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Genetics

H-2.28, an alloantigenic marker allelic to H-2.1, is expressed on all three known types of H-2 molecules

(H-2K, H-2D, H-2L/private and public specificities/capping/genetic control)

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ABSTRACT **Each allele at the *K* or *D* region of the *H-2* complex produces two kinds of "allelic" or mutually exclusive antigenic characteristics: its unique private specificity and a public specificity(ies) of either the H-2.28 or H-2.1 family. The private specificities of the *K* and *D* regions are expressed on H-2K and H-2D molecules, respectively. The *D* region produces another molecule, H-2L, which lacks the H-2K and H-2D private specificity but exhibits the H-2.28 or H-2.1 specificity. We analyzed the expression of the H-2.28 determinants on H-2K, H-2D, and H-2L molecules. When an antiserum against H-2.28 is used to sensitize cells where it can react with only H-2K molecules or H-2D molecules, by subsequent elution antibodies against H-2.28 are recovered that can also react with H-2L molecules. Hence, determinants reactive with antibodies against H-2.28 are present on H-2L as well as on H-2K and H-2D molecules. The expression of the H-2.28/H-2.1 polymorphism on all three known types of H-2 molecules, without some obvious relation to the private specificities, suggests that the antigenic determinants of these two kinds of allelic systems (private in contrast to H-2.28/H-2.1) may be controlled by separate genes, even when they are expressed on the same molecule.**

The H-2 antigens are highly polymorphic cell-surface glycoproteins controlled by the *K* and *D* regions of the *H-2* gene complex (1–6). According to the generally known two-locus model for the *H-2* antigenic specificities, the *K* and *D* regions each contain one gene, *H-2K* and *H-2D*, respectively, each encoding one polypeptide chain that carries all the antigenic specificities of this region. These include one private (7) and several public (5, 6, 8) specificities. Each private specificity is unique for one *K* or *D* region allele, while each public specificity is present in products of several different alleles of *K* or *D* region or both (5, 6, 8–12). The prediction of the two-locus model, that the antigenic specificities controlled by different regions are expressed on different molecules, was corroborated by immunoprecipitation (13) and antibody-induced redistribution (14, 15) studies.

Recently a further insight into genetics of H-2 antigens was achieved through analysis of two groups of public specificities, H-2.28 and H-2.1. H-2.28 and H-2.1 are not simple serological entities, but complexes or families of several antigenic specificities with very similar strain distribution (10, 16). They are present in both the *K* region and the *D* region products, and the product of each *K* region or *D* region allele is of either the H-2.28 or the H-2.1 type (6, 10, 16). Thus, both the *K* and *D* region products exhibit two types of "allelic" or mutually exclusive variation. One of these involves the private specificities, since usually each *K* or *D* region allele produces only one private specificity; the second involves the H-2.28 and H-2.1 families, since each *K* and *D* region allele produces either an H-2.28 or H-2.1 specificity but never the two in combination (6, 10).

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This pointed to the possibility that the specificities belonging to the two apparently separate allelic systems, i.e., the private specificities on the one hand and the H-2.28/H-2.1 specificities on the other hand, may actually be under the control of the different genes (17). We therefore investigated the molecular relationship between H-2.28 and the other *D* region specificities. Using antibody-induced redistribution (capping) we have shown that the sera against the specificities of the H-2.28 family detect in the products of the D^d region a previously unknown type of H-2 molecule that lacks the *D* region private specificity and most of the public specificities (18–20). These findings were confirmed by immunoprecipation of detergent-solubilized H-2 antigens (21, 22). The locus controlling the new molecules was named *H-2L* (23, 24). It is closely linked to the *H-2D* locus in the *D* region (17–26). Recently, we have demonstrated that in the products of the D^k region (H-2.28 negative, H-2.1 positive) antisera against H-2.1 detect a molecule lacking the *D* region private specificity and some public specificities (23). This is in accordance with the postulated allelism of the H-2.28 and H-2.1 specificities and suggests that the molecule detected by antisera against H-2.1 is controlled by the $H\text{-}2L^k$ allele. The H-2L molecules, like H-2K and H-2D, have molecular weights of approximately 45,000 (21, 22, 25) and possess a β-2-microglobulin chain (17).

