THREE GENES FOR LUPUS NEPHRITIS IN NZB × NZW MICE

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NZB × NZW hybrid mice provide an excellent model of spontaneous autoimmune disease, showing a very high incidence of a renal disorder which closely resembles lupus nephritis (1). The occurrence of an autoimmune condition, causing early death from renal failure, in the hybrid of two in-bred strains which do not themselves show the disorder provides a unique opportunity for elucidating the genetic basis of inherited autoimmune disease. The NZB × NZW renal disease must depend on the action of at least two genes, one from each strain, and these genes must be dominant or codominant in that they express their effect in the heterozygous state.

In an earlier paper (2) we reported the results of a study of the genetic contribution of the NZB mouse to the renal disease of the NZB × NZW hybrid, demonstrating that the NZB strain contributes a single gene or cluster of closely-linked genes to the disorder. In this paper we report a study of the genetic contribution of the NZW strain to the renal disease of the hybrid.

Materials and Methods

Mice. All mice were derived from the Otago University inbred colonies. Virgin female mice were used in all the studies described. Female (NZB × NZW)F_1 hybrid mice were backcrossed to NZB mice and 150 offspring were *H-2* typed and monitored for the onset of renal disease by using the criteria described below. In addition (NZB × NZW)F_1 mice were out-crossed to NZC mice and 190 offspring were similarly studied. Three control groups, comprising 143 (NZB × NZW)F_1 hybrids, 75 NZB, and 40 NZW mice were also monitored for the occurrence of renal disease.

H-2 Typing. The H-2 phenotype of each (NZB × NZW)F_1 × NZB mouse and of 100 of the (NZB × NZW)F_1 × NZC mice was determined by using a modification of Stimpfling's PVP hemagglutination technique (3) as described previously (2). Anti-H-2.4 and anti-H-2.5 antisera, kindly supplied by Dr. J. G. Ray, Transplantation and Immunology Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. were used to differentiate between $H\text{-}2^d$ and $H\text{-}2^z$ haplotypes.

Monitoring Renal Dysfunction

MEASUREMENT OF PROTEINURIA. The onset of renal disease was monitored by fortnightly testing for proteinuria by using the method described previously (2). Briefly, 10-μl samples of urine were spotted onto strips of chromatography paper, fixed in ethanol, and stained with bromophenol blue. The degree of proteinuria in each mouse was assessed by visually comparing the color intensity of the urine spots with that of spots of bovine serum albumin standards (3,000, 1,000, 333, 111, and 37 mg/100 ml that had been similarly treated.

MEASUREMENT OF RENAL CLEARANCE. Detection of heavy proteinuria (333 mg/100 ml) was followed by the assessment of glomerular function with a single injection ^{51}Cr-EDTA clearance method (2). Studies on healthy 12-wk old mice established the normal half-value time as 14.0 ± 3.1 min. A half-value time of greater than twice the normal value was arbitrarily chosen as indicative of severe renal impairment (3).

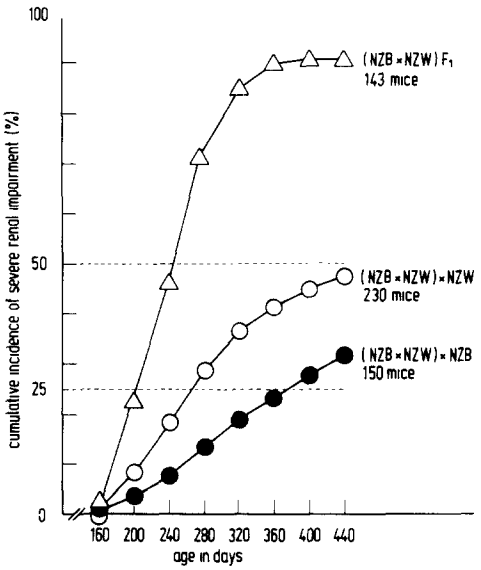

FIG. 1. A comparison of the cumulative incidence of severe renal impairment in NZB × NZW mice and in the reciprocal back-crosses.

