Tissue Antigens (1975), **6**, 353–365
Published by Munksgaard, Copenhagen, Denmark

Studies on Recombination within the Mouse *H-2* Gene Complex. III. Further Serological Analyses of the *H-2*^{t} Haplotypes*

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The five *H–2*^{t} haplotypes are all intra-*H–2* recombinants which have identical *K* and *D* regions, but differ in the central regions of the complex. Analyses of H–2, Ia and Ss-Slp types indicate that the crossover in *H–2*^{t1} took place between the *K* and *I* regions, in the *H–2*^{t2} between *G* and *D*, in *H–2*^{t3} and *H–2*^{t4} between the *I–B* and *I–C* subregions and in *H–2*^{t5} between *I* and *S*. The set of recombinants will be very valuable for studies of traits which map in the *I* and *S* regions.

Received for publication 24 June, accepted 14 August 1975

The mouse histocompatibility–2 (*H–2*) gene complex has been separated into four major regions on the basis of intra-*H–2* recombination (Klein et al. 1974b). The *H–2K* and *H–2D* loci, in the *K* and *D* regions respectively, control cell membrane specificities which are the classical histocompatibility antigens (Klein & Shreffler 1972). These specificities are also the target antigens in cell mediated lympholysis (Nabholz et al. 1974). In the middle of the complex, the *S* region contains the *Ss–Slp* genes. The Ss gene controls the quantitative level of a specific serum protein (Shreffler & Owen 1963), while the Slp gene controls the presence or absence of a sex-limited allotypic variant of that protein (Passmore & Shreffler 1970, 1971). The *I* region codes for several traits: *Ir* genes control immune responses to a wide variety of antigens (Benacerraf & Katz 1974); *Lad* genes determine stimulation in the mixed leucocyte reaction (MLR) (Bach et al. 1972, Meo et al. 1973a) and graft versus host reaction (GVHR) (Klein & Park 1973); the *Ia* genes control serologically detectable antigens associated with the *I* region (Shreffler et al. 1974), and the *H–2 I* locus determines a histocompatibility antigen (Klein et al. 1974a). Recently, genes necessary for successful cooperation between T and B lymphocytes,

* Supported by U.S.P.H.S. grants GM 15419 and AI 11962.

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designated *Ci,* have been found also to be located in the *I* region (Katz et al. 1975). A fifth region, designated *G,* between the *S* and *D* regions contains the *H–2G* locus which controls erythrocyte antigens (David et al. 1975a).

Recombination between the *H–2K* and *H–2D* loci has been calculated to occur with a frequency of about 0.5 percent. The *I* region has been further divided into subregions by several crossovers *within* the *I* region (Shreffler & David 1975). The *I–A* subregion controls responses to a number of antigens. The response to (H,G)--A–L, Ia specificities 1, 2, 8, 9 and 11 and a *Lad* determinant for strong MLR stimulation have all been specifically mapped to this region. The I–B subregion controls responses to antigens IgG and LDH_B, as well as the Ia.3 specificity. The I–C subregion determines Ia specificities 6 and 7 and possibly also contains a *Lad* gene. Recently, a new *I* subregion, designated *I–E,* between *I–B* and *I–C,* has been postulated on the basis of a new Ia specificity, Ia.15 (David, in preparation).

In this paper, we describe the H–2 genetic constitution of five intra-*H–2* recombinants which are all K^sD^d, i.e. are identical for the *K* and *D* region antigens. These have been designated as $H\text{–}2^t$ haplotypes ($H\text{–}2^{t1}$, $H\text{–}2^{t2}$, $H\text{–}2^{t3}$, $H\text{–}2^{t4}$, $H\text{–}2^{t5}$). The crossovers which gave rise to these haplotypes occurred at various positions between the *K* and *D* regions, such that they differ from one another in the *I* region and/or the *S* region. These five recombinants constitute a set of haplotypes which are invaluable for studies of the traits which map between the *K* and *D* regions.

Materials and Methods

Mice: Strain A.TL was derived by backcrossing onto the A/WySn background the recombinant haplotype $H\text{–}2^{t1}$, which resulted from a crossover between haplotypes $H\text{–}2^{a1}$ and $H\text{–}2^s$ (Shreffler & David 1972). The A.TH strain is an A/WySn congenic carrying the $H\text{–}2^{t2}$ crossover, derived from recombination in a heterozygous parent $H\text{–}2^a/H\text{–}2^s$ (Stimpfling & Reichert 1970). This crossover was initially established and is still also carried in Stimpfling's strain B10.S(7R). Strain B10.HTT carries recombinant haplotype $H\text{–}2^{t3}$, which was derived from a recombination between haplotypes $H\text{–}2^{t1}$ and $H\text{–}2^s$ (Meo et al. 1973b). The $H\text{–}2^{t4}$ haplotype of B10.S(9R) was derived from a crossover between $H\text{–}2^a$ and $H\text{–}2^s$, found by Stimpfling et al. (1971). Strain BSVS is a long-established inbred strain (Webster 1937) recently shown to carry a distinct haplotype, $H\text{–}2^{t5}$ which is *presumed* to be a recombinant between the $H\text{–}2^a$ and $H\text{–}2^s$ haplotypes (Rose et al. 1973).

