	–	–	–	.	8	–	–	–	h	a
	h	k	–	–	–	25	–	–	23	–	–	–	11	–	–	.	8	5	3	1	h	a, o
	i	b	–	–	–	–	–	–	–	–	–	33	–	36	35	.	–	5	–	–	h	a, o
	m	k	–	–	–	25	–	–	23	–	–	–	11	–	–	.	8	5	3	1	*l*	o
	o	d	–	–	–	–	–	–	–	–	31	–	–	–	–	.	8	–	–	–	h, *l*	a, o
	t	s?	–	–	19	–	–	–	–	–	–	–	–	–	–	.	–	5	–	1	$\frac{h}{l}$	$\frac{a}{o}$
	y	q	–	–	–	–	17	–	–	–	–	–	11	–	–	.	–	5	–	.	h	a
Suspected duplicates	*l* (= j?)		–	–	–	–	–	–	–	15	–	–	–	–	–	.	–	–	–	–	h	a
	w (= j?)		–	–	–	.	–	.	–	15	–	–	–	–	–	.	–	.	.	–	h	a
	n (= p?)		.	.	–	.	–	16	.	–	–	–	.	–	–	34	8	–	–	–	h	a

(–) = absence of an antigen.
(.) = not tested.
(*) = modified form of an antigen.
(c) = weak cross-reactivity.
(?) = questionable result.

Since the *Ss-Slp* determinant maps between the *H-2K* and *H-2D* regions, since there is no evidence for functional relationship between the Ss-Slp and H-2 traits, and since the Slp structural variation strongly suggests that *Ss-Slp* is a structural locus for the Ss protein, it seems almost inescapable that the *H-2* complex must be composed of *at least* three independent genes corre-

I
traits arranged by region

Ir region			*H-2D* region																			
				Public														Private					
(T G)-A--L	(H G)-A--L	(P G)-A--L	*H-2D* region symbol	1	3	5	6	7	13	27	28	29	35	36	41	42	43	2	4	9	32	30	18
h	l	h	b	–	–	5	6	–	–	27	28	29	–	–	–	–	–	2	–	–	–	–	–
m	m	h	d	–	3	–	6	–	13	27	28	29	35	36	41	42	43	–	4	–	–	–	–
l	*l*	h	f	–	–	–	6	7	–	27	–	–	–	–	–	–	–	–	–	9	–	–	–
l	m	h	j	–	–	5*	6	7	–	–	28	29	–	–	–	–	–	2	–	–	–	–	–
l	h	h	k	1*	3*	5*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	32	–	–
l	*l*	h	p	1*	3*	5*	6	7	c	–	–	–	35	–	41	–	–	–	–	–	–	–	–
l	*l*	h	q	1*	3*	.	6	–	13	27	28	29	c	c	c	c	c	–	–	–	–	30	–
l	.	.	r	1*	3*	.	6	–	c	–	–	–	–	–	c	c	–	–	–	–	–	–	18
l	*l*	*l*	s	1*	3*	.	6	7	c	–	–	–	c	36	c	42	–	–	–	–	–	–	–
.	.	.	v	1*	3*	.	.	.	c	–?	28?	.	–	–	–	c	43	–	–	–	–	30*	–
.	.	.	d	–	3	–	.	–	13	27?	28	.	35	36	41	42	43	–	4	–	–	–	–?
l	h	h	d	–	3	–	6	–	13	27	28	29	35	36	41	42	43	–	4	–	–	–	–
m	*l*	h	b	–	–	.	6	–	–	27	28	29	–	–	–	–	–	2	–	–	–	–	–
.	h	.	b	–	–	.	6	–	–	27	28	29	–	–	–	–	–	2	–	–	–	–	–
.	*l*	.	d	–	3	–	6	–	13	.	.	.	35	36	41	42	43	–	4	–	–	–	–
l	h	h	q	1*	3*	.	6	–	13	27	28	29	c	c	c	c	c	–	–	–	–	30	–
.	m	.	k	1*	3*	5*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	32	–	–
.	*l*/h	.	d	–	3	–	6	–	13	.	.	.	35	36	41	42	43	–	4	–	–	–	–
l	h	h	d	.	3	–	6	–	.	.	.	.	.	.	.	.	.	–	4	–	.	–	–
l	*l*	h	*l* (= j?)	–	–	?	6	7	–	–	–	?	–	–	–	–	–	2	–	–	–	–	–
l	*l*	h	w (= j?)	–	–	.	.	.	–	.	.	.	–	–	.	–	.	2	–	–	–	–	–
l	*l*	h	n (= p?)	1*	3*	.	6	7	–	–	–	–	.	.	.	.	.	–	–	–	.	–	–

sponding to the *H-2K*, *Ss-Slp*, and *H-2D* regions. However, it remains to be established whether these regions in fact represent single genes or complexes of related genes.

