ISOANTIGENS OF THE *H-2* AND *Tla* LOCI OF THE MOUSE

INTERACTIONS AFFECTING THEIR REPRESENTATION ON THYMOCYTES*

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The arrangement of the various components of the cell surface, or what one might call the topography of cell membranes, is relatively unexplored. A great deal is known, for example, about the inheritance of isoantigens, their tissue distribution, and their influence on histocompatibility, but little about where the components on which these specificities are carried fit into the cell membrane. Although it is an assumption that these components are situated in defined interrelated patterns, this appears self-evident from the facts of differentiated cell function and organogenesis, for example, which can scarcely be dissociated from topographical details of cell surfaces.

Two features of the H-2 (1) and TL (2) isoantigens suggested an approach to this question. First, *H-2* (*Histocompatibility-2*) and *Tla* (*Thymus leukemia antigen*), both apparently compound loci, are closely linked to one another (2, 3) suggesting the possibility of related features, including perhaps location of H-2 and TL antigens on the cell. Some similarity was suggested by the finding that TL antigens can be isolated by methods previously applied with success to H-2 (4), although they are, at the same time, largely separated from H-2. Secondly, mice which we classify as TL− because they lack the TL antigens expressed in the strains classified TL+ (TL.2 or TL.1,2,3) are thought also to lack antigens genetically homologous with TL.1 and TL.2,[1] and possibly

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[1] The existing basis for this view (which is substantiated in this communication) is the occurrence of TL.1 and TL.2 antigens in certain leukemias of probably all mouse strains, including TL− strains (5). The explanation put forward to account for the presence of TL.1 and TL.2 in leukemias of TL− mice is activation of TL.1 and TL.2 structural genes that normally are repressed in TL− mice; it follows that normal thymocytes of TL− mice cannot possess components directly homologous with TL.1 and TL.2 in the sense of being specified by alleles of TL.1 and TL.2 structural genes (2, 6, 7). (Notational obscurity that might arise from this genetic situation has been avoided in this report by the use of TL phenotype symbols rather than *Tla* genotype symbols.)

TL.3. This permits comparison of cells possessing antigen specified by a particular locus with cells of the same type lacking such antigen, an opportunity that does not arise with systems of isoantigens other than TL, except in rare instances such as the Bombay blood type in man (8). These were the reasons for examining the antigens of the *H-2* and *Tla* loci in this context. The study was necessarily limited to thymocytes because these are the only normal cells on which TL antigens are expressed.

TABLE I

Cytotoxic Test Systems Used in Quantitative Absorption Tests

Serum	Test cell	Specificity detected in absorption test* (designation in text)
A *anti*-C57BL ascites leukemia EL4	C57BL/6 lymphocytes H-2H lymphocytes H-2I lymphocytes	H-2^b H-2^b(D region) H-2^b(K region)
C57BL/6 *anti*-A strain spontaneous leukemia ASL1	H-2I lymphocytes H-2H lymphocytes	H-2^a(D region) H-2^a(K region)
(C57BL/6 × A)F_1 *anti*-C3H ascites sarcoma BP8	C3H/An lymphocytes	H-2^k(D region)
C57BL/6 *anti*-BP8	H-2H lymphocytes	H-2^k(K region)
B10.A(3R) *anti*-B10.A(H-2^a) lymphoid tissue‡	H-2I lymphocytes	H-2.4, 13
B10.A(3R) *anti*-B10.A(H-2^a) absorbed in vivo in B10.AKM (H-2^m)‡	H-2I lymphocytes	H-2.4
C57BL/6 *anti*-ASL1	C57BL/6 radiation-induced leukemia ERLD	TL.1
C57BL/6 *anti*-129 thymocytes	129 thymocytes	TL.2
(BALB/c × C3H/An)F_1 *anti*-ASL1 absorbed in vivo in (C57BL/6 × A)F_1 ♂♂ bearing advanced transplants of C57BL/6 leukemia ERLD	A thymocytes	TL.3

* The notation "D region" or "K region" is here preferred to identification by naming all the specificities that may be represented in polyvalent H-2 antisera so designated, because under the conditions of quantitative absorption with diluted serum only antibodies of high titer contribute to the result, low-titer antibody to "weak" components having been diluted out.

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Materials and Methods

Antisera.—See Table I.

