SEROLOGICAL DISTINCTION OF MUTANTS B6.C-H(z1) AND B6.M505 FROM STRAIN C57BL/6

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Two mutations affecting the *K* end of the *H-2*^{b} haplotype have been reported: *H-2*^{ba}, carried by a congenic line B6.C-H-*2*^{ba}, also called B6.C-H(z1) and abbreviated Hz1 (1), and *H-2*^{bd}, present in a congenic line B6.M505 (abbrev. M505, cf. ref. 2). The *H-2*^{ba} mutation has attracted attention of immunologists because it is capable of eliciting graft rejection (1), mixed lymphocyte reaction (MLR, cf. ref. 3), and cell-mediated lymphocytotoxicity (CML, see ref. 3, 4) in combination with the parental haplotype *H-2*^{b}, and yet the two haplotypes are serologically indistinguishable. The *H-2*^{da} mutation has recently been demonstrated to behave the same way (5, 6). In this respect, it has been difficult to accommodate the two mutations into the prevailing view of the functional differentiation of the *H-2* complex. Many investigators believe that the *H-2K* and *H-2D* gene products, in addition to inducing serologically detectable antibodies, cause skin graft rejection but are ineffective in eliciting MLR, which is thought to be an affair of the *I* region. For the development of effective CML, cooperation between the *K*(*D*) and *I* region has been postulated (7). According to this concept, the two mutations should be either serologically detectable and should cause no MLR, or should be serologically identical and should stimulate MLR; they should not cause CML. The fact that they are not serologically detectable and yet elicit MLR and CML could mean either that each of them affects more than one locus, or alternatively, that the present concept of the functional subdivision of the *H-2* complex is an oversimplification. In this report we describe evidence that the latter alternative is probably correct.

Materials and Methods

Mice. The breeding pairs of the Hz1 and M505 strains were obtained through the courtesy of Doctors D. W. Bailey and I. K. Egorov, respectively. Breeders of the C57BL/6 (or B6) strain were supplied to us by the Jackson Laboratories, Bar Harbor, Maine. All other mice were obtained from our colony at the University of Texas.

Serological Tests. Hemagglutination tests were performed according to the method of Stimpfling (8) in a modification described previously (9). Cytotoxic test in microplates was carried out as described elsewhere (10) using splenocytes as target cells and normal guinea pig serum as a source of complement, and estimating the percentages of killed cells by means of phase contrast microscope. Quantitative absorptions were performed by the techniques described by Snell et al. (11). Briefly, lymphocytes from spleen and lymph nodes were distributed into a series of five tubes in such a way that the first tube contained 8×10^6 (or in some cases 32×10^6) cells, and the subsequent tubes doubling dilutions of the initial suspension. Antiserum was added to each tube in a volume that gave a final concentration corresponding to that giving 80% of dead cells in a regular titration of the antiserum. The mixtures were incubated for 30 min, then centrifuged and the supernates tested for residual cytotoxicity. The concentration of cells in the initial suspension was determined by three

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independent hemocytometer counts. Each absorption was run in triplicate and each cytotoxic test in duplicate.

Results

Bailey et al. (1), as well as other investigators, made several attempts to produce alloantisera using the congenic pair of B6 and B6.C-H(z1), but failed. We failed to produce antibodies in the following strain combinations: B6 → M505, M505 → B6, M505 → (B10 × C3H)F_1, using different immunization regimens and a variety of serological tests. We also failed to differentiate the *H-2*^{ba} and *H-2*^{bd} haplotypes from the *H-2*^{b} haplotype using a battery of oligospecific antisera against known *H-2* antigens. In all instances, antigens present in the *H-2*^{b} haplotype were found to be present in the two mutant haplotypes, and vice versa. However, in tests involving antiserum K-30, a slight quantitative difference in cytotoxic reactivity was observed between *H-2*^{b} on the one hand and *ba* and *bd* on the other hand. The suggested difference was then explored in detail using the quantitative absorption technique.