As noted above, the Ir-1 trait also very probably maps inside the *H-2* complex, between the *K* and *Ss-Slp* regions. Questions about the functional relationship between *Ir-1* and the H-2 antigens might also be raised here. It would be helpful to have skin grafting data involving *H-2*-identical but *Ir-1*-distinctive combinations, as was the case for *Ss-Slp*. However, thus far such

a test has not been possible. It can only be stated that there is no demonstrable correlation between Ir-1 type and any defined H-2 specificities. Furthermore, recombination between Ir-1 type and most of the known H-2 specificities has been demonstrated (C. Grumet & H. McDevitt, personal communication). Therefore it is questionable whether the Ir-1 differences reflect simple H-2 alloantigenic differences. Although further testing of this point will be necessary, at the moment it would appear that there is no direct interrelationship and that, like Ss-Slp, the Ir-1 trait represents an independent genetic determinant or set of determinants which have in some way come to be located within the *H-2* gene complex.

The question of the functional relationship of the *K* and *D* regions has been considered previously (Shreffler & Klein 1970). We will only reiterate that the weight of evidence supports functional independence of the genes in these two regions.

In summary, while many points remain to be clarified, a number of positive statements can be made about the genetic organization of the *H-2* complex. First, it can be definitely subdivided by recombination into at least four regions, *H-2K*, *Ir-1*, *Ss-Slp*, *H-2D*, and these have been positioned with respect to each other and to other markers in the 9th linkage group. The frequency of recombination within the complex ranges between 0.1 and 1 per cent. The different regions appear to be functionally unrelated and therefore probably represent distinct, independent genetic loci or complexes of genetic loci. There is evidence that the products of the *K* and *D* regions are serologically related, suggesting that they may have evolved through duplications of a primitive ancestral *H-2* gene. The position of *Ss-Slp* and *Ir-1* within this complex chromosomal region probably reflects a chance intercalation of unrelated loci into the complex during the duplication process.

POLYMORPHISM OF THE H-2 SYSTEM

Since 1951 when it first became apparent that the H-2 antigens are complex (Snell 1951), the serology of the H-2 system has been in a state of constant modification and revision. New antigens have been discovered and new *H-2* chromosomes identified, old antigens have been split and new mouse strains added to the growing list of those typed. It has become customary to summarize current knowledge of H-2 serology in the form of a so-called 'H-2 chart' listing all the typed strains, their *H-2* chromosomes, and the antigens determined by the chromosomes.

The H-2 chart published in 1964 (Snell *et al.* 1964) contained 18 *H-2* chromosomes (a, b, c, d, e, f, g, h, i, j, k, l, m, n, p, q, r, s) and 25 H-2 anti-

gens (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 16, 17, 19, 22, 25, 27, 28, 29, 30, 31, 32, 33). Since that time 6 more *H-2* chromosomes have been added to the chart: *H-2*^{w} (Snell & Stimpfling 1966), *H-2*^{o} (Shreffler *et al.* 1966), *H-2*^{t} (David *et al.* 1969), *H-2*^{y} (Klein *et al.* 1970), *H-2*^{u} and *H-2*^{v} (Snell *et al.* 1971a, Démant *et al.* 1971b). At the same time, the number of H-2 antigens has increased by 14, with the following antigens added to the list: 18 (Shreffler & Snell 1969), 15 (Snell *et al.* 1971c, J. Klein, unpublished data), 34 (Davies 1969), 20, 21, 23, 35, 36, 41, 42, 43 (Snell *et al.* 1971a, Démant *et al.* 1971b), 37, 38, and 39 (Démant *et al.* 1971a). This makes a total of 24 known *H-2* chromosomes and 39 known H-2 antigens. However, several *H-2* chromosomes and H-2 antigens should probably now be dropped from the chart because they are proven or suspected duplicates of other *H-2* chromosomes or antigens. The following *H-2* chromosomes are proven to be identical: *H-2*^{c} of D1.C and *H-2*^{d} of DBA/2 (J. Klein & D. C. Shreffler, unpublished data); *H-2*^{e} of STOLI and *H-2*^{q} of DBA/1 (Shreffler & Snell 1969). The following *H-2* chromosomes are thought but not yet proven to be identical: *H-2*^{n} of F/St and *H-2*^{p} of P; *H-2*^{l} of I/St, *H-2*^{w} of WB/Re, and *H-2*^{j} of JK/St. This leaves only 19 of the original 24 chromosomes. Of the 39 H-2 antigens, H-2.10, 14, and 22 should probably also be deleted from the chart because the antisera which identified these antigens have not been reproducible. Thus the number of known H-2 antigens would be reduced to 36.