Cytotoxic Test.—The cytotoxic test was performed according to Gorer and O'Gorman (9) modified (see reference 10); diluent Medium 199. Details concerning the use of thymocytes as test cells in the cytotoxic test are given elsewhere (10). The most important precaution is absorption of the guinea pig serum (C′ source) with mouse cells. This removes its natural toxicity for thymocytes (11) and is performed in the cold to minimize loss of C′.

Estimation of H-2 and TL Antigens on Viable Thymocytes by Quantitative Absorption of Cytotoxic Sera.—Aliquots (0.06 ml) of diluted antiserum were absorbed with viable thymocytes in numbers ranging from 2×10^6 to 2×10^7, preliminary tests being performed to determine for each antiserum the dilution at which absorption with 5×10^6 thymocytes (for 30 min at 6°C with shaking) reduced its cytotoxicity for the selected standard test cells indicated in Table I to approximately 50% (count of dead cells). A stock of this serum dilution was stored frozen in aliquots for use throughout the entire study. In instances where this dilution was greater than 1/20, 2% normal mouse serum (NMS) was added to the diluent (199). The results of cytotoxic tests with the absorbed aliquots of serum were expressed as per cent viability of the standard test cells, and plotted vs. number of thymocytes used for absorption. This absorption curve gave, for each thymocyte-type, the number of thymocytes required to lower the cytotoxic activity of the diluted serum to the level at which 50% of the standard test cells (Table I) remained viable. Two thymocyte-types were always tested concurrently, to give the comparative content of antigen (absorption capacity) of the pair. The indexes obtained by these pair-by-pair comparisons were finally converted to per cent antigen content of the selected standard thymocyte, according to the groups shown in the tables.

Mice.—These were obtained from our colonies. The two cross-over stocks C57(TL+) and A(TL−) were derived by serial backcrossing (to C57BL/6 and A respectively) of two mice in which crossing-over between *H-2* and *Tla* was observed in the course of linkage tests (2). The C57(TL+) cross-over stock differs from C57BL/6 in being TL.1,2,3 like the A strain. The A(TL−) cross-over stock differs from the A strain in being TL− like the C57BL/6 strain.

RESULTS AND DISCUSSION

Influence of the TL Phenotype on the Representation of H-2 Antigens on Cell Surfaces.—Table II summarizes the measurements of H-2 representation on thymocytes of a variety of different *H-2* and *Tla* genotypes. (The lines and columns in the table have been given numbers and letters, respectively, for reference in the text.) Comparison of pertinent entries in the Table illustrates the considerable accuracy of the absorption method used for quantitative determination of antigen, an experimental error of more than 3% being exceptional.

The H-2 antigen content of *H-2* heterozygous cells (lower entries in Table II) is expressed as the percentage of a standard *heterozygous* cell rather than of a standard homozygous cell for the reason that under the conditions stated heterozygous cells do not show the expected 50% absorption capacity of homozygous cells, but a higher figure (Table III). (This is true of H-2 but not of a number of non-H-2 systems, including TL itself, as will be seen in Table IV.) These data may imply that the *H-2* locus actually produces more antigenic determinants in heterozygous cells, but we think it more likely that

the discrepancy is inherent in methods of quantitative absorption and has to do with the relative densities of H-2 antigenic sites on cells of the two types. According to this view, the pattern and density of H-2 antigenic determinants on the cell surface entails competition of antibody molecules for specific attachment sites. This competition we assume to be greater with *H-2* homozygous

TABLE II

Reduced Representation of H-2(D Region) Isoantigens on Thymocytes Carrying TL Antigens

Thymocytes: parental *H-2* genotypes and TL phenotypes* *H-2* TL/*H-2* TL	H-2 Antigens							
	$H\text{-}2^b$		$H\text{-}2^a$				$H\text{-}2^k$	
	Region D	Region K	Region D	Region D (4, 13)	Region D (4)	Region K	Region D	Region K
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
	relative antigen content (% standard)							
H-2 homozygotes								
(1) *b*−/*b*−	100(s)‡	100(s)						
(2) *b* 1, 2, 3/*b* 1, 2, 3	58	101						
(3) *a*−/*a*−			100(s)	100(s)	100(s)	100(s)		
(4) *a* 1, 2, 3/*a* 1, 2, 3			77	65	47	100		
(5) *k*−/*k*−							100(s)	100(s)
(6) *k* 1, 2, 3/*k* 1, 2, 3							78	97
H-2 heterozygotes								
(7) *b*−/*a*−	100(s)		100(s)					
(8) *b*−/*a* 1, 2, 3	66	100(s)	72			100(s)		
(9) *b* 1, 2, 3/*a*−	64	102	74			98		
(10) *b* 1, 2, 3/*a* 1, 2, 3	34		52					

The lines in the table have been numbered and the columns lettered for reference in the text.