Antisera against H-2.28 react with both the *K* and *D* region products of H-2.28-positive alleles (6, 10) and cap H-2D molecules (17–20). It has not been known, however, whether their reactivity with H-2D or H-2K molecules is due to expression of the H-2.28 antigenic determinants on these molecules as well as on the H-2L molecules, or whether these sera contain several populations of antibodies, some of them reactive with H-2L molecules and others with H-2D (or H-2K) molecules.

In this paper we show, by antibody-induced redistribution, that there are antibodies against H-2.28 that react with H-2L molecules as well as with H-2K or H-2D molecules. An antiserum against H-2.28 was incubated with cells where it can react with only H-2K molecules or H-2D molecules. The adsorbed antibodies were subsequently eluted and their reactivity with H-2L molecules was demonstrated by immunofluorescence or cytotoxicity on cells where H-2K molecules do not carry H-2.28 and the H-2D molecules were capped. Absorption tests confirmed that these antibodies have anti-H-2.28 specificity. These results demonstrate that antigenically related determinants of H-2.28 type are present on all three known types of H-2 molecules (H-2K, H-2D, and H-2L).

MATERIALS AND METHODS

Inbred and Congenic Mouse Strains. The inbred and congenic mouse stains C3H/HeA ($H\text{-}2^k$), C3H-$H\text{-}2^o$ ($H\text{-}2^{o2}$), *C3H.B10* ($H\text{-}2^b$), BALB/cBy ($H\text{-}2^d$), BALB/c-$H\text{-}2^{db}$ ($H\text{-}2^{db}$), B10.A ($H\text{-}2^a$), B10.A(2R) ($H\text{-}2^h$), B10.AKM ($H\text{-}2^m$), A.SW

(*H-2*^{s}), B10.WB (*H-2*^{ja}), B10.RIII (*H-2*^{r}), and B10.M (*H-2*^{f}) were maintained at the Netherlands Cancer Institute. Strain BALB/c-*H-2*^{db} was received from K. Melief. The serologically defined H-2 antigenic specificities relevant for this study are as follows (private specificities italicized) (10, 23, 25, 26):

B10.A	K: *23*, "1"	D: *4*, "28"	L: "28"
BALB/c-*H-2*^{db}	K: *31*, "28"	D: *4*, "28"	L: negative
B10.AKM	K: *23*, "1"	D: *30*, "28"	L: "28"
C3H-*H-2*^{o}	K: *31*, "28"	D: *32*, "1"	L: "1"

H-2 Antisera. The specificity of the antisera was controlled by tests on an appropriate panel of inbred strains. The antisera against H-2.28 were also extensively tested by absorption (16) and elution experiments. The D-23, D-23b, D-30, and D-31 sera were from the National Institutes of Health serum bank; the ASA sera were produced by P.D.

Antiserum against H-2.28: anti-H-2.28: ASA-1, C3H anti-C3H.B10.

Antisera against private specificities: anti-H-2.4: ASA-3, (B10.AKM × C3H.B10)F_1 anti-B10.A; anti-H-2.23: D-23b, (B10.D2 × SJL)F_1 anti-B10.A; anti-H-2.30: D-30, (B10.A × LP.RIII)F_1 anti-B10.AKM; and anti-H-2.31: D-31, (B10 × A)F_1 anti-B10.D2.

Eluates. Acid elution of antibodies absorbed specifically on lymphocytes was used (27). Briefly, 120 × 10^6 lymphocytes were incubated with 0.25 ml of antiserum (10 min at 37° and 20 min at 23°). This procedure was repeated once. Absorbed antibodies were eluted by resuspending the cells in 0.1 M HCl in 0.83% NaCl solution at pH 3.0 for 10 min at 23°. After centrifugation the pH of the supernatant was adjusted to 7.0 by 40 mM NaOH in 0.83% NaCl.

Target Cells. Splenic T lymphocytes were separated on a column of degalan beads coated with mouse Ig and rabbit anti-mouse Ig (28). The suspension contained less than 5% of membrane Ig-bearing lymphocytes, as detected by direct immunofluorescence test. Capping of antigenic sites was tested by induction of resistance to antibody-mediated cytotoxicity (15) and/or by immunofluorescence, using the differential redistribution method (14, 18).