The complexity of the present H-2 chart can be further reduced when the origins of the individual *H-2* chromosomes are considered, if two assumptions based upon the discussion in the preceding section are made: First, there are two genes or gene complexes in each *H-2* chromosome, the *H-2K* region and the *H-2D* region, and these two regions are separated by a 'gap' occupied by genes not related directly to H-2 antigens, e.g. *Ss* and *Slp* (Shreffler 1971), *Ir-1* (McDevitt & Tyan 1968), and perhaps others. As noted above, this 'gap' between *H-2K* and *H-2D could* be occupied by several hundred genes (Shreffler & Klein 1970). Second, these two *H-2* genes (or gene complexes) were derived during evolution from a single 'primordial' *H-2* gene by duplication (Shreffler *et al.* 1971).

Of the 19 known *H-2* chromosomes, 8 or 9 are known or suspected to be derived from other *H-2* chromosomes by recombination (*H-2* chromosomes g, h, i, o, t, and y are known cross-overs, chromosomes a, m, and possibly also u are suspected cross-overs). With the two above assumptions in mind, the *H-2* cross-overs can be interpreted as derived by recombination *not within* the *H-2* genes but in the 'gap' *between H-2K* and *H-2D*. If this interpretation is correct, then the number of different *H-2* chromosomes making up the H-2 chart is still 19 but the number of different 'alleles' at the *H-2K* and *H-2D* 'loci' is 10 and 11 respectively. (The *H-2*^{u} chromosome is interpreted as the

result of a recombination between an unknown *H-2* chromosome and chromosome *H-2*^{d}.) A simplified H-2 chart based upon these assumptions is shown in Table I. (A somewhat similar chart has been presented by Snell *et al.* (1971b).) Thus, if our underlying premises are correct, the serological analysis of the available inbred strains does not provide as complex a picture of H-2 polymorphism as usually thought. This is undoubtedly a reflection of the fact that most of the presently known inbred strains are related in origin (Staats 1966). Under these circumstances it is questionable whether a search for new *H-2* chromosomes by typing more inbred strains would really be worth the effort.

A more realistic estimate of H-2 complexity can be achieved through studies of wild mice. Such studies are already under way both in our laboratories and in the laboratory of Dr. Pavol Iványi in Prague. A complicating factor in these studies is the peculiar structure of the natural populations of the house mouse, *Mus musculus*. Evidence gathered for some time by several investigators indicates that wild mice live in more or less closed colonies (demes), isolated by virtual absence of migration between these units (for references, see Klein & Bailey 1971). As a consequence of this isolation, a certain degree of inbreeding can be expected and is indeed being found within a deme. Variation of any genetic trait within these breeding units is therefore strongly decreased. Our calculations based on skin graft survival between C3H × Wild hybrids show that mice in any given deme segregate for only about 4 to 9 *H*-genes (Klein & Bailey 1971), as compared to some 29 *H*-genes segregating in crosses between two inbred lines (Bailey & Mobraaten 1969). A similar conclusion was also reached by Iványi & Démant (1970). This, of course, means that many mice caught at the same locality and presumably belonging to the same deme will have identical or very similar H-2 phenotypes (Klein 1970) and thus provide little information about H-2 polymorphism. The search for different *H-2* chromosomes must, therefore, be carried out on mice from as many different localities as possible. We have thus far tested over 150 wild mice from 25 different localities (Klein 1970, 1971a) and have found more than 30 different H-2 phenotypes. Of these, we have isolated 20 *H-2* chromosomes which are now being transferred onto the inbred background of strain C57BL/10ScSn, to produce a series of B10.Wild congenic resistant lines. Preliminary evidence indicates (J. Klein, unpublished data) that all 20 chromosomes are different, not only from the known inbred *H-2* chromosomes, but also from each other; the present H-2 chart will thus soon be expanded by another 20 chromosomes. The evidence is based on direct typing with monospecific H-2 reagents produced in inbred strains as well as reagents produced against wild mice. Four of the 20 wild *H-2* chromosomes have been studied in greater detail by absorption analysis (Klein,