* Source: *b*−, C57BL/6; *b* 1, 2, 3, C57(TL+); *a*−, A(TL−); *a* 1, 2, 3, A; *k*−, C3H/An; *k* 1, 2, 3, C58.

‡ (s) = standard. Determinations appearing below (s) in each column expressed as % of standard value.

cells than with *H-2* heterozygous cells, when antiserum against one of the parental determinants is being absorbed (Fig. 1). Homozygous cells would then appear to possess less antigen than in fact they have, in tests where measurement of antigen content depends upon uptake of antibody molecules. To avoid this uncertainty, all the measurements of H-2 antigen recorded in Table II were based only on comparisons between pairs of *H-2* homozygous cells or between pairs of *H-2* heterozygous cells.

It seems unlikely that a heterozygous cell would produce more antigenic determinants related to a particular locus simply because the two alleles happen to be specifying dissimilar products. Where such a conclusion is suggested by absorption data, e.g. as in the case of tests with Rh(D) antigen on human red cells (see reference 8), it might be well to consider that deviation from expected 2:1 ratios could be the result of less efficient absorption by the homozygous cell, as illustrated in Fig. 1. (It is true that the TL system provides an example where the heterozygote has > 50% of the quantity of antigen found in the homozygote—see Table IV and text below concerning amounts of TL.1 and TL.3 on TL.1,2,3/TL.1,2,3 thymocytes vs. TL.1,2,3/TL.2 thymocytes; but the unique features of the TL system suggest that it should not be taken as representative of isoantigenic systems.)

TABLE III

Some Representative Examples of H-2 Antibody Absorption by Homozygous vs. Heterozygous Thymocytes

Antiserum*	Thymocytes used for absorption		Relative absorption capacity‡ of heterozygous thymocytes (% absorption capacity of homozygous thymocytes)
	Homozygous	Heterozygous	
$H\text{-}2^b$	C57BL/6	(C57BL/6 × A)F_1	74
$H\text{-}2^b$ (D region)	C57BL/6		64
$H\text{-}2^a$ (D region)	A		72
$H\text{-}2^a$ (K region)	A		74
" "	A(TL−)	[C57(TL+) × A(TL−)]F_1	72

* See Table I.

‡ On a "per cell" basis, see Material and Methods. If heterozygous thymocytes contained half the number of relevant determinants possessed by the homozygous thymocyte, and if antibody were absorbed in direct proportion to the number of determinants, this figure would be 50 in all instances. A possible explanation of the higher figures actually obtained is illustrated in Fig. 1.

The most outstanding feature of Table II is the reduction in demonstrable H-2(D) antigen on cells of TL+ phenotype (columns a, c, d, e, and g). The apparent suppression of H-2(D) by TL was greater in *H-2* heterozygotes than in *H-2* homozygotes in both instances where this was tested. Column a shows reduction to 58% of the TL− standard in *H-2* homozygotes (lines 1 and 2) vs. reduction to 34% of the TL− standard in *H-2* heterozygotes (lines 7 and 10). The second example, in column c, shows reduction to 77% of the standard for *H-2* homozygotes (lines 3 and 4) vs. 52% of standard for *H-2* heterozygotes (lines 7 and 10). This difference, we think, is apparent rather than real, for reasons similar to those advanced above in explanation of the unexpectedly high absorption capacity of heterozygous cells observed with certain H-2 antisera; i.e. the effect of TL upon H-2 may be to some extent masked in *H-2* homozygotes by greater competition of antibody molecules for H-2 determinant