Typing: Both the H–2 antigens and the Ia antigens were detected by the ^{51}Cr release cytotoxic assay (Snell et al. 1971). In some cases, for H–2 antigens, PVP hemagglutination was also used (Stimpfling 1961). Ss and Slp serum antigens were classified by a quantitative radial immunodiffusion assay (Hansen et al. 1974). This method is sensitive enough to detect very small quantitative differences in Ss and Slp levels associated with different haplotypes.

Antisera: Details of the procedures used in producing H–2 antisera have been described (Shreffler et al. 1966). In some cases, antisera obtained from the Transplantation and Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, were used. Anti-Ia antisera were produced by reciprocal immunizations of strains which are identical at the *H–2K* and *H–2D* loci but which differ in the central regions of the

H–2 complex (David et al. 1973). More restricted anti-Ia sera were produced by using F_1 recipients (David & Shreffler 1974). Specificities Ia.8 and Ia.9 were tested for by an anti-H–2K^{b}I^b serum (Sachs et al. 1975).

Rabbit anti-Ss was prepared by immunizing with partially purified Ss protein from pooled mouse serum (Shreffler & Owen 1963). This heteroimmune serum was absorbed with pooled Ss–L serum to remove contaminating antibodies to other serum proteins. The anti-Slp alloantiserum was prepared by immunizing C3H.Q recipients with partially purified Slp antigen from DBA/2J♂ serum (Passmore & Shreffler 1970).

Absorptions: In vivo absorptions of anti-H–2 and anti-Ia sera were carried out as previously described (Shreffler et al. 1966). Briefly, 0.2 ml antiserum was injected intraperitoneally, the mice were bled 3 h later, and the sera were assayed for residual antibody activity either by the hemagglutination or cytotoxic test.

Immunizations: Reciprocal immunizations were done between strains B10.S(7R), B10.S(9R) and B10.HTT. Four F_1 crosses were also made (A.CA × B10.HTT; A.TH × B10.HTT; A.TL × B10.HTT; A.BY × B10.HTT) and these F_1's were immunized with A.TL and/or A.TH tissues. Approximately 10^7 lymph node, spleen and thymus cells were injected once a week for three weeks; the recipients were tested two weeks, given a booster and bled one week later. The sera were checked for antibody activity by the cytotoxic test and by PVP hemagglutination.

Results

H–2 Analyses

The ^{51}Cr cytotoxic titers of 11 anti-H–2

Table 1

Reactions of H–2^a and H–2^s parents and H–2^t recombinants with anti-H–2 sera[a]

Antiserum				Target strain (haplotype)						
Recipient	Donor	Antibodies against (H–2)	Relevant spec.	A.AL B10.A (H–2^a, H–2^{a1})	B10.S (H–2^s)	A.TL (H–2^{t1})	A.TH B10.S(7R) (H–2^{t2})	B10.HTT (H–2^{t3})	B10.S(9R) (H–2^{t4})	BSVS (H–2^{t5})
(C3H.Q×B10.D2)F$_1$	C3H	1, 23	1	1600	200	1600	200	400	800	200
(B10×A.CA)F$_1$	B10.D2	4, 3	3	1600	400	800	1600	400	1600	400
(B10×C3H.OL)F$_1$	B10.A(5R)	4, 3	4	1600	0	1600	400	200	400	400
(B10.D2×HTG)F$_1$	B10	5, 33	5	1600	1600	1600	1600	1600	1600	1600
C3H	C3H.B10	6, 2, ...	6	3200	3200	3200	3200	3200	3200	3200
(B10×A.SW)F$_1$	A.CA	8, 9	8	400	0	0	0	0	0	0
(Balb/c×B10.P)F$_1$	B10.RIII	11, 18	11	1600	0	0	0	0	0	0
(C3H×B10)F$_1$	C3H.Q	13, 30	13	50	0	50	50	50	100	50
(B6×A)F$_1$	B10.P	16	–	0	0	0	0	0	0	0
(B10×A)F$_1$	A.SW	19	19	0	3200	3200	3200	3200	3200	3200
AKR	AKR.M	27, 28, 29..	27, 28, 29	1600	400	1600	1600	1600	3200	1600

reagents against cells of the five $H\text{-}2^t$ haplotypes and the three parental types are shown in Table 1. Results of H-2 typings of $H\text{-}2^{t1}$, $H\text{-}2^{t2}$, and $H\text{-}2^{t5}$ have been reported previously (David & Shreffler 1972, Rose et al. 1973) but these haplotypes were also repeated in these studies for comparison with $H\text{-}2^{t3}$ and $H\text{-}2^{t4}$.