TABLE II

Antigens determined by four H-2 chromosomes derived from wild mice

H-2 chromosome	H-2 antigens*												Strain
	2	4	6	9	15	16	19	23	101	102	103	104	
wa	–	–	–	–	–	–	–	–	101	–	–	–	B10.KPA42
wb	–	–	–	–	–	–	–	–	–	102	–	–	B10.BAA77
wc	–	–	–	–	–	–	–	–	–	–	103	–	B10.SAA148
wd	–	–	–	–	–	–	–	–	–	–	–	104	B10.GAA20

* Only antigens which have been tested by *in vitro* absorption are included in the chart. Others tested by direct test await confirmation by absorption analysis and therefore have not been included.

to be published). Their H-2 antigenic composition is shown in Table II. These four lack all of the known H-2 antigens of inbred strains thus far tested, but each has a new antigen not present in inbred strains. Linkage tests show that these antigens belong to the H-2 system. We suggest that all H-2 antigens detected in wild mice and absent in inbred strains be designated by numbers above 100, and that all new *H-2* chromosomes derived from wild mice be denoted by a letter symbol beginning with w, *H-2*^{wa}, *H-2*^{wb}, *H-2*^{wc}, etc. Each of the antigens, H-2.101 through H-2.104, has been found in a number of wild mice trapped in a single locality, but not in mice from other localities. Thus, for instance, antigen H-2.101 was found in 4 out of 6 mice captured at locality KPA, but is absent from mice from 24 other localities tested. This restricted occurrence of the wild H-2 antigens is undoubtedly a consequence of the deme structure of mouse populations.

When tested with monospecific antisera against H-2 antigens of inbred strains, the wild mice divide these antigens into two classes (Klein 1971) – private and public (Figure 3). Private H-2 antigens occur with very low frequency in the local populations of wild mice. Some of them (e.g. H-2.4 or H-2.23) seem to be missing from wild populations completely. In inbred strains, the private antigens are restricted to a single *H-2D* or *H-2K* allele. The four new antigens detected in the wild mice (H-2.101 to H-2.104) probably also belong to this class. The public antigens, on the contrary, occur with high frequencies both in wild mice and in inbred strains. An example of these antigens is H-2.5, which is present in 95 per cent of wild mice trapped in the Ann Arbor area and is missing in only two out of 11 known *H-2D* or *H-2K* alleles. Antigens H-2.1, H-2.3, and others also belong to this class. The genetic factors for at least some of the public antigens seem to be present in both the *H-2K* and *H-2D* gene complexes (Shreffler *et al.* 1971, Snell *et al.* 1971a, Démant *et al.* 1971b). Furthermore, the public antigens as op-

posed to the private ones, seem to be complex, with a certain degree of cross-reactivity (Snell *et al.* 1971, Démant *et al.* 1971b, Klein 1971). These differences in properties of the private and public antigens could be explained by the assumption that the *H-2K* and the *H-2D* regions are each divisible into at least two genetic units, one for public and one for private antigens (Klein 1971). It should be stressed, however, that no genetic evidence for such duality of the two *H-2* regions is thus far available. Furthermore, the division into private and public antigens may be somewhat arbitrary; intermediate forms between these two classes will probably be found. The private-public distinction may also possibly relate in some way to the problems of serological cross-reactivity discussed in the next section.

In summary, although the inbred H-2 chart can be greatly simplified by the introduction of two simple premises, the studies of wild mice indicate a complexity of the H-2 system far exceeding complexity of other serological systems, perhaps with the exception of the B system in cattle (Stormont 1962). This serological complexity implies extensive genetic polymorphism of the H-2 system. The polymorphism can be explained either by a relatively few multiallelic genes or by multiple genes each with a few alleles. Although we favor the former explanation, further chemical and genetic data will be required to resolve this question.

