

ENHANCING EFFECT OF H-2-LINKED NZW GENE(S) ON THE
AUTOIMMUNE TRAITS OF (NZB × NZW)F1 MICE*

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New Zealand Black (NZB) mice spontaneously produce a variety of autoantibodies, including those to nucleic acids, T cells, and erythrocytes (1, 2), and show a high serum level of IgM that is probably due to the spontaneously occurring polyclonal activation of B cells (3, 4). They also develop immune complex-type glomerulonephritis resembling human lupus nephritis (1). Additional abnormalities found in NZB mice are the productions of a large amount of gp70, a major constituent of C-type retroviral envelope glycoprotein, and the antibodies to gp70, resulting in the formation of gp70 immune complexes (gp70 ICs) (5). These gp70 ICs, as well as DNA-anti-DNA ICs (6), have been implicated in the pathogenesis of renal disease in NZB and their progeny (5, 7-9). All these immunological abnormalities are under the control of multiple genes of NZB mice, and a specific genetic mechanism regulates the expression of each of the various traits (10).

As compared with this NZB strain, (NZB × NZW)F1 (B/W F1) hybrids show an earlier onset and a higher incidence of proteinuria associated with increased serum levels of anti-DNA antibodies, gp70 ICs and IgG (1, 9, 11). These findings can be explained by the involvement of a New Zealand White (NZW) gene(s) that acts to intensify the expression of relevant autoimmune NZB gene(s) in B/W F1 hybrids. Either one or two dominant NZW gene(s) have been implicated in the increased incidence and severity of the renal disease observed in B/W F1 hybrids (9, 12). Maruyama et al. (9) suggested that a single dominant NZW gene acts to intensify the production of anti-gp70 antibodies, which in turn results in the formation of a greater amount of gp70 ICs in B/W F1 hybrids. Our recent studies showed that increments in the serum level of anti-dsDNA antibodies in the F1 hybrids can be attributed to the combined effect of two independently segregating dominant NZW genes.¹ All these genetic studies in B/W F1 × NZB backcrosses revealed that each one of these three traits, the increased severity of renal disease and the enhanced productions of gp70 ICs and anti-DNA antibod-

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¹ Kohno, A., H. Yoshida, K. Sekita, N. Maruyama, S. Ozaki, S. Hirose, and T. Shirai. 1983. Genetic regulation of the class conversion of anti-dsDNA antibodies in (NZB × NZW)F1 hybrid. Manuscript submitted for publication.

ies, is significantly associated with the inheritance of H-2^z haplotype.

To investigate the possible effect of H-2 complex of NZW strain on the production of autoantibodies and the renal disease observed in B/W F1 mice, we developed the ZWD/8 strain, a NZW congenic line carrying the H-2^d haplotype, produced (NZB × ZWD/8)F1 (B/WD8 F1) mice, and examined the difference in several immunological abnormalities between the H-2^d/H-2^z heterozygous B/W F1 and the H-2^d/H-2^d homozygous B/WD8 F1 mice.

Materials and Methods

Mice and Sera. ZWD/8 mice, a NZW congenic line carrying the H-2^d haplotype, were developed by backcrossing B/W F1 mice to NZW for eight generations. The H-2^d typing in each generation was done by a cytotoxicity test against peripheral blood lymphocytes using the anti-H-2^d antiserum produced by immunizing NZW with the spleen cells of NZB mice. Only female mice were used in this study.

Anti-DNA Antibodies. Measurement of antibodies to dsDNA and ssDNA was performed by the Farr assay with slight modification, as described previously (11). The immunoglobulin classes of anti-dsDNA antibodies were determined by *Crithidia luciliae* kinetoplast immunofluorescence (KIF) test (13). The specificity of the KIF test for anti-dsDNA antibodies was confirmed by staining of the *C. luciliae* with mouse monoclonal antibodies to DNA (14). The mouse sera that showed a positive KIF at 1:10 dilution or more were regarded as positive.

Serum gp70 ICs. Serum gp70 ICs were measured by the inhibition radioimmunoassay, as described elsewhere (9).