H-2.4 is a private $H\text{-}2D^d$ specificity determined by haplotypes $H\text{-}2^a$ and $H\text{-}2^{a1}$. All five $H\text{-}2^t$ haplotypes were positive for this determinant, indicating the presence of the $H\text{-}2D^d$ allele. H-2.11, which is a marker for the $H\text{-}2K^k$ allele, was negative in all haplotypes, while the private $H\text{-}2K^s$ specificity, H-2.19, was positive in all.

Public specificities H-2.1, 3, 5, and 6 are determined by both the parental types and, as expected, were present in products of all five $H\text{-}2^t$ haplotypes. Specificity H-2.8 is determined by the $H\text{-}2^a$ haplotype and associated with the K region. It was found to be absent from products of all five recombinant haplotypes. Specificity H-2.13, present in $H\text{-}2^a$ and associated with the D region, was present in all $H\text{-}2^t$ haplotypes.

The five $H\text{-}2^t$ haplotypes thus exhibit identical reactivities for K^s and D^d region antigens. Variations in titer are due to the presence of antibodies against I region antigens. Absorption analyses showed that the five haplotypes absorb anti-D^d and anti-K^s sera completely for each other, indicating identity in these regions.

Ss and Slp Analyses

The origins of the S regions in the $H\text{-}2^t$ haplotypes were determined by Ss and Slp typing. Strains A.TL($H\text{-}2^{t1}$) and B10.HTT($H\text{-}2^{t3}$) are Ss-L, like $H\text{-}2^k$, thereby indicating the presence of an S^k region.

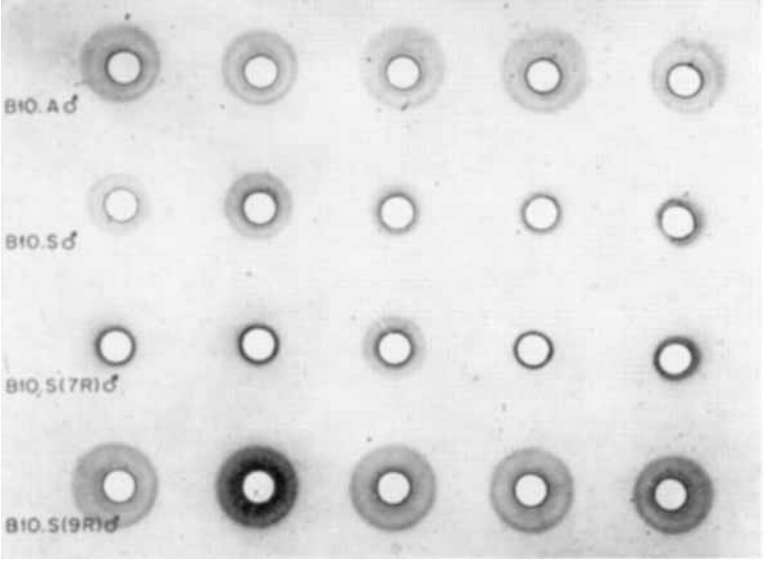

Figure 1. Radial immunodiffusion plate comparing Slp levels in sera of males of strains B10.A, B10.S, B10.S(7R) and B10.S(9R). Serum samples from five different adult male mice of the indicated strain were added to each of the four rows. Note similarity of Slp levels in B10.A and B10.S(9R) and similarly in B10.S and B10.S(7R).

Strains A.TH($H\text{-}2^{t2}$), B10.S(9R)($H\text{-}2^{t4}$) and BSVS($H\text{-}2^{t5}$) were Ss–H and therefore could be either S^d or S^s, depending upon the position of the crossover.

Table 2
Slp levels determined by $H\text{-}2^t$ and parental haplotypes[a]

Strain	S-regions	Slp units	(No.)
A	S^d	1.01±0.03	(18)
A.SW	S^s	0.35±0.06	(12)
A.TH	S^s	0.45±0.04	(10)
B10.A	S^d	0.68±0.04	(25)
B10.S	S^s	0.18±0.03	(25)
B10.S(7R)	S^s	0.17±0.04	(15)
B10.S(9R)	S^d	0.69±0.05	(10)
B10.BSVS(N_2)	S^d/S^b	0.46±0.03	(6)
(B10.S × B10)F_1	S^s/S^b	0.05±0.02	(4)
(B10.A × B10)F_1	S^d/S^b	0.33±0.05	(7)

[a] All sera were from male mice older than 12 weeks.