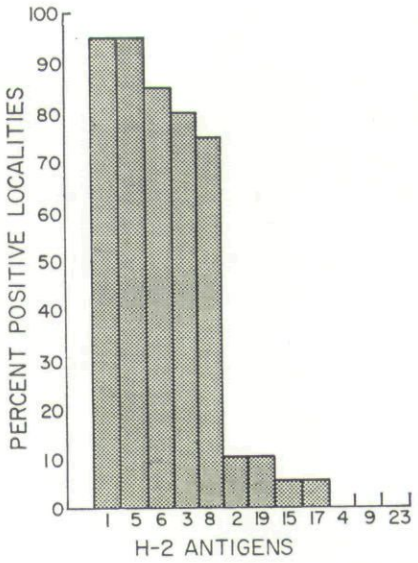

Figure 3. Phenotypic frequencies of some H-2 antigens in wild mice from 20 different localities in the Ann Arbor area. The antigens were tested by direct hemagglutination with monospecific H-2 antisera.

HOMOLOGY OF H-2 AND HL-A SYSTEMS

The fact that every species of mammals seems to have one major H-system might indicate that all these systems evolved from one original gene, i.e. that all are genetic homologues. If this is the case, one would expect a high degree of serological, genetic, and biochemical similarity between major H-systems of different species. Unfortunately, in most species knowledge about these systems is in such a rudimentary state that a realistic comparison of their properties is impossible. The only two exceptions are the H-2 system of the mouse and the HL-A system of man. Having briefly reviewed the genetic and serological aspects of the H-2 system, we would like now to make a few comparisons with the genetics and serology of the HL-A system.

The HL-A system (for a review and references, see Kissmeyer-Nielsen & Thorsby 1970) consists of 11 antigens which are officially accepted by the WHO Nomenclature Committee and some 10 or more additional antigens recognized thus far only by individual laboratories. The antigens have been divided into two groups, the so-called first (LA) and second (Four) segregant series. Six officially accepted, plus two or more additional antigens, have been assigned to the first series; five officially accepted, plus 8 or more additional antigens, have been assigned to the second segregant series. The antigens of each series are mutually exclusive and are believed to be controlled by multiple alleles of the same gene (sublocus). According to present dogma, each *HL-A* chromosome (haplotype) carries two subloci and can determine a maximum of two antigens (one by each sublocus). The gene frequencies at each sublocus vary from less than 0.01 for some alleles to more than 0.3 for others. When added up, the gene frequencies for known antigens at each sublocus total close to 1.0, at least for Caucasian populations. This is interpreted by many investigators as evidence that the serology of the two HL-A series is basically fairly well established and that almost all the HL-A antigens are known and their properties rather well-defined. Several cases of apparent recombination between antigens of the two segregant series have been reported. These are interpreted as further evidence supporting the concept that the two segregant series of antigens represent the products of two separate genes or subloci.

There are, however, some difficulties with this beautifully simple picture. In many instances, antigens of each segregant series are associated in so-called *inclusion groups*. In general, antigen Y is considered to be included in antigen X if X+Y+, X+Y– and X–Y–, but not X–Y+ individuals are found in the population. The inclusion phenomenon is explained by most HL-A investigators by unidirectional cross-reactivity of the corresponding antibodies. For example, anti-Da-15 (= Ba*) is assumed to react with Da-15 and cross-

react with HL-A2, while anti-HL-A2 reacts only with HL-A2 and does not cross-react with Da-15. In the population, all Da-15-positive and all HL-A2-positive individuals react with anti-Da-15 but only HL-A2-positive individuals react with anti-HL-A2. The two antisera thus form an inclusion group in which HL-A2 antigen is included in antigen Da-15. Cross-reactivity of HL-A antibodies seems to be a very common phenomenon. However, at the moment, it would seem that every inconsistency in the HL-A system is attributed to cross-reactivity, often with no experimental basis. The actual extent of cross-reactivity can be assessed only through appropriate absorption and immunization studies.

Some HL-A antigens do not fit into the two-segregant series scheme. The 4a/4b system of van Rood is a typical example. One difficulty with this system is that almost none of the anti-4a/4b antisera gives identical or even reasonably similar reaction patterns. Furthermore, 4a/4b antigens of different investigators show different phenotypic frequencies and the population usually shows a surplus of 4a+4b+ heterozygotes. No agreement has yet been reached on how to interpret this system.

Other non-conforming HL-A antigens have been used by different investigators for construction of additional segregant series. Unfortunately, the investigators disagree as to whether these additional series are real or illusory.

