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ANALYSIS OF THE *HISTOCOMPATIBILITY-2* (*H-2*) LOCUS OF NZB MICE[1]

SUMMARY

NZB strain of mice has been shown to have the allele $H\text{-}2^d$ by both hemagglutination and skin grafting studies. Snell's F_1 and component tests were used.

Since the discovery of autoimmune disease in aging NZB/Bl mice this strain has become widely used in many laboratories (*1*). Many experiments carried out on NZB mice involve tissue transplants in the acceptance or rejection of which a major role is played by the *histocompatibility-2* (*H-2*) locus (*2*). Experiments described in this report were therefore designed to determine the *H-2* genotype of NZB mice.

Two sublines of NZB mice have been used, one kept at Stanford University and the other kept at the Institute of Experimental Biology and Genetics in Prague. Both sublines were derived from the original colony of NZB mice kept by Dr. M. Bielschowsky of the University of Otago (Dunedin, New Zealand) and delivered via the Walter Eliza and Hall Institute (Melbourne, Australia). The mice underwent approximately 40 generations of brother-sister matings at the University of Otago, 50 generations at the Eliza and Hall Institute and 10 generations at Stanford University and Prague. Since there was no difference between the mice from these two sources, the results were pooled.

In order to obtain some initial information about the serological reactivity of NZB mice, their erythrocytes and erythrocytes of some other strains were tested against a panel of 10 different anti-*H-2* sera. The antisera were prepared by spleen cell injections as described previously (*4*, *5*). Hemagglutinations were performed by the polyvinylpyrrolidone method of Stimpfling (*9*). Titrations were done starting at an antiserum dilution of 1/20. The titer is expressed as the reciprocal of the last antiserum dilution showing 1+ agglutination on a scale from 0 to 4+. The results of the hemagglutination are summarized in Table 1. The conspicuous similarity between NZB and B10.D2 erythrocytes seen in hemagglutination reactivity suggests that NZB has the $H\text{-}2^d$ genotype. This similarity was therefore further studied by the F_1 test of Snell (6–8).

C57BL/10ScSn (=B10) and NZB mice were crossed, and the F_1 hybrids were grafted with skin from B10.D2 donors. B10 and B10.D2 are congenic lines, which were bred to differ at the *H-2* locus only carrying the alleles $H\text{-}2^b$ and $H\text{-}2^d$ respectively. If NZB resembles B10.D2 in having the $H\text{-}2^d$ allele, then these grafts should permanently survive. Or, more strictly, the grafts will permanently survive only if B10.D2 has no *H-2* antigenic components not present in the (B10 × NZB) F_1. The method of Billingham and Medawar (*3*) was used for the skin grafting. Twenty-one (B10 × NZB)F_1 hybrids of both sexes, aged 3 to 4 months, were grafted with B10.D2 skin transplants from donors of the same sex. Graft survival was checked daily from day 8 to day 21 after transplantation and once a week thereafter. At the time of writing this report (20 February 1967) the grafts have survived about 150 days without any sign of rejection. Therefore we conclude that NZB has all the $H\text{-}2^d$ antigenic components with the possible exception of those determined by both $H\text{-}2^b$ and $H\text{-}2^d$. These latter antigens can

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TABLE 1

Hemagglutination of red blood cells of NZB and some other strains with various sera

Antiserum	H-2 combination	Hemagglutination titer with RBC from strains—					
		NZB (H-2^d)	B10.D2 (H-2^d)	A/J (H-2^a)	CBA (H-2^k)	DBA/1 (H-2^q)	B10 (H-2^b)
B10 anti-A/J	*b* anti-*a*	640	1280	2560	640	1280	0
C3H/Sn anti-A/J	*k* anti-*a*	640	640	640	0	160	0
C3H/Sn anti-DBA/2	*k* anti-*d*	1280	2560	1280	0	160	80
DBA/2 anti-RIII/J	*d* anti-*r*	0	0	640	640	80	160
C3H.SW anti-DBA/2	*b* anti-*d*	1280	1280	2560	320	1280	0
C3H/HeJ anti-DBA/1	*k* anti-*q*	320	320	160	0	320	40
A/J anti-B10	*a* anti-*b*	0	0	0	0	0	320
RIII anti-BALB/c	*r* anti-*d*	320	1280	1280	0	640	640
B10 anti-C3H	*b* anti-*k*	0	0	320	320	80	0
DBA/2 anti-B10	*d* anti-*b*	0	0	320	80	80	160

be tested for by means of the "component test" (*6*, *7*).

The component test (*6*, *7*) is based on the finding that the H-2^d and H-2^k alleles together specify all H-2 components which are specified by the H-2^a allele. Therefore, only if NZB has the entire set of H-2^d components will H-2^a grafts be accepted by (NZB × H-2^k)F_1 hybrids.