Natural Thymocytotoxic Autoantibody (NTA). NTA was measured by a two-step cytotoxicity test against BALB/c thymocytes, as described previously (2). The sera that showed 50% or more cytotoxicity at 1:2 dilution were regarded as positive in this study.

Anti-erythrocyte Autoantibody (AEA). AEA was examined by direct Coombs' test.

Serum IgG and IgM Levels. Solid phase inhibition radioimmunoassay was performed to determine the serum levels of IgG and IgM. A mixture of ¹²⁵I-labeled myeloma proteins, MOPC 21 (γ_1, κ), RPC 5 (γ_{2a}, κ) and MOPC 195 (γ_{2b}, κ), were used for IgG assay and ¹²⁵I-labeled MOPC 104E (μ, λ_1) for IgM assay.

Proteinuria. The onset of renal disease was monitored by biweekly measurement of proteinuria (12). The proteinuria of 111 mg/100 ml or more was regarded as positive.

Statistical Analysis. Statistical analysis was performed using χ^2 Yates test and Student's *t*-test. Probability values (*P* values) of <5% were considered as significant.

Results

As in the case of NZW, the ZWD/8 strain, a NZW congenic line carrying the H-2^d haplotype, shows no immunological abnormalities. We compared the immunological abnormalities between the H-2^d/H-2^z B/W F1 and the H-2^d/H-2^d B/WD8 F1 mice.

Anti-DNA Antibodies. As shown in Fig. 1 A and B, the B/WD8 F1 mice showed markedly lower serum levels of dsDNA- and ssDNA-binding activities as measured by the Farr assay than did the B/W F1 mice at 7 months ($t = 3.253, P < 0.01$ for dsDNA and $t = 4.450, P < 0.001$ for ssDNA) and 9 months of age ($t = 4.232, P < 0.001$ for dsDNA and $t = 3.738, P < 0.001$ for ssDNA). Fig. 2 shows the data of the KIF test, indicating that as compared with B/W F1 mice, B/WD8 F1 mice showed a significantly lower incidence of IgG anti-dsDNA antibodies ($\chi^2 = 15.631, P < 0.001$). A lack of significant difference, however, was observed in the incidences of IgM anti-dsDNA antibodies between these two hybrid strains of mice ($\chi^2 = 2.011, P > 0.10$).

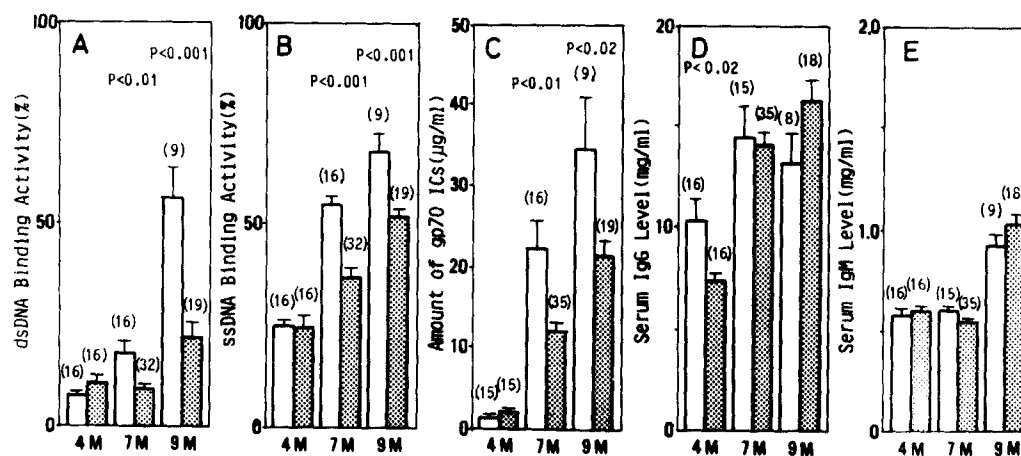


FIGURE 1. Comparisons of (A) dsDNA-binding activities, (B) ssDNA-binding activities, (C) the amounts of serum gp70 ICs, (D) serum IgG levels, and (E) serum IgM levels between B/W F1 (□) and B/WD8 F1 (▨) mice at 4, 7, and 9 months of age. Each vertical column shows the mean value and bars represent the SE. The number of mice tested is shown in the parenthesis. *P* values are estimated by Student's *t*-test at each month.