Results

Incidence of Renal Disease in NZB, NZW, and NZB × NZW Mice. By using the criteria outlined above we observed 6 of 75 NZB mice (8%) and 1 of 40 NZW mice to have severe renal disease before the age of 440 days. In striking contrast, 130 of 143 (NZB × NZW)F_1 mice (92%) had severe renal disease before 440 days. We observed a continuing occurrence of renal disease in older NZB mice, the incidence reaching 16% by 540 days.

Incidence of Renal Disease in (NZB × NZW)F_1 × NZB Backcross Mice. Fig. 1 shows that the incidence of renal disease in the group of 150 (NZB × NZW) × NZB backcross mice reached 32.7% by 440 days. As we have observed a small, but significant, incidence of early renal disease in NZB mice we consider it more likely that this represents an over-estimate of 25% than an under-estimate of 50% incidence of early renal disease in this backcross. This view is strengthened by the observation that there was a marked difference in the distribution of ages of onset of renal disease in the mice of this backcross as compared to the NZB × NZW animals, with some of the backcross mice showing a tendency towards later onset of renal disease as occurs in the NZB strain. Thus 14 of 49 backcross mice developed renal disease when older than 360 days as compared to 2 of 130 NZB × NZW mice ($\chi^2 = 28.7$, $P < 0.001$).

These data, together with those of our earlier study (2), are therefore compatible with the conclusion that the renal disease of the NZB × NZW hybrid is determined by three genes, all dominant or codominant, one contributed by the NZB strain and two, unlinked, contributed by the NZW strain. We have designated these three genes LN-1 (lupus nephritis-1),[1] LN-2, and LN-3, respectively (see Fig. 2).

[1] *Abbreviation used in this paper:* LN, lupus nephritis.

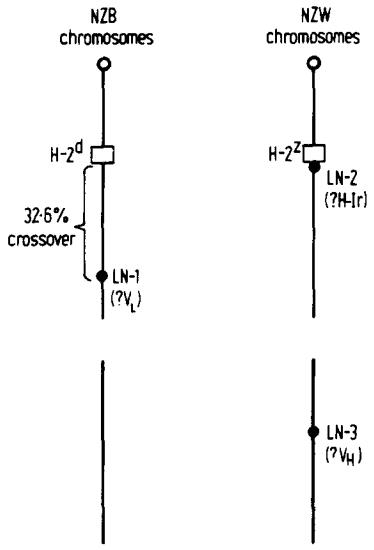

FIG. 2. Three genes for lupus nephritis (LN-1, LN-2, and LN-3), all dominant, in NZB × NZW mice. LN-1, LN-2, and LN-3 could represent clusters of closely linked genes rather than single loci.

TABLE I

Segregation of Renal Disease and H-2 Type in (NZB × NZW)F_1 × NZB Back-cross Mice

	Histocompatibility type		Total
	H-2^d/H-2^z	*H-2^d/H-2^d*	
Mice with renal disease	43	6	49
Mice without renal disease	35	66	101
Total	78	72	150

Linkage between the H-2 Complex and One of the NZW Renal Disease Genes. The mice of the (NZB × NZW) × NZB backcross would be expected to be either homozygous (*H-2^d/H-2^d*) or heterozygous (*H-2^d/H-2^z*) with respect to the *H-2* complex (NZBs are *H-2^d*, NZWs are *H-2^z*) (4). Table 1 shows that, as expected, the 150 mice were nearly equally divided between the two genotypes. However, a great preponderance of mice which developed renal disease were heterozygous. This difference is highly significant ($\chi^2 = 35.2$, $P < 0.001$) and indicates that one of the genes contributed by the NZW strain is linked to the *H-2* complex. The occurrence of renal disease in *H-2^d/H-2^d* mice could indicate that there is a crossover frequency of approximately 8% between the renal disease gene and the *D* end of the *H-2* complex, the side on which the new gene lies being undetermined. However, as already indicated, a small proportion (8%) of NZB mice develop early severe renal disease and these are indistinguishable from NZB × NZW mice on the basis of the criteria used in this study. Therefore it seems more probable that the six homozygous mice showing renal disease are not the result of crossover and that this NZW renal disease gene, which we have designated LN-2, is very closely linked to the *H-2* complex as indicated in Fig. 2.