The component test was carried out both by tumor and skin grafting. In the first case the A/J strain tumor Sarcoma I, kindly provided by Dr. G. D. Snell, was transplanted by the trocar method to (C3H × NZB)F_1 hybrids. Controls used were (C3H × BALB/c)F_1 hybrids, A/J, C3H/He, and NZB mice. A/J, C3H/He, and BALB/c mice bear H-2 alleles *a*, *k*, and *d* respectively. The results of this experiment are summarized in Table 2. The A/J tumor was accepted by 4/11 (H-2^k × NZB)F_1 mice. The rejections could mean either that some H-2^d antigens are missing in NZB mice or that the non-H-2 barrier between NZB and Sarcoma I is relatively strong. A decision between these two possibilities was made by the performance of the same experiment with congenic lines and by the use of skin grafts instead of tumor grafts.

Fourteen (NZB × B10.BR)F_1 hybrids were transplanted with B10.A skin grafts and the survival of the grafts checked starting 10 days after transplantation. At the time of writing this report the grafts have survived for more than 70 days (last line, Table 2). Strains B10.A and B10.BR are congenic differing at the H-2 locus (H-2^a and H-2^k respectively). The long-term survival of these grafts proves that NZB mice have all the H-2^d antigenic components which were not tested for in the F_1 test.

The results from the F_1 test and the component test taken together prove that NZB mice have all the known H-2^d antigens. But the possibility remains that they may have some new H-2 antigenic components. To test this possibility, immunization experiments were performed.

Fourteen B10 mice were injected i.p. once a week with a mixture of spleen, liver, and thymic cells from these NZB mice, then bled 10 days after the fourth injection and the sera titrated individually with a red blood cell panel in the hemagglutination assay. The reaction of some of the sera is shown in Table 3. All the antisera behaved as though they contained only anti-H-2^d antibodies.

In similar fashion 14 B10.D2 mice were immunized against NZB tissues. None of the sera agglutinated NZB red blood cells, obtained from animals 2 months of age or younger. Red cells from older NZB mice were agglutinated with B10.D2 anti-NZB sera, but it was proven that the sera contain anti-allotype (anti-γG_{2a}) antibodies

TABLE 2
Acceptance of grafts

Graft	Recipient	No. mice accepting graft / No. mice grafted
Sarcoma I[a]	(C3H × NZB)F1	4/11
	(C3H × BALB/c)F1	6/6
	A/J	6/6
	C3H	0/7
	NZB	0/4
B10.A skin	(NZB × B10.BR)F1	14/14

[a] Strain A(*H-2*^{a}) tumor.

TABLE 3
Titration of B10 anti-NZB sera with various red blood cells

B10 anti-NZB serum	Reciprocal of hemagglutinin titer with RBC from strains—[a]			
	NZB (*H-2*^{z})	B10.D2 (*H-2*^{d})	A/J (*H-2*^{a})	DBA/1 (*H-2*^{q})
1	160	160	160	160
2	320	320	160	80
3	160	320	320	80
4	320	320	320	160
5	160	160	320	160

[a] No hemagglutination was noted for RBC of the following strains: CBA(*H-2*^{k}), RIII (*H-2*^{r}), and B10 (*H-2*^{b}) (control).

and that these antibodies can be absorbed by older NZB erythrocytes (unpublished results). It can be concluded therefore that the B10.D2 anti-NZB sera do not contain anti-*H-2* antibodies. Thus, NZB has been shown to carry an *H-2* allele indistinguishable from *H-2*^{d}. We suggest the *H-2*^{d} allele be assigned to NZB.

At the present time eight strains carrying *H-2*^{d} allele are known (*8*): BALB/c, C57BL/Ks, B10.D2, DBA/2, ST.T6, Wh, YBL/Rr, and YBR/W. It seems very unlikely that NZB mice have a lineal relationship to any of these strains. The NZB mice were developed from the mixed mouse colony kept in the Animal Department of the Medical School of the University of Otago. This stock had been brought to Dunedin from the laboratories of the Imperial Cancer Research Fund, Mill Hill, London, in 1930. "In 1948 several pairs of mice with similar coat color were chosen from this colony and systematic brother-sister matings were started. Coat color was the guiding principle for selection in the early generations. Selection . . . for agouti (led) to the NZB strain. . . . In one litter of a pair of F_3 agoutis, black mice were present. From one pair of these black mice . . . NZB, was obtained" (*1*). As no animals from other sources have been introduced to the mixed colony of the Animal Department between the years 1930 and 1948 (personal communication of Dr. M. Bielschowsky) and as it is unlikely that some of the above mentioned eight *H-2*^{d} strains were kept at Mill Hill before 1930, the *H-2*^{d} allele of NZB mice may be of independent origin.

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