

## HL-A Model of the H-2 system?

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In a recent article, Thorsby (1971a) proposed what he called an "HL-A interpretation of the H-2 system". According to this interpretation, each allele of the two H-2 genes (*H-2D* and *H-2K*) controls only one "true" antigen; the rest of the specificities presently listed in the H-2 chart are assumed to be artifacts caused by cross-reactive antibodies. In a review of the H-2 system, we briefly criticized this view (Klein & Shreffler 1971) for the following reasons: (1) The *general* idea that at least some of the H-2 specificities may be due to cross-reactive antibodies is not at all new; (2) The *specific* model proposed by Thorsby is confusing and misleading, and contributes nothing to our understanding of the H-2 system; (3) The traditional interpretation of the H-2 system (several specificities determined by each H-2 chromosome) better suits present needs; (4) There is no way to definitively prove or disprove, by serological methods, either of the two alternative interpretations of com-

plex immunogenetic systems such as H-2 or HL-A, and it therefore seems premature to take a *dogmatic* position on the matter.

In a reply to our criticisms, Thorsby (1971b) has reiterated the concept that a "complex-simple" interpretation of serological data may be applied to the H-2 system (a point which we do not contest), but has, in our view, confused and confounded the *interpretation* with the *system of notation* used to represent serological findings. We would like to further amplify and clarify our objections to Thorsby's H-2 model, and then to deal with the specific new points and criticisms of our review raised in his letter.

Ever since Landsteiner's classical experiments on cross-reactive antibodies against simple haptenic groups (Landsteiner 1945), the concept, which much later came to be called the "complex-simple" interpretation of serological systems (Hirschfeld 1965), has existed in immuno-

Clearly, the interpretation and the notation applied fail to recognize substantial incompatibilities and have very little predictive value with respect to intra-*H-2* recombination. This is hardly an improvement over the present interpretation.

The primary basis for these difficulties is that the approach which was employed in assigning a "single antigen" designation for each *H-2D* and *H-2K* allele was arbitrary and artificial, and not even consistent with the approach employed for HL-A. In general, as we understand it, a number of *narrow*, mutually exclusive HL-A antigens may be included in a "family", cross-reactive with one or more *broadly* reactive antisera. In cases in which a "piece" of a broadly reactive specificity is not covered by a narrow specificity this is given a (\*) designation, as IH\* in Thorsby's example (1971a). Thus, a better approach to an "HL-A interpretation of H-2" is the one taken by Snell et al. (1971) in assigning haplotypes composed of "private" H-2 specificities. The statement by Thorsby (1971b) that "very few antisera which clearly recognized private antigens of the H-2D and H-2K regions were published at the time the tentative model was developed," is simply not correct. The fact is that most of the private H-2 antigens have been recognized for more than a decade and only three new private specificities were added recently. A simple examination of the past H-2 literature would have revealed this. For example, Amos (1962), clearly identified certain of the private H-2 specificities. Beyond this objection, even if the private specificities were not recognized, the usual HL-A interpretation would have assigned a *distinctive* symbol to each antigen in the inclusion group. Thus the antigen of the D-series for *H-2* chromosomes *f*, *g*, *l* and *n* should at least have been 6\*, rather than 6, by HL-A convention, etc.

Furthermore, we feel no temptation to abandon the traditional notation for the H-2 system and replace it by such a rigid complex-simple model for two additional reasons. The first one is historical. The H-2 and HL-A systems have developed under different circumstances. The H-2 system was constructed on the basis of very thorough serological analysis of a limited number of *H-2* chromosomes, extensive cross-immunization studies, and almost no population analysis. The HL-A system, on the other hand, has been based, at least in its first phase, almost exclusively on population analysis, with very limited intentional immunization studies. Thus, there has been almost no limitation on the complexity in the case of the H-2 system, while in the case of the HL-A system some simplifications have been essential to bring order into the very complex data. To reinterpret the H-2 system and assign a new notation at this stage of development just to make it look more like HL-A would be most unwise. However, as we have suggested earlier (Klein & Shreffler 1971), further development of H-2 toward population studies and HL-A toward intentional cross-immunization analysis, coupled with better chemical definition of the antigens, might bring the two systems together on the same platform quite naturally.