The H-2 system presents a different picture. The number of known H-2 antigens is already greater than the number of HL-A antigens and probably no one in the field believes that we are anywhere near the point of complete knowledge of H-2 serology. Quite on the contrary, the studies of wild mice seem to imply that we are just at the door-step of understanding of H-2 complexity. Also, contrary to HL-A, a single *H-2* chromosome determines more than 2 antigens, in some cases ($H\text{-}2^a$) up to 19 antigens! Only private antigens controlled by the two *H-2* complexes show mutual exclusiveness (Snell *et al.* 1971b), but since these antigens are so rare in the mouse population and the population studies are limited, it does not necessarily mean that they are controlled by allelic series. No antithetical relationships are thus far apparent in the group of public H-2 antigens.

The genetic structure of the H-2 and HL-A systems is seemingly similar. In both cases the chromosomal region controlling the two systems can be divided into two subregions – *H-2K* and *H-2D* in the case of H-2, and LA and Four in the case of HL-A. However, the basis for this division is different for the two systems. The division of the *H-2* complex is based on clearly established recombination plus the fact that a gene or genes (*Ss-Slp*) which apparently has nothing to do with H-2 antigens is inserted between the two subregions. The division of the *HL-A* chromosomal region is primarily based on the mutual exclusiveness of antigens controlled by the same subregion and

random association of antigens controlled by different subregions. In both systems, recombinations among antigens consistent with genetic crossing-over have been reported. More than 40 have been found in *H-2* (Shreffler 1970, Stimpfling & Reichert 1970), almost all confirmed by progeny test, and some 20 subjected to detailed serological and immunization analyses. The findings have been summarized in a preceding section. Even with this body of data, there are many unanswered questions. A total of six putative HL-A recombinants has been reported (see Kissmeyer-Nielsen & Thorsby 1970). None of these has yet been confirmed by progeny test. While the *most probable* explanation for these cases is certainly genetic recombination, the proof of this hypothesis in presently inadequate. It does seem a bit premature to calculate a recombination frequency and the number of *cistrons* encompassed, based on two cases (Bodmer *et al.* 1970), and to conclude that this '*clearly* (italics ours) indicates that the *LA* and *Four* genetic determinants occur in different cistrons.' For both systems, the chemical evidence is consistent with two separate genetic regions, but cannot yet be regarded as conclusive (see Shreffler & Klein 1970). Hence the genetic bipartity of the H-2 system is based upon an adequately verified separation of antigens by crossing-over plus a physical separation by an intercalated gene and can be considered as an established fact. The bipartity of the HL-A system involves only statistical separation and a few cases of recombination still requiring verification, and should therefore be considered as a reasonable hypothesis.

Thus not only parallels but also important differences exist between the H-2 and HL-A systems. In general, the HL-A system appears to be serologically and genetically simpler than the H-2 system. The discrepancies between the serologies of the H-2 and HL-A systems can be explained in several different ways. One way is to assume that the two systems are not truly homologous, the similarities between them being merely coincidental. Such an explanation seems, however, rather unlikely. There are several arguments against it. The H-2 and HL-A antigens behave similarly in ontogenesis (Möller & Möller 1962, Klein 1965, Seigler & Metzgar 1970), they have the same tissue distribution (Basch & Stetson 1962, Berah *et al.* 1970), the same subcellular localization (Aoki *et al.* 1969, Silvestri *et al.* 1970) and perhaps most important, they have similar chemical compositions (for a review, see Nathenson 1970). A second explanation for the current discrepancies between the serologies of the H-2 and HL-A systems is to assume that the two systems are truly homologous but that they are *interpreted* differently. According to Hirschfeld (1965), any composite immunogenetic system can be interpreted on the basis of two different models, which he calls simple-complex and complex-simple. In the simple-complex model it is assumed that the antibodies are simple (they react specifically with a single antigenic determinant) and the

antigens are complex (one molecule carries several antigenic determinants). According to the complex-simple model, the antibodies are complex (they cross-react with more than one antigenic determinant) and the antigens are simple (each molecule carries only one antigenic determinant). 'Both models give oversimplified and conceptually different pictures of reality. The conventional simple-complex model is idealized (and thus falsified) in one direction in that simple (specific) antibodies are assumed. The new complex-simple model is idealized (and thus falsified) in the opposite direction, in that simple antigens (antigens with only one kind of antigenic determinant) are assumed. Both of these models are consequently restricted and complementary' (Hirschfeld 1965). Until the chemical basis for the antigenic differences in an immunogenetic system is known, it will be very difficult, probably impossible, to decide which of the two models is closer to reality for that system.