Antiserum K-30 was produced in combination (B10.D2 × A)F_1 anti-HTI or *d/a* anti-*i* and should contain antibodies against the private antigen controlled by the *H-2K*^{b} allele (antigen *H-2.33*). It could also contain antibodies against non-*H-2* antigens; however, since the critical strains used in our experiments were of the same genetic background, only *H-2* antibodies could be detected. Theoretically, the antiserum could contain antibodies against the *I*-region-associated (Ia) antigens. Since the Ia antigens are expressed strongly on one population of lymphocytes (usually B cells), the antiserum was absorbed with different numbers of T cells (to remove *H-2* antibodies) and then tested against B cells (to detect Ia antibodies), or vice versa. However, in all experiments absorption by T (B) cells always removed all activity against B (T) cells, suggesting that the only antibodies present in the antiserum were those against *H-2* antigens. Panel test of the antiserum (Table I) revealed presence of two antibodies, one against antigen *H-2.33* and another against unidentified antigen shared by strains C57BL/10Sn(*H-2*^{b}), B10.S(*H-2*^{s}) and probably also DBA/1 (*H-2*^{q}). Absorption by B10.S cells removed the B10.S and DBA/1 reactivity but did not significantly affect the B6, Hz1, or M505 reactivity.

Quantitative absorption of antiserum K-30 by lymphocytes from B6, Hz1, M505, and B10.D2 strains was repeated 6 times, always with similar result: approximately 2.5 times more Hz1 or M505 than B6 cells were required to reduce the cytotoxicity of the antiserum against B6 cells to 50% (Fig. 1). This effect was specific, since antibodies against antigen *H-2.2*, the private antigen of the *H-2D*^{b} gene unaffected by the two mutations, were absorbed to an equal extent by *b*, *ba*, and *bd* cells (Fig. 2).

Discussion

These observations indicate that the anti-*H-2.33* absorptive capacity of the *ba* and *bd* cells is lower than that of the *b* cells. The decrease in the absorptive capacity of the mutant cells can principally be explained in two ways: first, the

two mutations were of a regulatory type and as such merely decreased the content of *H-2.33* on the cell surface; second, the mutations occurred outside of the *H-2.33* site and modified this site only indirectly. The first explanation is contradicted by the fact that both mutations elicit graft rejection, MLR and

TABLE I

Reactivity of antiserum K-30 PI/ (B10.D2xA)F_1 anti-HTI with panel of cells

Strain	H-2 haplotype	Reactivity of antiserum*	
		Unabs.	Abs. by B10.S
B10.A	*a*	—	—
C57BL/10Sn	*b*	++++	++++
B6-*H-2*^{ba}	*ba*	+++	+++
B6.M505	*bd*	+++	+++
B10.D2	*d*	—	—
B10.M	*f*	—	—
B10.D2(R103)	*g3*	—	—
B10.A(4R)	*h4*	—	—
B10.A(3R)	*i3*	++++	++++
B10.A(5R)	*i5*	++++	++++
B10.A(R107)	*i7*	++++	++++
B10.A(R106)	*ia*	+++	+++
B10.WB	*ja*	—	—
B10.BR	*k*	—	—
B10.AKM	*m*	—	—
DBA/1	*q*	++	—
DA	*qp1*	—	—
B10.RIII(71NS)	*r*	—	—
B10.S	*s*	++	—
A.TL	*t1*	—	—
B10.S(7R)	*t2*	—	—
B10.HTT	*t3*	—	—

* ++++ = 50% titer 132, maximum kill of 95–100%; +++ = 50% titer 116, maximum kill of 95–100%; ++ = 50% titer 1:16, maximum kill 50%; – = less than 10% dead cells at antiserum dilution of 1:12 or higher.

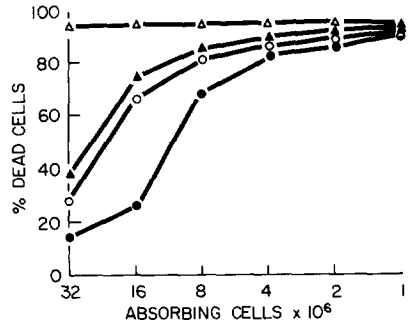

FIG. 1. Absorption of antiserum K-30: (B10.D2 × A)F_1 anti-HTI (anti *H-2.33*) by spleen and lymph node cells from B10.D2 (Δ—Δ), M505 (▲—▲) Hz1 (O—O) and B6 (●—●) mice.

