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

BY SACHIKO HIROSE, RYUJI NAGASAWA, IWAO SEKIKAWA, MASARU HAMAOKI, YASUO ISHIDA, HIDETOSHI SATO, AND TOSHIKAZU SHIRAI*

From the Department of Pathology, Juntendo University School of Medicine, Tokyo 113, Japan

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. 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 antibo-

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* Address reprint requests to Prof. T. Shirai, The Department of Pathology, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113, Japan.
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.

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ies, is significantly associated with the inheritance of H-2d 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-2d haplotype, produced (NZB × ZWD/8)F1 (B/WD8 F1) mice, and examined the difference in several immunological abnormalities between the H-2d/H-2d heterozygous B/W F1 and the H-2d/H-2d homozygous B/WD8 F1 mice.

**Materials and Methods**

**Mice and Sera.** ZWD/8 mice, a NZW congenic line carrying the H-2d haplotype, were developed by backcrossing B/W F1 mice to NZW for eight generations. The H-2d typing in each generation was done by a cytotoxicity test against peripheral blood lymphocytes using the anti-H-2d 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 Thymocyte Toxic 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 (γ1, k), RPC 5 (γ2a, k) and MOPC 195 (γ2b, k), were used for IgG assay and 125I-labeled MOPC 104E (μ, λ) 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 χ² 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-2d haplotype, shows no immunological abnormalities. We compared the immunological abnormalities between the H-2d/H-2d B/W F1 and the H-2d/H-2d B/WD8 F1 mice.

**Anti-DNA Antibodies.** As shown in Fig. 1A 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 (χ² = 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 (χ² = 2.011, P > 0.10).
Serum gp70 ICs. Fig. 1C shows that the average amounts of serum gp70 ICs in B/W D8 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 1, 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/W D8 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/W D8 F1 mice \((t = 2.495, P < 0.02)\). However, B/W D8 F1 mice showed as high a serum level of IgG as did B/W F1 mice at 7 and 9 months of age (Fig. 1D). There was no significant difference in the mean serum levels of IgM between these two hybrid mice (Fig. 1E).
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 (%) |
| 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.

![Graph showing cumulative incidences of proteinuria and mortality between 17 B/W F1 and 34 B/WD8 F1 mice.](image-url)

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. 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-2d 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-2z-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-27 haplotype of their own antigen-presenting cells, may be relevant.

Finally, it is noteworthy that the H-2z-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. 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-2z 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-2d haplotype, produced (NZB × ZWD/8)F1 (B/WD8 F1) mice, and examined the difference in several immunological abnormalities between the B/W F1 (H-2d/H-2d) and the B/WD8 F1 (H-2d/H-2z) mice. In comparison with B/W F1 mice, the B/WD8 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/WD8 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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