Since there is a sizeable quantitative difference in Slp level between the S^d and S^s types (Hansen et al. 1974), Slp typing by the quantitative radial immunodiffusion method was employed to determine the origins of the S regions. A radial diffusion plate showing the levels of Slp antigen in sera from males of the B.10.S(7R), B10.S(9R), and B10.BSVS strains is presented in Fig. 1, along with control sera from B10.S(S^s) and B10.D2(S^d) males. B10.S(7R) males and B10.S males were found to have very similar mean concentrations of serum Slp. This indicated that the $H\text{-}2^{t2}$ haplotype has the S^s region. Previous tests revealed comparable similarity in Slp levels between strains A.TH and A.SW (Hansen et al. 1974). B10.S (9R) males were found to have a mean Slp concentration like that of the B10.D2 strain (S^d), indicating the presence of the S^d region in the $H\text{-}2^{t4}$ haplotype. Evidence from sera of two heterozygous animals of the partially congenic B10.BSVS strain, compared with sera from S^s/S^b and S^d/S^b F_1 hybrids, clearly indicates that the S region of this strain was also derived from the $H\text{-}2^a$ (S^d) haplotype. The relative Slp levels in all of these strains are shown in Table 2.

Ia Analyses

The various $H\text{-}2^t$ haplotypes could have derived their I region antigens either from $H\text{-}2^a$ or $H\text{-}2^{a1}$ or from $H\text{-}2^s$. Haplotypes $H\text{-}2^a$ expresses specificities Ia.1,2,3,6,7 and 15, $H\text{-}2^{a1}$ expresses Ia.1,2,3,7 and 15, and $H\text{-}2^s$ expresses Ia.4,5,9 and 12. We will describe the analysis of Ia antigens in the $H\text{-}2^t$ haplotypes by groups of Ia specificities (Table 3).

Ia.1,2,3: Antiserum (A.TH × B10.D2) anti-A.TL (anti-Ia.1,2,3) react with cells of the donor strain (A.TL) but not with the other four $H\text{-}2^t$ strains. The failure of cells from B10.S(9R), B10.HTT and BSVS to react with this antiserum indicates the absence of these specificities from them. Since these specificities map in the $I\text{-}A^k$ and $I\text{-}B^k$ subregions (David & Shreffler 1974) this indicates the absence of these regions in haplotypes $H\text{-}2^{t3}$, $H\text{-}2^{t4}$ and $H\text{-}2^{t5}$. The absence of the $I\text{-}A^k$ region from all but the $H\text{-}2^{t1}$ haplotype is also indicated by the reaction pattern of antiserum (C3H.OH × A.SW) anti-C3H (anti-Ia.1,2).

Ia.4,5: Antiserum A.TL anti-A.TH determines the I^s specificities Ia.4 and Ia.5. All the $H\text{-}2^t$ haplotypes except A.TL reacted with the antiserum, suggesting that they shared at least part of the I^s region. Antiserum (A.TL × A.TB)F_1 anti-A.TH contains only anti-Ia.4 antibodies. Ia.4 has been mapped in the $I\text{-}A$ subregion (David & Shreffler 1974). A.TH, BSVS, B10. HTT and B.10.S(9R) were all positive for this specificity indicating that these haplotypes carry the $I\text{-}A^s$ region.

Table 3

Reactions of H-2^a and H-2^s parental and H-2^t recombinant cells with anti-Ia antisera[a]