The second reason for maintaining the traditional interpretation of the H-2 system is more pragmatic. One can, of course, trim the H-2 chart and leave in it only the private antigens; but this will not stop investigators from using reagents made against public H-2 specificities. To preserve some order in the system the specificities should have symbols assigned to them and if the symbols are maintained, there is no reason to exclude the public specificities from the H-2 chart. Even if the complex-simple interpretation is closer to reality, it would still be desirable to keep

genetics. H-2 investigators have been aware of the two alternative explanations of the H-2 system essentially from the time of recognition of H-2 complexity. The possibility of a complex-simple interpretation of the H-2 system was most explicitly expressed by Owen (1959a, b) and reiterated by one of us in 1966 (Shreffler 1966). The "segregant series" in the H-2 system was first suggested by Gorer in 1956 (although, at that time, they were given more prosaic terms such as "alleles" and "antithetical antigens"). The bipartite structure of the H-2 system has been a clearly established fact for some time (Shreffler 1965). Under these circumstances the development of an "HL-A interpretation of the H-2 system" seems to us to reflect an unfamiliarity with the history of immunogenetics and particularly of the H-2 system. It is perhaps worthwhile to point out that immunogenetics as a scientific discipline did exist before the discovery of HL-A.

Our strongest objection is to the specific model and notation proposed by Thorsby. In his model, Thorsby chose two of the several specificities determined by each H-2 chromosome and designated them as the "true" antigens with the rest being

ascribed to cross-reactivity. In this way he assigned 34 antigens to 17 H-2 chromosomes. In our opinion, of these 34 assignments, 28 are entirely incorrect. Specificities H-2.1,3,5,6,8,13 and 28, which Thorsby variously assigned to chromosomes b,d, f,g,h,i,j,k,l,m,n,o,p,q,r and s, are all "public" antigens broadly distributed among different H-2 chromosomes. H-2.6, for instance, is one of the broadest H-2 specificities known, shared by all the known H-2 chromosomes except one. None of these specificities fulfills the criterion on which Thorsby based his classification into two segregant series, namely mutual exclusiveness. If any, *these* specificities are the most likely ones to be artifacts of cross-reactivity.

As a consequence, Thorsby's assignments are full of internal inconsistencies which create paradoxical situations. We have already noted that H-2 chromosomes *f* and *g* were assigned the same haplotype, even though they differ by 7 specificities. Thorsby's "revised" H-2 chart also includes four haplotypes derived by recombination involving other haplotypes in the chart. In every case the assignments are inconsistent, as shown below:

Recombinant chromosome	Parental chromosome	Parental haplotypes		Haplotype expected		Haplotype assigned	
		D locus	K locus	D locus	K locus	D locus	K locus
g	$\frac{d}{b}$	4	$\frac{\quad}{5}$	28	8	6	8
h	$\frac{a}{b}$	4	$\frac{\quad}{5}$	28	23	3	23
i	$\frac{a}{b}$	4	$\frac{\quad}{5}$	4	5	4	1*
o	$\frac{d}{k}$	4	$\frac{\quad}{23}$	3**	8	3**	1

the public specificities in the chart, because this is a very useful mechanism for showing the cross-reactivities among the various H-2 antigens. In fact, it has recently been suggested by Ceppellini (1971) that it would be of value to include in the HL-A haplotype designations, the broad specificities such as 4a and 4b. To take Thorsby's example (1971a), perhaps it would be *useful* to have an "HL-A chart" which shows the antigenic profile of haplotype HL-A2,5 as HL-A2, Da 2, HL-A5, 4c, SL, 4a, 4a1, 4a2, 4A\*, because it *could* be that factors Da2 or SL or 4a, etc. are more important in transplant survival or disease association than are HL-A2 or HL-A5. One could still *use* such a chart under *whatever serological interpretation* he might like.

Thorsby suggests some experiments which he thinks could test the complex-simple model of the H-2 system. For example, he would immunize H-2<sup>b</sup> mice with H-2<sup>a</sup> cells and search for antibodies which would react with antigen H-2.4 and cross-react with antigens H-2.3 and 13. Such immunizations have been done many times in several laboratories and have not proven anything. Antisera obtained from such immunizations react with all strains possessing H-2.4 as well as with strains possessing H-2.3 and 13. Thorsby would interpret this as evidence that the antisera contain only one antibody (anti-H-2.4) which cross-reacts with H-2.3 and 13. It can also be postulated that the antiserum contains at least three antibodies, anti-H-2.3, 4 and 13 and that these antibodies react differentially with the corresponding antigens. Thus the same data can be interpreted in two different ways. We see no way that this can be resolved by serology alone. One interpretation may be more *convenient* for a particular system but that does not prove that it is correct. The ambiguity of such serological data is not new. It has been

repeatedly stressed in the past by many immunogeneticists.