Incidence of Renal Disease in (NZB × NZW)F_1 × NZC Mice. NZC mice have not been found to show any incidence of autoimmune lupus nephritis (5), so the incidence of renal disease in the (NZB × NZW) × NZC out-cross was studied as an independent test for linkage between renal disease genes from the NZB and the NZW strains. If the gene contributed by the NZB strain is on the same chromosome as one from the NZW strain then renal disease would only occur if there was crossingover between them. We observed 10 of 190 mice (5.3%) of this out-cross to develop renal disease. This figure approximates the 7.5% incidence which one would expect if the renal disease were determined by two dominant unlinked genes (A and B) from one strain and one dominant gene (C) from the other strain, linked to gene A but separated from it by a crossover frequency of 30%.

Only 100 of the 190 mice were *H-2* typed before some mice died of renal failure. However all six of the mice which developed renal disease and which had been typed were *H-2*$^{d/z}$, (NZC mice are *H-2*^{d}) (6) which suggests that (one of) the gene(s) from the NZW is closely linked to the *H-2* complex. Thus the data obtained from this out-cross provide independent confirmation of the data obtained from the reciprocal backcrosses.

Discussion

In cross-breeding studies of the NZB mice with other inbred New Zealand strains, Helyer and Howie (7) observed that NZB × New Zealand Yellow F_1 hybrids showed only a very low incidence of hemolytic anemia and positive Coombs tests (4%) but died prematurely with renal lesions similar to those seen in lupus nephritis. Subsequently Helyer and Howie (8) reported that NZB × NZW mice die even more prematurely from a similar lupus nephritis. Positive lupus erythematosus cell tests were observed in both hybrids (8). It was shown by fluorescence techniques that NZB × NZW hybids have accumulations of immunoglobulins in the glomeruli (9, 10), together with complement (10), and elution studies have shown that the immunoglobulins have specificity for nuclear antigens in accord with the concept that the renal lesions are caused by immune complexes of nuclear antigens and their complement-fixing autoantibodies which become trapped in the glomeruli (10).

NZB and NZW mice from the Otago colonies do not show the early renal failure characteristic of the NZB × NZW hybrids. Howie and Helyer (1) found no heavy proteinuria in 270 virgin female NZB mice at 1 yr of age and we observed an incidence of only 4% in a similar group of 75 NZB mice aged 1 yr. Similarly we have observed only a very low incidence (2.5%) of early renal disease in 40 NZW mice.

It appears that renal disease and/or antibodies directed against nuclear components are found much more commonly in colonies of NZB and NZW mice elsewhere in the world. Thus reports on anti-nuclear antibody incidence in NZB mice have varied from almost 0 to 100% (1, 11–16). It is highly relevant that many of the authors reporting a high incidence of anti-nuclear antibody (ANA) in NZB mice have also observed considerable frequencies of ANA in unrelated strains, suggesting that environmental factors could be largely responsible, as indicated by the studies of Barnes and Tuffrey (17) and Shulman et al. (18).

Dixon et al. (19) demonstrated that virus infections could cause nephritis in normal mouse strains as a result of deposition of viral-anti-viral-antibody complexes in glomeruli. The presence of C-type particles indicative of murine leukemia virus in NZB mice appears to be widely accepted as a normal attribute of the strain. However, C-type particles have never been observed in mice from the Otago colony despite extensive electron microscope studies (20) and serological testing of serum samples from the Otago colonies has failed to reveal the presence of active murine leukemia virus, T. Maguire, personal communication. Differences in viral infestations could well explain many of the discrepancies in the literature on the NZB mouse and its hybrids.