The H-2 system has traditionally been interpreted according to the simple-complex model. It has generally been treated as though each *H-2* chromosome controls several antigenic determinants and the H-2 antibodies react specifically with each determinant. The possibility of alternative interpretations has been pointed out, however (Shreffler 1967). The HL-A system, on the other hand, is generally interpreted according to the complex-simple model. It is believed that each *LA* and *Four* allele determines only one antigen and that the HL-A antibodies can cross-react with antigens determined by different alleles of the same sublocus. It is obvious that the serological discrepancies between the two systems might be less striking if both were interpreted on the basis of the same model. The problem is, of course, to decide which of the two interpretations more nearly approaches reality.

According to Thorsby (1971), it is the H-2 system which is interpreted erroneously. Thorsby suggests that each *H-2K* and *H-2D* allele actually determines only one antigen and that all the other antigens associated with the particular allele are caused by cross-reacting antibodies. As supporting evidence for this interpretation, Thorsby cites two inclusion groups depicted by Snell and coworkers (Snell *et al.* 1971a, Démant *et al.* 1971b). One of the two groups is the following:

H-2K allele	Inclusion group antigens	Other antigens
k	23, 25, 11, 5,	8, 3, 1
r	25, 11, 5,	8
q	11, 5,	17
b	5,	33, 35, 36
s	5,	19
v	5,	21
u	5,	8, 20

Thorsby explains the inclusions by unidirectional cross-reactivity of the H-2 antibodies: Anti-H-2.5 cross-reacts with H-2.23, 25, and 11; anti-H-2.11 cross-reacts with H-2.23 and 25 but not with H-2.5; anti-H-2.25 cross-reacts with H-2.23 but not with H-2.11 or 5, etc. According to this concept, $H\text{-}2K^{k}$ would determine H-2.23 but not H-2.25, 11, and 5, these latter antigens being an artifact of the cross-reactivity; $H\text{-}2K^{r}$ would determine H-2.25 but not H-2.11 and 5, etc.

Serious objections can be raised against such an interpretation of the H-2 system. Firstly, since the inclusion groups are based on a limited number of *H-2* alleles, there is no guarantee that they are real. Secondly, even if they are real, they can be explained differently, for instance by linkage disequilibrium or by structural features of the H-2 molecule (Shreffler & Klein 1970). There is no evidence for cross-reactivity between H-2.23, 25, 11, and 5. On the contrary, H-2.11 of $H\text{-}2K^{k}$ is, for instance, a serologically much stronger antigen than H-2.11 of $H\text{-}2K^{q}$ although, according to the cross-reactivity model, it should be just the opposite. Thirdly, even if the inclusion groups were the result of cross-reactivity, there are additional antigens determined by each allele which do not fit into the inclusion scheme and which must somehow be accounted for. Finally, the scheme leads to *H-2* 'haplotype' assignments which are clearly inaccurate when the *full* serological data are considered. For example, $H\text{-}2^{f}$ and $H\text{-}2^{g}$ are both assigned the 'simplified' haplotype 6, 8, yet $H\text{-}2^{f}$ is, in fact, H-2.6, 7, 8, 9, 27 and $H\text{-}2^{g}$ is H-2.2, 6, 8, 22, 27, 28, 29, 31; they are totally dissimilar. [One might perhaps relate this to the observation that in unrelated individuals, there is no correlation between HL-A compatibility and skin graft survival time (Kissmeyer-Nielsen & Thorsby 1970). Possibly the *HL-A* haplotype assignments in these cases are likewise too highly simplified.] Another example is the $H\text{-}2^{o}$ recombinant, derived from $H\text{-}2^{k}/H\text{-}2^{d}$ (3**,23/4,8). It was assigned, by Thorsby, the haplotype 3**,1, but should be 3**,8. A number of similar examples could be cited. It seems fair to say that this application of the complex-simple interpretation leads to no very obvious improvement in our picture of the H-2 system. Despite these difficulties, a complex-simple interpretation of the H-2 system is certainly possible. However, it would have to be done in a more sophisticated way. One possibility is, perhaps, to begin with the private antigens and to attempt to arrange the rest of the H-2 chart around them (Snell *et al.* 1971c).