Induction of Resistance to Lysis (Lysostrip). RPMI medium with 50 μg of cycloheximide per ml, containing 10% heat-inactivated normal mouse serum, was used throughout the experimental procedure. Antisera against H-2 and rabbit antisera against mouse Ig were diluted in the same medium without normal mouse serum. Purified T lymphocytes (1 × 10^6) were incubated with 50 μl of antiserum against H-2 for 20 min at 0°. After three washes the cells were incubated in 50 μl of rabbit antibody against mouse Ig (Nordic) for 30 min at 37°, then washed three times and incubated for 30 min at 37° in medium. In order to induce complete resistance to lysis, the whole procedure was once repeated. Cells treated in the presence of medium instead of antisera against H-2 were used as controls. After the lysostrip procedure the cells were tested by the cytotoxicity test.

Cytotoxicity Test. The target cell suspension (1 μl, 1 × 10^6 cells per ml) was incubated with 1 μl of antiserum and 1 μl of appropriately diluted rabbit complement in Terasaki plates for 50 min at 25°. The cells were stained by eosine (1.3%) and fixed by formaldehyde (9%); the percentage of dead cells was determined by using an inverted phase-contrast microscope.

Immunofluorescence Assay of Antibody-Induced Redistribution (Capping). This assay was performed as described (14, 18, 20). Briefly, purified spleen T lymphocytes were incubated with an antiserum against H-2. Aggregation and capping of the antigenic sites on the cell surface was achieved at 37° by addition of a goat antiserum against mouse Ig conjugated to fluorescein isothiocyanate. This procedure was repeated twice in order to induce complete capping of the antigenic sites. The suspension was then incubated at 0° with another antiserum against H-2 followed by goat antiserum against mouse I_g conjugated with tetramethylrhodamine isothiocyanate. If the antibodies in the second antiserum against H-2 reacted with molecules that were not redistributed after treatment with the first antiserum, the cell surface exhibited diffuse red staining outside of the green caps. If, however, the second antiserum against H-2 reacted only with the molecules that were already redistributed by the first antiserum against H-2, no red labeling was observed on the cell surface outside the green caps. The cytotoxicity (lysostrip) and immunofluorescence assays gave the same results in parallel tests.

RESULTS

Antibodies against H-2.28 eluted from H-2D^d molecules can react with H-2L^d molecules

Specificity of "H-2D^d Eluate." Antiserum against H-2.28 (ASA-1) was incubated with BALB/c-*H-2*^{db} cells, which carry a mutant haplotype, *H-2*^{db} (K^d I^d S^d G^d D^d L^{neg}), characterized by the loss of expression of H-2.28 determinants (26) normally present on the H-2L molecule (25). The absorbed antibodies were subsequently eluted and antibodies against H-2K^d, Ia, or H-2G were removed by exhaustive absorption with C3H-*H-2*^{o} (K^d I^d S^d G^d D^k L^k) cells. The reagent obtained in this way, called the "H-2D^d eluate," should contain only antibodies reactive with the H-2D^d molecules of BALB/c-*H-2*^{db}. This was confirmed by antibody-induced redistribution (Table 1, Exps. 1–10). When the H-2D^d molecules on BALB/c-*H-2*^{db} cells were redistributed by antibodies against H-2.4, the "H-2D^d eluate" as well as the antiserum against H-2.28 absorbed by C3H-*H-2*^{o} cells no longer bound diffusely to the cell surface or induced lysis by complement, respectively. The redistribution of H-2D^d by antiserum against H-2.4, however, did not affect the reactivity of BALB/c-*H-2*^{db} cells with unabsorbed antiserum against H-2.28 since this serum also reacts with H-2K^d molecules (23). The redistribution of H-2D^d molecules by antiserum against H-2.4 did not induce the redistribution of H-2K^d molecules, and redistribution of H-2K^d molecules by antiserum against H-2.31 did not induce the redistribution of the molecules reactive with antiserum against H-2.4, with the "H-2D^d eluate," or with antiserum against H-2.28 (data not shown). These tests show that the "H-2D^d eluate" contains antibodies reactive on BALB/c-*H-2*^{db} cells only with the H-2D molecules.