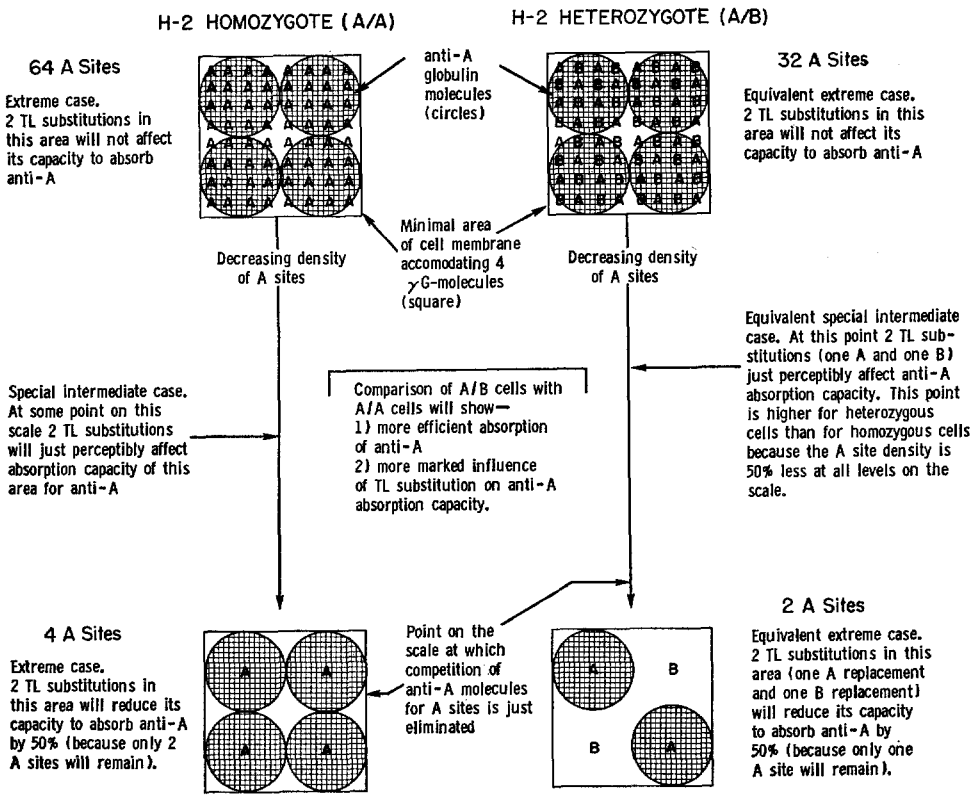

FIG. 1. Diagram to show how a higher density of A antigenic sites on homozygous (A/A) cells, as compared with heterozygous (A/B) cells, might give rise to (*1*) spuriously low values for the quantity of A antigen on A/A cells, and (*2*) spurious differences in the suppression of A antigen by TL in A/A cells vs. A/B cells, as measured by quantitative absorption of A antibody by intact viable cells.

A and B represent alternative isoantigens determined by alleles in the D region of *H-2*; the pattern is imaginary. It is assumed (*a*) that the true density of A sites lies somewhere on the scale whose extremities are represented in the upper and lower drawings, (*b*) that the number of A sites on A/A cells = the number of A sites plus the number of B sites on A/B cells, and (*c*) that consequently the actual A density on the left-hand scale must be at the same level as the actual A + B density on the right-hand scale. The area of thymocyte membrane selected for illustration is a square into which 4 7S γG-molecules can just fit. At densities of A antigen above the point indicated on the left and right scales there is competition among *anti*-A molecules for antigen sites, and therefore not all A determinants can be engaged by antibody. The effect of increasing the density of sites is to narrow the difference in *anti*-A absorption capacity of A/A and A/B from *50%* (lower drawings, where there is no competition) to *0* (upper drawings, where A/B cells would absorb the same quantity of *anti*-A as A/A cells). At all levels A/B cells are the more efficient absorbers of *anti*-A and consequently will appear to contain more than 50% of the A content of A/A cells. (It may be that antibody competition

sites. The possibility is illustrated in Fig. 1. Accordingly, the reduction of H-2 by TL is probably more accurately revealed by *H-2* heterozygotes. H-2(K) is not influenced by TL (columns b, f, and h), which raises the question whether certain H-2 components are affected to the full extent and others not at all, or whether there is a decreasing gradient of reduction from the D end to the K end of the locus. When different H-2 sera are used for absorption there are marked apparent differences in the extent of H-2 reduction (cf. columns a, c, d, e, and g) which seem to speak for a gradient, supposing that these sera are distinguishing D region components specified within *H-2* at various distances from *Tla*. On these grounds the genetic order *Tla*:*H-2.4*:*H-2.13* seems the most likely (cf. columns d and e) because *anti*-4 shows more reduction than *anti*-4,13, but this is only a tenuous suggestion. The four heterozygotes in Table II represent the four possible combinations of TL.1,2,3 vs. TL− with $H\text{-}2^a$ vs. $H\text{-}2^b$, all derived from the C57BL/6 and A strains or the reciprocal cross-over stocks C57(TL+) and A(TL−). Columns a and c show that in TL−/TL.1,2,3 thymocytes, H-2 is reduced by only half as much as in TL.1,2,3/TL.1,2,3 thymocytes (cf. lines 7–10), and that the reduction of H-2 is similar for both *cis* and *trans* position of *Tla* in relation to *H-2* (cf. lines 8 and 9).