We have stressed above that both the complex-simple and the simple-complex interpretations are to some degree idealized and therefore false. The question which is more appropriate for the genetic evaluation of a given system is essentially academic, since we would maintain that *no mode* of inter-

pretation can reliably achieve the desired goal of relating serological properties to the molecular structure of the gene products and in turn to the properties of the controlling genes (Shreffler 1967). While serological methods provide extremely sensitive means for detection of genetic differences, they are of little value in defining the molecular structures of the antigens with which they react. That is a chemical problem. On the other hand, if it is found that an antigenic difference now interpreted according to the complex-simple model as due to cross-reactivity can actually induce transplantation immunity, then the question of interpretation of the HL-A system becomes of great practical importance. As far as the H-2 system is concerned, there seems thus far to be no compelling need for gross changes in its interpretation.

Another explanation for the serological discrepancies between the two systems is that the present HL-A serology is greatly oversimplified. One might predict that as it becomes better defined it will resemble more closely the current picture of the H-2 system. There are several important differences in the ways the two systems are analyzed which justify such a prediction.

Many H-2 antigens can be detected only by highly hyperimmune antisera. Such antisera are obtained by repeated injections of lymphoid cells in amounts equivalent to an entire mouse spleen. The HL-A antigens are detected mostly by antisera from multiparous women or polytransfused patients. Only recently have intentional immunizations of volunteers been widely used. In none of these cases does the intensity of immunization approach that possible in the mouse. It is therefore likely that many HL-A antigens escape detection simply because they are poor stimulants of antibody formation. From the clinical standpoint, there is no evidence to indicate that a serologically weak antigen could not be a strong transplantation antigen.

The process of selection of donor-recipient combinations for immunizations is also different in the H-2 and HL-A studies. In the mouse, the tendency is to test systematically as many donor-recipient combinations as possible so that it is more or less guaranteed that few serological differences will escape detection. In man, until recently, no such system existed in antiserum production. The investigators were dependent on random immunizations during pregnancies or transfusions. The more recently introduced, intentional immunizations of volunteers do not much improve the situation, since the aim of such immunizations is usually to produce antisera to antigens already known.

The selection of HL-A typing antisera is also biased. It is guided by two principles: The antiserum must be strong and, when tested with a panel of cells, it must show a statistically significant correlation in positive and negative reactions with at least one additional antiserum. Such selection will, of

course, eliminate the weak and more complex antisera, leading to detection only of the more dominant antigens of the system. This may account in particular for the failure to detect complex and broadly reactive sera comparable to those defining the 'public' antigens of the H-2 system. HL-A serology is likewise biased in the process of 'splitting up' broad antigens into more narrow ones. If an antigen which was originally assumed to be homogeneous proves to be heterogeneous instead, only those antisera with the most restricted reaction pattern are used to redefine the antigen, again with loss of potentially useful information.

It is customary in H-2 serology to study each new antigen or new allele, not only by direct tests and absorption, but also by immunization analysis. HL-A serology was for a long time based mainly on the results of direct tests. Only recently have absorption tests been more or less routinely used (although not usually published). Systematic immunization analyses are still lacking in most HL-A studies.

As these methodological limitations in HL-A serology are gradually overcome, the evidence mounts that the simple, single allele-single antigen concept is no longer tenable. The confusion about the additional HL-A subloci, the difficulties with the 4a/4b system, the frequent occurrence of 'extra' reactions even in supposedly monospecific antisera, and such anomalies as production of antibodies against antigens absent from the immunizing cells, production of antibodies to antigens present on the recipient's cells – all suggest that it will eventually be necessary to abandon the present simple HL-A model in favor of a more complex (more H-2-like?) one. Sentiment that even the established HL-A antigens are not simple entities, but rather antigenic complexes, has recently been expressed by several investigators (Iványi & Dausset 1966, Batchelor & Sanderson 1970). We would predict that it will sooner or later be shown that: (1) there are only two genetic regions (subloci) in the *HL-A* complex; (2) each region determines more than one antigen; (3) many of the currently defined HL-A antigens will be shown to be complex and will be subdivided into several components; (4) serologically weaker antigens will be discovered with improved methods of immunization.

In conclusion, it would appear at the moment that the H-2 system has in the past been somewhat overcomplicated and is undergoing a degree of simplification, while the HL-A system has been rather oversimplified and is now entering a phase of more detailed definition. At some intermediate point, they may be expected to meet. We take it as an article of faith that the two systems are homologous and are likely to be very similar in their general properties. The key issue thus becomes, not which system is the 'model' for the other, but how the investigations of these two systems (and others in other

species) can complement each other to yield maximum information, on the one hand, about practical problems of transplantation and, on the other hand, about the genetic and molecular mechanisms and the biological significance of the most complex mammalian genetic systems presently known.

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