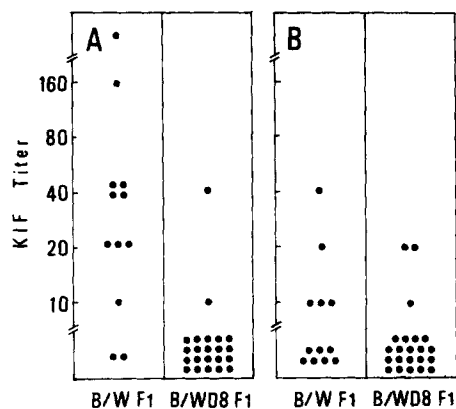


FIGURE 2. (A) IgG anti-dsDNA antibodies and (B) IgM anti-dsDNA antibodies measured by the KIF test in B/W F1 and B/WD8 F1 mice at 7 months of age. KIF titer is expressed as the reciprocals of the highest serum dilution giving positive kinetoplast immunofluorescence.

Serum gp70 ICs. Fig. 1 C shows that the average amounts of serum gp70 ICs in B/WD8 F1 mice were significantly lower than in B/W F1 mice at 7 months ($t = 3.470$, $P < 0.01$) and 9 months of age ($t = 2.627$, $P < 0.02$).

NTA and AEA. As shown in Table I, there was a lack of significant difference in both the percentage of cytotoxicity and the incidence of NTA, as well as in the prevalence of AEA between B/W F1 and B/WD8 F1 mice.

Serum Immunoglobulins. The serum levels of IgG were estimated by the amounts of IgG₁ and IgG₂. At 4 months of age, B/W F1 mice showed a higher mean serum level of IgG than did B/WD8 F1 mice ($t = 2.495$, $P < 0.02$). However, B/WD8 F1 mice showed as high a serum level of IgG as did B/W F1 mice at 7 and 9 months of age (Fig. 1 D). There was no significant difference in the mean serum levels of IgM between these two hybrid mice (Fig. 1 E).

TABLE I
Appearances of Natural Thymocytotoxic Autoantibody and Anti-erythrocyte Autoantibody in B/W F1 and B/WD8 F1 Mice

Mouse	NTA			AEA	
	Age	% Cytotoxicity	No. positive/No. tested (%)	Age	No. positive/No. tested (%)
	<i>mo</i>			<i>mo</i>	
B/W F1	7	67.1 ± 14.8%*	22/24 (92)	9	3/12 (25)
B/WD8 F1	7	63.4 ± 10.3%	22/24 (92)	9	4/19 (21)

* The mean value of percent cytotoxicity ± 1 SD to BALB/c thymocytes at 1:2 serum dilution.

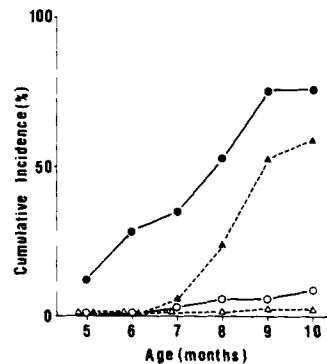


FIGURE 3. Comparisons of the cumulative incidences of proteinuria and mortality between 17 B/W F1 and 34 B/WD8 F1 mice. ●, proteinuria in B/W F1; ○, proteinuria in B/WD8 F1; ▲, mortality in B/W F1; △, mortality in B/WD8 F1 mice.

Proteinuria and Mortality. Fig. 3 shows that in B/W F1 mice, proteinuria first appeared at 5 months of age, and the cumulative incidence reached 76% by 10 months of age. The cumulative mortality of B/W F1 mice had reached 59% by the same time. In B/WD8 F1 mice, proteinuria first appeared at 6 months and this incidence reached only 9% by 10 months of age. The cumulative mortality remained in only 3% at the same time.

Discussion

Our findings provide good evidence that the H-2-linked NZW gene(s) acts to intensify the expression of NZB autoimmune disease genes in B/W F1 hybrids. This NZW gene action was related to the traits, anti-DNA antibodies, circulating retroviral gp70 ICs and the IC-type glomerulonephritis, but not to NTA, AEA, and the serum levels of IgG and IgM.