Previous results having shown equal depression of H-2 by the two known phenotypes TL.2 and TL.1,2,3 (6), it may be presumed that the effects of TL.2 alone on H-2 are generally similar to those of TL.1,2,3.

The question whether TL affects H-2 by an influence on synthesis or by steric masking of H-2 sites is not one that can profitably be discussed at length before more evidence is available. The latter explanation was entertained by Stimpfling in connection with observations on H-2 hemagglutination (12) and it provides a plausible hypothesis for the TL:H-2 interaction. Whatever the explanation, it can be stated that the TL phenotype rather than the *Tla* genotype influences H-2, because cells that have undergone *antigenic modulation* (loss of the TL+ phenotype from cells exposed to TL antibody in the absence of lytic complement [13, 14]) show an increase in H-2 antigen (6, 14).

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is particularly marked with complex antisera that are recognizing groups of determinants rather than one, whether the serological data reveal this or not.)

With regard to substitution of TL for H-2, which may be equated for the present purpose with steric masking of H-2 by TL, we may consider the effect which substitution of TL for A or B in two positions will have on the absorbing capacity for *anti*-A. At high A density there is no effect on either A/A or A/B cells. At low A density, where there is no competition of antibody for A sites, both A/A and A/B cells show 50% reduction of A. At intermediate levels, because of the lower density of A sites on A/B cells, the effect of TL substitutions on *anti*-A absorption capacity is greater with A/B cells than with A/A cells. Thus the TL+ phenotype appears to reduce H-2 antigen more in thymocytes heterozygous for *H-2* than in thymocytes homozygous for *H-2*.

The purpose of this diagram is to emphasize that where uptake of antibody globulin is used as a measure of the content of antigen (number of antigen sites) on cell membranes, it is necessary to recognize that the concentration of antigen sites may have a bearing on the results. There is a particular need to consider this in comparisons between homozygous and heterozygous cells.

Interactions within the Tla System Affecting the Quantitative Surface Representation of the Three TL Antigens (Table IV).—Thymocytes of the progeny of TL.1,2,3 × TL.1,2,3 matings (lines 1–3) have double the quantity of TL.1 and TL.3 antigens present in thymocytes of TL−/TL.1,2,3 progeny (lines 7–10). (This would be anticipated from the finding that reduction of H-2 in the former is double that in the latter, as discussed above, see Table II.) This shows that the inert TL.1 structural gene of TL− mice (2, 7) is not activated by an accompanying chromosome of the type specifying the TL.1,2,3 phenotype.

TABLE IV

Quantitative Representation of TL Antigens on Thymocytes of Different Tla Genotypes

Mouse*	Parental TL phenotypes	TL antigen		
		1	2	3
		relative antigen content (% standard)		
(1) A	1,2,3/1,2,3	100(s)‡	100(s)	100(s)
(2) C58	1,2,3/1,2,3	98		
(3) A × C58	1,2,3/1,2,3	100		
(4) A × 129	1,2,3/2	100	79	98
(5) A × BALB/c	1,2,3/2	100	80	99
(6) A × DBA/2	1,2,3/2	100	80	99
(7) A × C3H	1,2,3/−	52		
(8) A × I	1,2,3/−	53		50
(9) A × AKR	1,2,3/−	50		
(10) C57(TL+) × C57BL/6	1,2,3/−	50		50
(11) BALB/c	2/2	0	60	0
(12) DBA/2	2/2	0	64	0
(13) 129	2/2	0	62	0
(14) BALB/c × C57BL/6	2/−		30	

* In the case of heterozygotes, tests with reciprocal hybrids gave similar results.

‡ (s), standard. Determinations appearing below (s) in each column expressed as % of standard.

These matings, therefore, reveal no interaction betweel *Tla* alleles on different chromosomes.