Antiserum		Antibodies against (Ia)	Target strain (haplotype)						
Recipient	Donor		A.AL B10.A (H-2^a, H-2^{a1})	B10.S (H-2^s)	A.TL (H-2^{t1})	A.TH B10.S(7R) (H-2^{t2})	B10.HTT (H-2^{t3})	B10.S(9R) (H-2^{t4})	BSVS (H-2^{t5})
(A.TH × B10.D2) F_1	A.TL	1, 2, 3	3200	0	3200	0	Tr	0	0
(C3H.OH × A.SW) F_1	C3H	1, 2	800	0	800	0	0	0	0
A.TL	A.TH	4, 5	0	640	0	2560	1600	1600	640
(A.TL × A.TFR3) F_1	A.TH	4	0	1600	0	1600	1600	1600	1600
B10.A(4R)	B10.A(2R)	6	640	0	Tr	0	Tr	320	0
A.TH	A.TL	1, 2, 3, 7	5120	0	10280	0	1600	1600	0
(B10 × HTI) F_1	B10.A(5R)	7	400	0	1600	0	400	200	0
(A.TH × B10) F_1	A.TL	1, 2, 7	800	0	3200	0	400	1600	0
(A.TH × B10.M) F_1	A.TL	2, 3, 7	100	0	400	0	10	40	0
(HTH × C3H.Q) F_1	C3H.B10	8	0	0	0	0	0	0	0
(A × B10.D2) F_1	B10.A(5R)	9	0	320	0	320	320	100	320
(B10.A(2R) × C3H.NB) F_1	B10.RIII	12	0	80	0	80	80	80	80
AQR	B10.T(6R)	10	0	0	0	0	0	0	0
(B6 × A) F_1	B10.D2	11, 16	0	0	0	0	0	0	0
(B6 × A) F_1	B10.P	13	0	0	0	0	0	0	0
(A.TH × A.AL) F_1	A.TFR4	14	0	0	0	0	0	0	0

[a] Titer by ^{51}Cr cytotoxic release assay.

Ia.6,7: Antiserum B10.A(4R) anti-B10.A (2R) defines specificity Ia.6 which is limited to the I^d strains and maps in the *I–C* region (David et al. 1974). Only B10.S (9R)was clearly positive with this antiserum, suggesting the presence of the $I\text{–}C^d$ region in this haplotype, but not the others. Antiserum A.TH anti-A.TL specifies Ia.1,2,3 and 7. Strains B.10.HTT and B10.S(9R) were positive with this serum. We have already shown above the absence of specificities Ia.1,2,3 from these strains, therefore this reaction must be due to expression of Ia.7. Several other antisera carrying anti-Ia.7 also react specifically with strains A.TL, B10.HTT and B10.S(9R) (Table 3). Antiserum (B10 × HTI)F_1 anti-B10.A(5R) contains only anti-Ia.7 antibodies, substantiating the specific expression of Ia.7 in these strains. This is in agreement with previous suggestions (Meo et al. 1973b) that B10.HTT and A.TL share a segment of the I^k region.

Ia.8,9: Antiserum (HTH × C3H.Q) F_1 anti-C3H.B10 contains anti-Ia.8, besides anti-H–2.33. All the $H\text{–}2^t$ haplotypes were negative with this antiserum. Antiserum (A × B10.D2)F_1 anti-B10.A(5R) contains anti-Ia.9, besides anti-H–2.33. Specificity Ia.9 has been mapped in the I–A subregion and is found in strains carrying I^b, I^s and I^q regions (Sachs et al. 1975). Only strains A.TL failed to react with this antiserum, again indicating that A.TH, B10.HTT, B10.S(9R) and BSVS have the $I\text{–}A^s$ subregion.

Ia.12: Antiserum (B.10.A(2R) × C3H. NB)F_1 anti-B10.RIII defines specificity Ia.12, as well as H–2.18. Ia.12 is expressed in I^s strains and maps in the *I–A* region (David, in preparation). Again only strain A.TL failed to react with this antiserum, indicating the presence of $I\text{–}A^s$ in $H\text{–}2^{t2}$, $H\text{–}2^{t3}$, $H\text{–}2^{t4}$ and $H\text{–}2^{t5}$.

Ia specificities 10, 11, 13, 14 and 16 are not expressed in any of the parental strains and as expected were not expressed by the $H\text{–}2^t$ haplotypes. Ia specificity 15 was first identified during immunizations conducted as part of this study and will be described in detail in a later publication.

Immunizations

Antisera from the following immunizations were assayed for anti-Ia activity. 1) B10.S (7R) anti-B10.S(9R); 2) B10.S(7R) anti-B10.HTT; 3) B10.S(9R) anti-B10.S(7R); 4) B10.S(9R) anti-B10.HTT; 5) B10.

Table 4

Strain distribution of reactivity with antisera B10.S(7R) anti-B10.HTT and B10,S(7R) anti-B10.S(9R) [a]

Target strain	Haplotype	Antiserum		
		B10.S(7R) anti-B10.HTT	B10.S(7R) anti-B10.S(9R)	Ia specificity
B10	a	0	0	–
B10.D2	d	80	80	7
B10.M	f	0	0	–
B10.K	k	160	80	7
B10.P	p	80	80	7
C3H.Q	q	0	0	–
B10.RIII	r	80	80	7
B10.S	s	0	0	–

[a] Titer by ^{51}Cr cytotoxic release assay.