The NZB strain contributes a single dominant locus (*Lpn-1*) or a cluster of closely linked loci to the development of renal disease and either one (*Lpn-2*) or a combined effect of two dominant loci (*Lpn-2*, *Lpn-3*) of the NZW strain is involved in the accelerated onset and the increased severity of renal disease in B/W F1 hybrids (9, 12). Related to this may be the findings that a major single dominant locus of NZB strain (*Agp-1*) determines the production of anti-gp70 antibodies and that the magnitude is to a great degree intensified by a single dominant locus of NZW strain (*Agp-3*) (9). The spontaneous production of anti-

dsDNA antibodies in the B/W F1 hybrids is determined by a combined effect of two dominant NZB loci (*Ads-1*, *Ads-2*) (11), and a combined effect of two dominant loci of NZW strain (*Ads-3*, *Ads-4*) acts to increase the amount of anti-dsDNA antibody production and to convert the class of the antibodies from IgM to IgG.¹ All these studies suggested that *Lpn-1*, *Agp-1*, and *Ads-1* loci are to some extent linked to H-2 complex of NZB and that *Lpn-2*, *Agp-3*, and *Ads-3* loci are linked to H-2 complex of NZW strain (9–12). The present studies presented good evidence that among these, *Lpn-2*, *Agp-3*, and *Ads-3* loci are located within or closely linked to the H-2 complex of NZW mice. Development of the H-2 congenic ZWD/8 line raises another important point. The H-2^d haplotype of ZWD/8 line was derived from the NZB strain, nevertheless, ZWD/8 developed no immunological abnormalities. Therefore, we concluded that neither *Lpn-1* nor *Agp-1* is located within the H-2 complex of the NZB strain. Close linkages among *Ads-1*, *Agp-1*, and *Lpn-1* on chromosome 17 of NZB strain (10) suggested that *Ads-1* is also outside of the H-2 complex.

One of the possible pathways by which the H-2^z-linked NZW gene promotes the autoimmunity in B/W F1 mice is through the role of I region in the H-2 complex. The findings of Papoian and Talal (15), that the response of B/W F1 mice to DNA occurs primarily through the NZW H-2^z haplotype of their own antigen-presenting cells, may be relevant.

Finally, it is noteworthy that the H-2^z-linked NZW gene(s) may be involved in the class conversion of anti-dsDNA antibodies from IgM to IgG. In contrast to the NZB mice in which the IgM is the predominant class of anti-dsDNA antibodies, the IgG class antibodies predominate in B/W F1 hybrids.¹ However, this gene action of NZW mice was proved to be unrelated to the increased serum level of polyclonal IgG that also characterized the B/W F1 hybrids.

Summary

To investigate the possible enhancing effect of the H-2^z haplotype of the New Zealand White (NZW) strain on the production of autoantibodies and renal disease observed in B/W F1 mice, we developed the ZWD/8 strain, a NZW congenic line carrying the H-2^d haplotype, produced (NZB × ZWD/8)F1 (B/W D8 F1) mice, and examined the difference in several immunological abnormalities between the B/W F1 (H-2^d/H-2^z) and the B/W D8 F1 (H-2^d/H-2^d) mice. In comparison with B/W F1 mice, the B/W D8 F1 mice showed markedly lower serum levels of the anti-DNA antibodies and the gp70 ICs, and a later onset and a lower incidence of proteinuria with a lower mortality. In contrast, there was no significant difference in the incidences and the amounts of both natural thymocytotoxic autoantibody and anti-erythrocyte autoantibody between these two hybrid strains. Further, the serum levels of IgG and IgM in B/W D8 F1 mice were as high as those in B/W F1 mice. These findings indicate that the gene(s) that is within or closely linked to the H-2 complex of NZW strain specifically acts to intensify the levels of anti-DNA antibodies and gp70 ICs, and to promote the severity of renal disease in B/W F1 mice. This gene may play a role in the class conversion of anti-dsDNA antibodies from IgM to IgG.

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