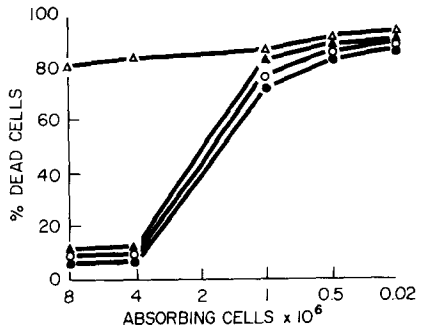

FIG. 2. Absorption of antiserum K-302: (B10.D2 × A)F_1 anti-B10.A(2R) (anti-*H-2.2*) by spleen and lymph node cells from B10.M (Δ—Δ), M505 (▲—▲), Hz1 (O—O) and B6 (●—●) mice.

CML when tested in combination with the *H-2*^{b} strain, indicating that the mutational change was qualitative rather than quantitative. Thus, the serological observations indicate quantitative, and the cellular immunity studies qualitative alterations caused by the two mutations. It seems to us that the simplest way to reconcile these seemingly contradictory results is to postulate that the serologically detectable site (recognized by B cells) and the site detectable by cellular reactions (recognized by T cells) are located in different portions of the same molecule. The mutations occurred in the site detectable by cellular immunity and changed the conformation of the *H-2* molecule in such a way that it lowered the capacity of the serologically detectable site to bind anti-H-2.33 antibodies (the change in the primary structure of the *H-2* molecule affected the cellular immunity site, and the change in the tertiary structure affected the serologically detectable site). Alternatively, one can postulate that in both *H-2*^{ba} and *H-2*^{bd} two mutations occurred simultaneously in two different genes, one coding for the serologically detectable antigens and another for antigens detected by cellular immunity. However, such an event is highly unlikely in the case of point mutations.

The findings on the *H-2* mutations challenge the commonly held view that the serologically detectable *H-2* antigens are identical with antigens eliciting cellular immunity. Such a view is probably a misconception (for discussion see ref. 6) and more likely the relationship between the two types of antigens can be interpreted in terms of hapten-carrier interaction (for discussion see ref. 12).

The absorption data indicate that both the *H-2*^{ba} and *H-2*^{bd} mutations occurred in the *H-2K*^{b} gene rather than in one of the genes located in the *I* region. From this observation two important conclusions can be drawn. First, the *H-2K* antigens are capable of causing significant MLR; second, induction of CML across the *H-2K* locus difference does not require simultaneous difference in the *I* region. These conclusions are supported by the findings with a third mutation, *H-2*^{da} (6); they contradict the hypothesis of SD-LD polarization of major histocompatibility complexes (13), as well as the hypothesis of specific *K* and *I* region cooperation in the development of CML (7).

While this work was being completed, a manuscript by Apt and co-workers (5)

reached us, in which the authors report that absorption of *d/a* anti-*i* serum with M505 spleen cells did not clear for $H\text{-}2^b$ and $H\text{-}2^{ba}$, and absorption with Hz1 cells did not clear for $H\text{-}2^b$ and $H\text{-}2^{bd}$ indicating a qualitative serological difference between the three *H-2* haplotypes. The discrepancy between these results and the results reported here can probably be explained by the fact that Apt et al. used only a single cell dose in their absorption studies and that this dose was insufficient to remove completely all the cross-reactivity.

Summary

A quantitative serological difference was found between strains Hz1 and M505 carrying mutant *H-2* haplotypes *ba* and *bd*, respectively, and the original strain B6($H\text{-}2^b$). The finding suggests that the mutations occurred in the $H\text{-}2K^b$ gene, and together with data on MLR and CML challenges the current concept of *H-2* regions' involvement in immune reactions.

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