HTT; anti-B10.S(7R); 6) B10.HTT anti-B10.S(9R). Antibodies were detected in only two combinations, B10.S(7R) anti-B10.S(9R) and B10.S(7R) anti-B10.HTT. In the ^{51}Cr-cytotoxic test, both antisera gave release of 15–20 % above base line and titers of 80–160 with the same strain distribution of reactivity (Table 4). Analyses of recombinant strains indicated that these antibodies are directed against specificity Ia.7. These findings are in agreement with the results presented above showing that B10.S(9R) and B10.HTT have the *I–C*^{d} and *I–C*^{k} regions respectively (which specify Ia.7), while B10.S (7R) has the *I–C*^{s} region (which does not express Ia.7, or any other Ia specificity identified thus far).

The immunizations in F_1 combinations yielded the following results (see Table 5). Antisera (A.TL × B10.HTT)F_1 anti-A.TH and (A.CA × B10.HTT)F_1 anti-A.TH gave no detectable antibodies against antigens specified by the *I–C*^{s} region. Antiserum (A.CA × B10.HTT)F_1 anti-A.TL produced a strong anti-Ia.2 and a very weak anti-Ia.3. Antiserum (A.BY × B10.HTT)F_1 anti-A.TL produced a strong anti-Ia.2 and a weak anti-Ia.1. Antiserum (A.TH × B10.HTT)F_1 anti-A.TL produced antibodies against specificities Ia.1,2 and 3. But the antiserum also reacted against *H–2*^{d} cells. This cannot be due to Ia.7 since B10.HTT carries this specificity; B10.S(9R), which expresses Ia.7, also failed to react with this antiserum. Further analyses of this serum have led to the identification of a new specificity, designated Ia.15, mapping in a new subregion, designated *I–E,* between the *I–B* and *I–C* subregions (David & Shreffler, in preparation).

Table 5

Strain distribution of reactivity of three anti-A.TL sera[a]

Strain	Haplotype	Antiserum			Relev. Ia specificity
		(A.CA×B10.HTT)F_1 Anti-A.TL [anti-Ia.2,3(?)]	(A.BY×B10.HTT)F_1 Anti-A.TL [anti-Ia.1,2]	(A.TH×B10.HTT)F_1 Anti-A.TL [anti-Ia.1,2,3,15]	
B10	b	0	0	50	3
B10.D2	d	0	0	50	15
B10.M	f	0	80	400	1
B10.K	k	400	800	1600	1, 2, 3, 15
B10.P	p	0	0	50	15(?)
C3H.Q	q	0	0	10	3(?)
B10.RIII	r	0	50	50	1, 3
B10.S	s	0	0	0	–

[a] Titer by ^{51}Cr cytotoxic release assay.

Ia Specificities in H–2^{t} *Haplotypes*

To summarize the above results, haplotype *H–2*^{t1} (A.TL) has a complete I^{k} region,

Table 6

Distribution of Ia specificities in the parental and H-2^t *haplotypes*

Strain	H-2 haplotype	Parental combination	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
C3H	k	–	1	2	3	–	–	–	7	–	–	–	–	–	–	–	15	–
DBA/2	d	–	–	–	–	–	–	6	7	8	–	–	11	–	–	–	15	16
A.SW	s	–	–	–	–	4	5	–	–	–	9	–	–	12	–	–	–	–
A	a	k/d[a]	1	2	3	–	–	6	7	–	–	–	–	–	–	–	15	–
A.AL	a1	k/d	1	2	3	–	–	–	7	–	–	–	–	–	–	–	15	–
A.TL	t1	s/a1	1	2	3	–	–	–	7	–	–	–	–	–	–	–	15	–
A.TH, B10.S(7R)	t2	s/a	–	–	–	4	5	–	–	–	9	–	–	12	–	–	–	–
B10.HTT	t3	s/t1	–	–	–	4	5	–	7	–	9	–	–	12	–	–	–	–
B10.S(9R)	t4	s/a	–	–	–	4	5	6	7	–	9	–	–	12	–	–	–	–
BSVS	t5	s/a[a]	–	–	–	4	5	–	–	–	9	–	–	12	–	–	–	–

[a] Presumed recombinants. Not observed in the laboratory.

expressing Ia specificities 1,2,3,7 and 15. The *H-2*^{t2} (A.TH, B10.S(7R)) and *H-2*^{t5} (BSVS) haplotypes appear to have a complete *I*^{s} region, expressing Ia.4,5,9 and 12. *H-2*^{t3} (B10.HTT) derived the *I-A* and *I-B* regions from *H-2*^{s} and the *I-C* region from *H-2*^{t1}, specifying Ia.4,9 and 12 of *H-2*^{s} and Ia.7 of *H-2*^{t1}. Haplotype *H-2*^{t4} (B10.S(9R)) derived the *I-A* and *I-B* regions from *H-2*^{s} and the *I-C* region from *H-2*^{a} and determines Ia.4,9 and 12 of *H-2*^{s} and Ia.6 and 7 of *H-2*^{a}. We had previously mapped Ia.5 in the *I-C* region (David & Shreffler 1974). Further absorptions with *H-2*^{t3} and *H-2*^{t4} cleared for Ia.5 suggested their expression in these two haplotypes and localization of Ia.5 in *I-A* or *I-B* region. Table 6 shows the Ia specificities present in the parental haplotypes and those now assigned to the *H-2*^{t} haplotypes.