"H-2D^d Eluate" Reacts with H-2L^d Molecules of B10.A. While the "H-2D^d eluate" reacts only with H-2D^d molecules on BALB/c-*H-2*^{db} cells that lack a detectable H-2L^d product, in tests with B10.A (*H-2*^{a}, K^k I^{kd} S^d G^d D^d L^d) cells the reactivity with H-2L^d molecules was clearly demonstrated (Table 1, Exps. 11–16). Redistribution of H-2D^d molecules by antiserum against H-2.4 did not cause redistribution of all molecules reactive with the "H-2D^d eluate." This is in contrast with the results obtained with BALB/c-*H-2*^{db} cells. Immunofluorescence and cytotoxicity controls show that the reactivity of B10.A cells with the "H-2D^d eluate" outside the H-2.4 caps was not due to an incomplete redistribution of the H-2D^d molecules by antiserum against H-2.4. The reactivity remaining after the redistribution of H-2D molecules was not due to reaction with the H-2K, Ia, or H-2G molecules, since B10.A shares the *K* region allele with the C3H strain that produced the antiserum

Table 1. Molecular relationships of specificities detected by "H-2D^d eluate" on BALB/c-*H-2^{db}* ($K^d I^d S^d G^d D^d L^{neg}$) cells and on B10.A ($K^k I^{kd} S^d G^d D^d L^d$) cells

Exp.	First antiserum*	Second antiserum†	Target cell	Immunofluorescence (capping)‡, % cells labeled outside green caps: None	Weak or intense	Complement cytoxicity (lysostrip),§ % dead cells at antiserum dilution: 1/5	1/10	1/20
1	None	"H-2D^d eluate"	BALB/c-*H-2^{db}*	0	100	75	87	75
2	None	Anti-H-2.28	BALB/c-*H-2^{db}*	ND	ND	75	87	75
3	None	Anti-H-2.28 abs. H-2^o	BALB/c-*H-2^{db}*	ND	ND	75	75	75
4	None	Anti-H-2.4	BALB/c-*H-2^{db}*	0	100	87	100	100
5	None	Anti-H-2.31	BALB/c-*H-2^{db}*	0	100	63	62	75
6	Anti-H-2.4	Anti-H-2.4	BALB/c-*H-2^{db}*	100	0	25	25	25
7	Anti-H-2.4	Anti-H-2.31	BALB/c-*H-2^{db}*	28	72	87	100	87
8	Anti-H-2.4	"H-2D^d eluate"	BALB/c-*H-2^{db}*	100	0		ND	
9	Anti-H-2.4	Anti-H-2.28	BALB/c-*H-2^{db}*	40	60		ND	
10	Anti-H-2.4	Anti-H-2.28 abs. H-2^o	BALB/c-*H-2^{db}*	ND	ND	12	25	12
11	None	"H-2D^d eluate"	B10.A	0	100	75	87	87
12	None	Anti-H-2.4	B10.A	0	100	62	87	87
13	None	Anti-H-2.23	B10.A	0	100		ND	
14	Anti-H-2.4	Anti-H-2.4	B10.A	100	0	12	12	12
15	Anti-H-2.4	Anti-H-2.23	B10.A	27	73		ND	
16	Anti-H-2.4	"H-2D^d eluate"	B10.A	20	80	87	100	100
17	None	"H-2D^d eluate" absorbed with B10.A, or B10.AKM, or B10.WB, or A.SW	B10.A	ND	ND	12	12	12
18	None	"H-2D^d eluate" absorbed with B10.M	B10.A	ND	ND	37	25	12
19	None	H-2D^d eluate" absorbed with B10.RIII	B10.A	ND	ND	25	37	50

* Followed by antibody against mouse Ig and incubation under capping conditions.
† Followed by tetramethylrhodamine isothiocyanate-conjugated antiserum against mouse Ig or by complement.
‡ Antibody-induced redistribution (capping). ND, not done.
§ Antibody-induced resistance to complement-mediated cytotoxicity (lysostrip). ND, not done.

against H-2.28 and anti-Ia of H-2G antibodies were removed from the "H-2D^d eluate" by absorption with C3H-*H-2^o* cells. In addition, the "H-2D^d eluate" cannot contain Qa-1,2,3 antibody activity (29, 30) since the antiserum against H-2.28 (ASA-1) does not react with C58/J and DBA/2J cells after absorption with BALB/cBy. Hence, these data show that the antibodies eluted from H-2D^d molecules react with H-2L^d molecules on the surface of B10.A cells. Correspondingly, redistribution of the H-2K^k molecules by antiserum against H-2.23 does not affect the reactivity of B10.A cells with the "H-2D^d eluate" or with antisera against H-2.28 or H-2.4 (data not shown), and redistribution of H-2D^d molecules does not affect the distribution of H-2K^k molecules.