In his reply to our criticism, Thorsby (1971b) argues that the complex-simple interpretation provides a better basis for explaining the work of Brondz & Goldberg (1969). The authors have shown that immune lymphocytes do not kill third-party target cells *in vitro* even though the third-party strain shares one public specificity with the donor. According to Thorsby, the lymphocytes fail to do so because the cross-reactivity is not recognized at the cellular level. This is certainly a possible explanation. However, we fail to understand how this has any bearing on the "code" used for the H-2 system. The fact is clearly established that there is serological cross-reactivity between the H-2 antigens of the H-2<sup>a</sup> and H-2<sup>b</sup> types. The relevant specificity in this case is given the designation H-2.5. The data of Brondz & Goldberg indicate that this cross-reactivity is not recognized in their particular test system. This certainly requires an explanation, but we do not understand the logic in assigning, to the H-2<sup>a</sup> and H-2<sup>b</sup> antigens, symbols which fail to show the cross-reactivity as a means for explaining the data. Nothing is gained in the understanding of the phenomenon by concealing the cross-reactivity. If Thorsby's notation were used for H-2, the phenomenon would not even have been recognized; in fact, the experiment would probably not even have been done in this way. Whether the basis for the effect lies in qualitative similarities and differences among single antigens or quantitative differences in number of antigenic sites, and whether the reactive lymphocytes are monovalent or polyvalent, are questions which must be answered at another level than that of serological interpretation. It is certainly possible to *recognize* these *alternatives* under *either type of notation*. One must be very careful not to *confuse*

*notation with serological interpretation.* In any case, the distinction between the serological alternatives must come from a *biochemical* analysis of the antigens. Preliminary results from one such analysis seem to support the simple-complex rather than complex-simple interpretation of the H-2 system.

At the molecular level, the two models can be visualized in a simplified form as follows: According to the complex-simple model, each H-2 molecule carries only a single antigenic site which is recognized by a heterogeneous population of cross-reactive antibodies. According to the simple-complex model, each H-2 molecule carries more than one antigenic site and the multiple sites are recognized by specific antibodies. If one could split the H-2 molecule into fragments and show either that the antigenic sites are always on one fragment or that different fragments carry different sites, this would show which of the two models is more realistic. This has not yet been achieved. However, Pancake & Nathenson (personal communication) used an indirect approach which tests the two models without the necessity of fragmenting the H-2 molecule. These investigators isolated the product of the *H-2K* locus in the *H-2<sup>b</sup>* chromosome. According to the complex-simple model, this molecule should carry only one antigenic site (H-2.33) and the reaction of anti-H-2.5 antibodies with the molecule should be due to cross-reactivity with the H-2.33 site. According to the simple-complex model, the H-2K<sup>b</sup> molecule could carry at least two antigenic sites, H-2.33 (private) and H-2.5 (public). Pancake & Nathenson subjected the molecules to formaldehyde treatment, followed by reductive alkylation, and then tested the preparations with anti-H-2.5 and anti-H-2.33 antisera. They found that after treatment the anti-H-2.33 antibodies no longer reacted with the pre-

paration while the reactivity of the anti-H-2.5 antibodies remained unchanged. Apparently, the treatment specifically inactivated the H-2.33 site and left the H-2.5 site intact. Therefore, there must be more than one antigenic site on the H-2K<sup>b</sup> molecule. Although this result is not completely unambiguous, it speaks strongly *in favor* of a multiple site interpretation of the H-2 system, at least with respect to these two specificities. This result should serve as a caution that we must keep an open mind about interpretation of serological data, and recognize the limitations of the serological approach. It seems to us quite possible that multiple H-2 specificities controlled by a single *H-2D* or *H-2K* allele will in some instances be found to be associated with a single antigenic site, in other instances with multiple sites on a single molecule. As we have stressed repeatedly (Shreffler 1966, Klein & Shreffler 1971), this is primarily a chemical problem.

Finally, we should emphasize that our purpose in the review (Klein & Shreffler 1971) was not to advocate the simple-complex over the complex-simple interpretation, or vice versa, but to show that many of the *apparent* serological differences between H-2 and HL-A stemmed from the differences in interpretation of the two systems. We indicated that a simple-complex interpretation of the HL-A system was possible, but recognizing our limited knowledge of the HL-A system, we did not feel it appropriate to offer a detailed "H-2 interpretation of the HL-A system." [Although we are not experts on the HL-A system, we would like to note that our statements concerning the HL-A system which were criticized by Thorsby as inaccurate are all based upon work of established HL-A investigators, *viz.* cross-reactivity between HL-A2 and Da15 (Walford et al. 1970, Figure 2; Dausset et al. 1970),

formation of antibodies to specificities not found on the immunizing cells (Amos 1970; see also Ceppellini 1971).] We agree with Thorsby that H-2 and HL-A are probably homologous and similarly organized – this is the point we tried to make. However, we believe that the true relationship of the two systems is more likely to be seen if one keeps an open mind toward all possible interpretations.

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