H-2 Composition of the H-2^t *Haplotypes*

The origins of each of the regions of the *H-2*^{t} haplotypes, as deduced from these and previous studies, are shown in Fig. 2. The *K* and *D* regions have been found to be identical in all five *H-2*^{t} haplotypes – *K* originating from *H-2*^{s} and *D* from *H-2*^{d} (via *H-2*^{a} or *H-2*^{a1}). Ss typing has clearly shown that A.TL and B10.HTT derived their *S* regions from *H-2*^{k}. Slp typing by the quantitative radial immunodiffusion technique has shown that B10.S(9R) and BSVS derived their *S* regions from *H-2*^{d} (via *H-2*^{a}), while A.TH (and B10.S(7R)) derived theirs from *H-2*^{s}. Previous typing for the H-2.7 specificity, which defines the *G* region, has shown that A.TL and B10.HTT have the *G*^{k} region, B10.S(9R) and BSVS have the *G*^{d} region and A.TH has the *G*^{s} region (David et al. 1975). Ia typing has shown that A.TL derived the *I* region from *H-2*^{k} (via *H-2*^{a1}) while A.TH and BSVS derived theirs from *H-2*^{s}. B10.HTT and B10.S

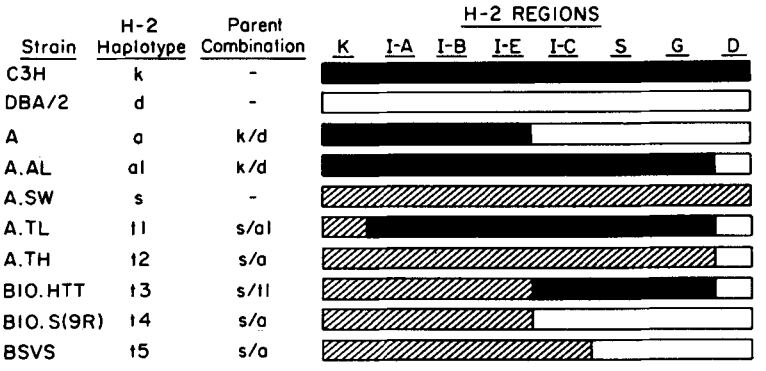

Figure 2. Composition of the H-2 gene complex of H-2^t haplotypes and their parents.

(9R) derived the *I-A* and *I-B* regions from *H-*2^s. B10.HTT derived the *I-C* regions from *H-*2^k (via *H-*2^{t1}) while B.10.S (9R) derived the *I-C* region from *H-*2^d (via *H-*2^a). Thus the crossover in A.TL took place between *K* and *I*, in A.TH between *G* and *D*, in BSVS between *I* and *S* and in B10.HTT and B10.S(9R) within the *I* region, between *I-B* and *I-C*.

Discussion

In this paper we have presented information on the H-2 specificities, Ia specificities and Ss-Slp types of the *H-*2^t haplotypes. The results on Ir typing with these strains agree in general with the crossover points deduced from the above results. Response to GAT maps in the *I-A* subregion and *H-*2^{t2}, *H-*2^{t3}, *H-*2^{t4} and *H-*2^{t5} are low like *H-*2^s while *H-*2^{t1} is high like *H-*2^k (Dorf et al. 1975). This is in agreement with our conclusion that *H-*2^{t2}, *H-*2^{t3}, *H-*2^{t4} and *H-*2^{t5} have the *I-A* subregion from *H-*2^s. The response to LDH_B maps in the *I-B* subregion and *H-*2^{t3} gives an intermediate response, in contrast to *H-*2^k (low) and *H-*2^s (high) (Melchers & Rajewsky, personal communication). It is possible that the response to LDH_B is controlled by two discrete genes in the *H-2* complex and that the crossover which gave rise to *H-*2^{t3} (B10.HTT) separated the two. Since the *H-*2^{t3} haplotype determines a low response to BGG, which maps in the *I-B* subregion (Shreffler & David 1975), it is like *H-*2^s, indicating presence of an *I-B*^{s} subregion. On the other hand, we cannot now entirely rule out the possibility that the crossover in *H-*2^{t4} occurred between *I-A* and *I-B*. Further results on the Ir typing of LDH_B antigen and IgG allotype in this haplotype might yield further information on this point. The data with the new specificity, Ia.15, and the new subregion *I-E* suggest crossing over to the right of *I-B* in *H-*2^{t3} and also in *H-*2^{t4} (David & Shreffler, in preparation). Haplotypes *H-*2^s, *H-*2^k and *H-*2^d differ in many responses. Using the *H-*2^t haplotypes, it may be possible to map some of these responses more precisely. B10 congenic strains carrying haplotypes *H-*2^{t2}, *H-*2^{t3} and *H-*2^{t4} are already available; *H-*2^{t1} and *H-*2^{t5} are currently being transferred to B10 background.