Antibodies in H-2D^d Eluate Reactive with H-2L^d Molecules Have H-2.28 Antibody Specificity. Antiserum against H-2.28 (ASA-1) could contain, besides antibodies against H-2.28, antibodies against other public specificities (H-2.35 or H-2.36) with a more limited strain distribution which could possibly react with the products of the *D* region of *H-2^d* and *H-2^a*. Absorption tests (16), however, performed with the "H-2D^d eluate" failed to reveal any antibody other than those of the H-2.28 family which could be responsible for its reactivity with the B10.A cells (Table 1, Exps. 17–19). The H-2.28-positive strains, B10.A, B10.AKM, B10.WB, and A.SW, completely removed the activity of the "H-2D^d eluate" with the B10.A cells, while B10.M and B10.RIII strains, which have a weak and incomplete expression of the H-2.28 family of specificities (10), were only partially effective in the removal of the activity of the H-2D^d eluate with B10.A cells.

Antibodies against H-2.28 eluted from H-2K^d molecules can react with H-2L^d and H-2L^q molecules

"H-2K^d Eluate." Antiserum against H-2.28 (ASA-1) was absorbed on C3H-*H-2^o* (*H-2^{o2}*, $K^d I^d S^d G^d D^k L^k$) cells and the absorbed antibodies were eluted. The reagent obtained, named the "H-2K^d eluate," could contain antibodies reactive with the products of the *K*, *I*, and *G* regions of *H-2^d*. We used, however, purified T cells, which express no or very small amounts of Ia or H-2G antigens (1–3); hence, the outcome of our assay is not influenced by them (18–20). The antiserum against H-2.28 (ASA-1) does not contain any antibodies against Qa-1,2,3 (see above). Since the antibodies against H-2.28 do not detect, in the *K*-region products, molecules other than H-2K (15, 23, 31), the antibodies in the eluate analyzed here are those that reacted with the H-2K^d molecules on the C3H-*H-2^o* cells.

"H-2K^d Eluate" Reacts with H-2L^d and H-2L^q Molecules. As shown by direct cytotoxicity (Table 2), the "H-2K^d eluate" reacted with B10.A cells (*H-2^a*, $K^k I^{kd} S^d G^d D^d L^d$). This reaction was with the *D* region products of B10.A since its *K* region is identical with that of the C3H strain which produced the serum, and possible antibodies against Ia or against H-2G are not detected in the assays used here. In order to know whether the antibodies in the "H-2K^d eluate" bind to the H-2L^d molecules, we tested its reactivity on B10.A cells where the H-2D^d molecules were capped. The positive reaction of the eluate with the B10.A cells was not removed after the redistribution of the H-2D^d molecules by antiserum against H-2.4

Table 2. Molecular relationships of specificities detected by "H-2K^d eluate" on B10.A ($K^k I^{kd} S^d G^d D^d L^d$) and B10.AKM ($K^k I^k S^k G^k D^q L^q$) cells

Exp.	Target cells	First antiserum*	Second antiserum†	Complement cytotoxicity (lysostrip), % dead cells at antiserum dilution 1/5	1/10	1/20
1	B10.A	None	Anti-H-2.4	62	75	87
2	B10.A	None	Anti-H-2.23	87	100	87
3	B10.A	None	"H-2K^d eluate"	87	87	87
4	B10.A	Anti-H-2.4	Anti-H-2.4	25	25	25
5	B10.A	Anti-H-2.4	Anti-H-2.23	87	87	100
6	B10.A	Anti-H-2.4	"H-2K^d eluate"	87	87	75
7	B10.AKM	None	Anti-H-2.30	100	100	100
8	B10.AKM	None	Anti-H-2.23	100	100	100
9	B10.AKM	None	"H-2K^d eluate"	87	87	87
10	B10.AKM	Anti-H-2.30	Anti-H-2.30	12	12	12
11	B10.AKM	Anti-H-2.30	Anti-H-2.23	100	100	100
12	B10.AKM	Anti-H-2.30	"H-2K^d eluate"	75	87	75

* Followed by antibody against mouse Ig and incubation under capping conditions.
† Followed by incubation with complement.