Matings of the type TL.1,2,3 × TL.2 (lines 4–6), however, do provide evidence of extrachromosomal interaction between different *Tla* alleles. In thymocytes of this type, the output of TL.1 and TL.3 antigens (but *not* of TL.2 antigen) equals that of TL.1,2,3/TL.1,2,3 homozygotes. There is no evidence to indicate whether both TL.1 structural genes are active in such thymocytes or whether only one is active. In the case of TL.3 it seems likely that the entire output comes from the one TL.1,2,3 chromosome. This may be concluded from the fact that TL.3 does not appear anomalously in leukemias of TL− mice (7), making it superfluous to propose that TL− mice have an

unexpressed TL.3 structural gene susceptible to activation in TL.1,2,3/TL.2 heterozygotes.

As the TL− chromosomes of C3H, I, AKR, and C57BL/6 mice do not induce the full output of TL.1 and TL.3 in heterozygotes (lines 7–10, Table IV), it can be inferred that these TL− strains contain no unrecognized *Tla* allele which, like the *Tla* allele of TL.2 strains, can influence *Tla* genes in the *trans* position.

Finally, TL.1,2,3/TL.1,2,3 thymocytes have a larger quantity of TL.2 antigen than do TL.2/TL.2 thymocytes (cf. lines 1 and 11–13, Table IV), indicating probably a second example of interrelated function by *Tla* genes.

Thus the quantitative control of TL antigens is complex and the data obtained so far appear insufficient to provide a satisfactory account of the mechanisms involved.

SUMMARY

H-2 and TL isoantigens of the mouse are specified by the closely linked genetic loci *H-2* and *Tla*. A study of their representation on thymocytes was performed in order to reveal any interactions between the determinant genes or their products affecting the synthesis or disposition of these components of the thymocyte surface. The method employed was quantitative absorption of cytotoxic antibody by viable thymocytes.

The phenotypic expression of TL antigens was found to reduce the demonstrable amount of certain H-2 antigens to as little as 34% of the quantity demonstrable on TL− thymocytes. A reduction was observed in all three H-2 types tested, ($H\text{-}2^b$, $H\text{-}2^a$, and $H\text{-}2^k$). As *antigenic modulation* (change of TL phenotype from TL+ to TL−, produced by TL antibody) is known to entail a compensatory increase in H-2(D) antigen, it is concluded that the TL phenotype, rather than the *Tla* genotype, influences the surface representation of H-2 antigens. The two known TL+ phenotypes of thymocytes (TL.2 and TL.1,2,3) depress H-2 equally. The H-2 specificities affected are those determined by the D end of the *H-2* locus, which is adjacent to *Tla*; antigens of the K end, which is distal to *Tla*, are not depressed. The reduction of demonstrable H-2 antigen on the thymocytes of TL+ × TL− progeny is half that of thymocytes of TL+ × TL+ progeny and the reduction affects equally the products of both *H-2* alleles (*cis* and *trans* in relation to *Tla*), indicating that the mechanism of H-2 reduction by TL is extrachromosomal. Whether it involves diminished synthesis of H-2 or steric masking by TL at the cell membrane is unknown, but in either case the reciprocal relation of TL and H-2(D) antigens implies that they probably occupy adjacent positions on thymocytes and that the gene order, *H-2*(K):*H-2*(D):*Tla* is reflected in cell surface structure.

Extrachromosomal interaction, apparently involving control of synthesis, occurs also *within* the TL system of antigens. Thymocytes of TL.2 × TL.1,2,3

progeny express the full homozygous quantity of antigens TL.1 and TL.3 (but not of TL.2), in contrast to the half-quantity present in thymocytes of TL− × TL.1,2,3 progeny. Another example of interaction is implicit in the finding that thymocytes of TL.1,2,3 × TL.1,2,3 progeny have more TL.2 antigen than thymocytes of TL.2 × TL.2 progeny, but in this instance there is nothing to indicate whether the mechanism is chromosomal or extrachromosomal.

Thus the quantitative surface representation of at least some H-2 and TL antigens is influenced by the cellular complement of *H-2*:*Tla* genes as a whole.

Comparison of *H-2* heterozygous thymocytes with *H-2* homozygous thymocytes in quantitative absorption tests shows (*a*) more than the expected 50% of each parental-type H-2 antigen on heterozygous cells, and (*b*) a greater suppression of H-2 by TL in *H-2* heterozygotes in comparison with H-2 homozygotes. Both results may be explained on the basis of differences in the density of H-2 antigenic sites and consequent differences in the efficiency of absorption of H-2 antibody. These considerations may be useful in other contexts, e.g. in estimating the representation of Rh antigens on the red cells of human subjects homozygous and heterozygous for Rh components.

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