Differences between the *H-*2^t haplotype with regard to specificity H-2.7 have recently been ascribed to a new locus *H-2G*, mapping between the *S* and *D* regions (David et al. 1975b). Specificity H-2.7 is expressed predominantly on erythrocytes

and can be detected only by the hemagglutination test. It exhibits three phenotypes: 1) positive by direct hemagglutination and absorption; 2) hemagglutination negative-absorption positive (HANAP); and 3) negative by both methods. $H\text{-}2^{t1}$ and $H\text{-}2^{t3}$ are positive only by absorption (like $H\text{-}2^k$), $H\text{-}2^{t2}$ is positive by direct test as well as absorption (like $H\text{-}2^s$) and $H\text{-}2^{t4}$ and $H\text{-}2^{t5}$ are negative by both methods (like $H\text{-}2^d$). This shows that strains A.TL and B10.HTT have the G^k region and strains B10.S(9R) and BSVS have the G^d region. Strains A.TH and B10.S(7R) have the G^s region, indicating crossing over between the *G* and *D* regions. On the basis of skin graft incompatibility between haplotypes $H\text{-}2^{t3}$ and $H\text{-}2^{t1}$, a histocompatibility locus designated *H-2I* was postulated in the *I* region (Klein et al. 1974a). Haplotypes $H\text{-}2^{t2}$ and $H\text{-}2^{t3}$ appear to be compatible for this locus, indicating localization in the *I-A* or *I-B* subregion.

Previous studies have shown that, in the MLR test, B10.HTT ($H\text{-}2^{t3}$) is more compatible with B10.S(7R) and A.TH ($H\text{-}2^{t2}$) than with A.TL ($H\text{-}2^{t1}$) (Meo et al. 1973b). This tends to map the major MLR determinant in the *I-A* subregion. MLR studies with haplotypes $H\text{-}2^{t2}$, $H\text{-}2^{t4}$ and $H\text{-}2^{t5}$ gave some interesting results (Dorf et al. 1975). Reciprocal cultures of BSVS and B10.S(7R) showed little or no stimulation in the MLR assay. Cells from BSVS and B10.S(7R) responded to stimulation with B10.S(9R) lymphocytes, but there was no activation of the B10.S(9R) responding cell population with either BSVS or B10.S(7R). This unidirectionality of response was also observed in our studies with regard to Ia antibody production. We were able to produce (B10.S(7R) anti-B10.S(9R) antibodies but not B10.S (9R) anti-B10.S(7R) antibodies. This suggests that the Ia specificity determined by the $I\text{-}C^d$ sugregion (Ia.7) might also be the Lad lymphocyte activating determinant. Studies in the MLR assay using purified T and B cells in combinations where only I-C region incompatibilities exist suggest that this Lad determinant may be expressed predominantly on T cells (Lonai & McDevitt, in preparation). Our studies indicate that Ia.7 is expressed on both T and B cells (David et al. in preparation).

Intra-I region crossing over, first shown by Lieberman et al. (1972) in strain B10.A(4R) with the separation of the *Ir-1* response from *Ir-IgG* response, seems now to be almost as frequent as crossing over in other parts of the H-2 gene complex. Ia typing, MLR studies and Ir typing have revealed a number of additional intra-*I* region recombinants viz. B10.A (David et al. 1974), B.10.A(5R) (David et al. 1975a), B10.HTT (Meo et al. 1973b) and D2.GD (Dorf et al. 1975). In this paper we have added another recombinant B10.S(9R) to this list.

Acknowledgements

The authors are greatly indebted to Ronald Jackson, John McCormick and John Wilson for technical assistance and Mrs. Mary Kellogg for secretarial help. We thank Dr. J. H. Stimpfling for providing the B10.S(9R) and BSVS breeding animals.

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