(Table 2, Exps. 1–6). Similarly, tests with B10.AKM cells ($H\text{-}2^m$, $K^k I^k S^k G^k D^q L^q$) have shown that the "H-2K^d eluate" also reacts positively after the redistribution of H-2D^q molecules by antiserum against H-2.30 (Table 2, Exps. 7–12). Since the haplotype of C3H and B10.AKM share the whole *KISG* segment, the "H-2K^d eluate" can here react only with the molecules that are controlled by genes located to the right of *H-2G*. Since the H-2D molecules were capped and no antibodies against Qa are present, the reaction has to be attributed to H-2L molecules. These results show that the antibodies eluted from H-2K^d molecules can react with H-2L^d and H-2L^q molecules.

Antibodies in H-2K^d Eluate Reactive with H-2L^d and H-2L^q Molecules Have H-2.28 Antibody Specificity. The reactivity of the antiserum against H-2.28 (ASA-1) with B10.A and B10.AKM cells could be theoretically due to, besides antibodies against H-2.28, antibodies against public specificities with a more limited strain distribution. In absorption tests, however, the reactivity of this serum with B10.A and B10.AKM cells was removed completely by absorption with B10.AKM, B10.A(2R), and BALB/cBy and B10.A, B10.A(2R), and BALB/cBy cells, respectively. This indicates that none of the known public specificities other than those of the H-2.28 family are responsible for these reactions.

DISCUSSION

The alloantibodies in the antiserum against H-2.28 that react with the H-2D^d or H-2K^d molecules were shown, after elution, to react also with H-2L^d or H-2L^d and H-2L^q molecules, respectively. The absorption analysis of the antiserum and of the eluted antibodies failed to reveal any known or new antigenic specificity other than those of the H-2.28 family which could account for these reactions. Without ruling out the possibility that some antibodies against H-2.28 may react with one type of H-2 molecule only, these results indicate that some specificities of the H-2.28 family are expressed on H-2D as well as on H-2L molecules and on H-2K as well as on H-2L molecules. Hence, the specificities of the H-2.28 family are alloantigenic markers of molecules controlled by at least three different loci: *H-2K*, *H-2D*, and *H-2L*.

The precise structural interpretation of the expression of H-2.28 specificity on three different H-2 molecules in terms of the pertinent antigenic determinants requires some caution since we do not know how closely similar the H-2.28 antibody-binding sites on H-2K and H-2D molecules are to the sites on H-2L molecules that bind the same antibody. Nevertheless, the similarities of the peptide composition and aminoacid sequences of *K* and *D* region antigens (4) suggest that the antigenic similarities between the products of these regions have their structural correlate.

The sera against the H-2.1 specificity behave in the same way as antisera against H-2.28. They react with *K* region products as well as with the *D* region products (5, 6, 8, 16); when reacted with the products of the D^k region, they can induce capping of both H-2L^k and H-2D^k molecules (23). It is therefore likely that specificities of the H-2.1 family are also expressed on all three types of H-2 molecules as an alternative marker to H-2.28.

The question raised by our data is how antigens of the same pair of allelic specificities (H-2.28/H-2.1) become expressed on three different polypeptide chains. It is generally accepted that the different genes of the *H-2* complex were derived by duplication (32). Thus, the H-2K, H-2D, and H-2L molecules may be controlled by structural genes that have developed through a series of duplications and mutations from ancestral (allelic or pseudo-allelic) genes of either the H-2.1 or the H-2.28 type.

Alternatively, an additional mechanism may be involved in the genetic determination of H-2 antigens (17), namely, that the two classes of antigenic specificities, H-2.1 and H-2.28 on one hand, and the private and most of the public specificities on the other hand, are under control of separate genes [provisionally named γ and α, respectively (6,17)]. According to such a model, the antigenic make-up of an H-2 molecule that carries both these classes of specificities is the result of an interaction between these two types of genes or between their products. This proposal is not without homologies, because the genes for the V and C portions of immunoglobulin molecules were shown to be separate (33) and several other viral and eukaryotic polypeptide chains were shown to be encoded by nonadjacent portions of the genome (e.g., 34 and 35). This model raises several questions. It is not known where the proposed γ genes are located and how they interact with the α genes. One possibility is that the γ and α genes for each molecule are adjacent to each other. An alternative possibility (17) is that a single region bearing the γ genes may be used for synthesis of several or all types of H-2 molecules.

Finally, it is not known whether the polymorphism of the

H-2.28/H-2.1 specificities and of the private specificities is due to the actual presence of different structural genes in the *H-2* haplotypes or to the polymorphism at the level of the control of expression determining which of the several structural genes present becomes expressed (36).

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