It now seems clear that the autoimmunity is not due to virus infection in that it cannot be transmitted by cell-free filtrates (21, 22) or enhanced by immune suppression (23, 24). On the other hand, the lymphoid tumours which occur with widely varying frequency in different NZB colonies (21) do appear to be caused by oncogenic viruses in that their frequency is greatly increased by immune suppression (23, 25) and they appear to have been transmitted by cell-free filtrates (26) as well as by living cells (27, 28). It seems likely that these lymphoid tumours are unrelated to the autoimmunity (23).

Our previous study (2) showed a 50% incidence of severe early renal disease in (NZB × NZW) × NZW backcross animals, indicating that the NZB strain contributes a single gene, or cluster of closely-linked genes, to the condition. This gene was found to be loosely linked to the *H-2* complex (see Fig. 2). The present study, showing a 25% incidence of early renal disease in (NZB × NZW) × NZB backcross animals, indicates that the NZW strain contributes two unlinked genes. However, additional genes, common to both the NZB and the NZW strains, could also be involved in the lupus nephritis. One of the NZW genes was found to be closely linked to the *H-2* complex (Fig. 2) and could be a histocompatibility-linked immune response (*H-Ir*) gene (29). Of interest in this regard is the observation of Lambert and Dixon (30) that NZW mice are high responders to heat denatured DNA coupled to methylated bovine serum albumin, and that high responder status is transferred in dominant fashion to the NZB × NZW hybrid (NZBs are low responders to DNA).

All three of the genes whose effect we have observed must be dominant or codominant in that their effect is expressed in the heterozygous state. From consideration of the features and pattern of inheritance of thyroid and other human autoimmune diseases one of us (31) has postulated that the genetic basis of inherited autoimmune disease lies in the specificity of V genes, the structural genes which code for the variable portions of the light and heavy polypeptide chains which combine to form antibody molecules (32). We consider that the genes LN-1 and LN-3 are most likely to be immunoglobulin V genes in accord with this concept. It is possible that LN-1 is a light chain V gene and LN-3 a heavy chain V gene which, in combination, code for an autoantibody in the NZB × NZW hybrid (Fig. 2). The crossover frequency between LN-1 and the *D* end of H-2, 32.6% (2), is very close to that (31.4%) for a gene observed by Lotzova and Cudkowicz (33) to control resistance to bone marrow grafts in mice. These two genes could be members of a single cluster of V genes.

A widely-held hypothesis proposes that autoimmune disease arises as a result

of an age-dependent loss of suppressor T cells, which are supposed to prevent production of autoantibodies in normal animals (34, 35). It has been claimed that suppression or prevention of autoantibody formation can be achieved in mice of strains which develop autoimmunity by injecting thymocytes from young syngeneic donors (36, 38). However, we have been unable to confirm these findings in NZB and NZB × NZW mice.[2] Furthermore, it is difficult to see how such a proposal could be compatible with present knowledge of the genetic basis of autoimmune diseases. The pattern of inheritance of human autoimmune diseases is dominant with incomplete penetrance and for thyrotoxicosis and diabetes there is evidence that at least two genes are involved, suggesting a basis in pairs of immunoglobulin V genes (31) and making an interesting parallel with the present data.

Summary

The occurrence of early severe lupus nephritis in (NZB × NZW)F_1 mice must depend on the action of at least two dominant or codominant genes (at least one gene from each parent) as neither of the inbred parental strains shows the disorder. Identifying affected animals by antemortem determinations of renal function, we have studied the incidence of the renal disease in 230 (NZB × NZW) × NZW backcross mice (an earlier study) and, in this study, in 150 (NZB × NZW) × NZB backcross mice. The data indicate that the NZB strain contributes only one gene and the NZW strain contributes two genes, or clusters of closely linked genes, to the renal disorder of the F_1 hybrid. One of the NZW genes was found to be linked to the *H-2* complex. All three genes must be dominant or codominant, as their effect is expressed in the heterozygous state.

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