

RETROVIRAL gp70 IMMUNE COMPLEXES IN
NZB × NZW F₂ MICE WITH MURINE LUPUS NEPHRITIS*

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NZB × NZW F₁ hybrid (NZB × W F₁) mice spontaneously develop a disease that closely resembles systemic lupus erythematosus (SLE)¹ in humans (1, 2). The characteristic immunologic abnormalities of these mice are the formation of diverse antibodies reactive with native or altered autologous antigens and immune complex (IC) glomerulonephritis (GN; 1-4).

The involvement of endogenous retrovirus-related antigens in murine SLE is a long-standing theory. In support is documentation that the major envelope glycoprotein, gp70, is preferentially expressed in sera of NZB × W F₁ mice (5-7) and is also found in IC deposits within their diseased glomeruli (4, 5). Retroviral gp70-anti-gp70 IC also become apparent in the circulation close to the onset of disease and their concentrations rise with the progression of nephritis (8), constituting good evidence that gp70-anti-gp70 IC are a potential source of renal injury. Additionally, the participation of DNA-anti-DNA IC in the pathogenesis of murine SLE was demonstrated when significant concentrations of anti-DNA antibodies were found in renal eluates of NZB × W F₁ mice (2, 4). The induction of tolerance to DNA in these mice delayed the development of anti-DNA antibodies and renal disease (9, 10), supporting the pathologic importance of DNA-anti-DNA IC in murine SLE.

Evidence is now accumulating that many individual autoimmune traits of the NZB × W F₁ mice may be determined by single genes or gene clusters segregating independently of each other (11-13). Analysis of the F₂ generation born of mating between several SLE-prone strains and normal mice yielded relatively little relationship among their various immunologic abnormalities such as IgM, anti-single-stranded DNA (ssDNA) antibodies, and anti-nuclear antibodies, suggesting independent segregation of the responsible genes (13). The F₂ generation proves to be an excellent subject for studying which immunologic abnormalities most closely relate to the development of renal disease in SLE-prone mice. We have now examined individual F₂ offspring of NZB × W mice to determine whether the development of fatal GN is associated with the presence of circulating antibodies to double-stranded DNA (dsDNA), ssDNA, or serum gp70. Our results indicate that in NZB × W F₂

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¹ *Abbreviations used in this paper:* dsDNA, double-stranded DNA; GN, glomerulonephritis; IC, immune complex; LDV, lactic dehydrogenase virus; PGE, prostaglandin E₁; SLE, systemic lupus erythematosus; ssDNA, single-stranded DNA.

mice, fatal GN correlates well with the presence of antibodies to serum gp70 measured as gp70-containing IC, but shows no significant association with free anti-DNA antibodies.

Materials and Methods

Mice. NZB and NZW mice were originally obtained from the Laboratory Animals Centre, Medical Research Council, Surrey, England, and since 1965 have been maintained by brother-sister matings at Scripps Clinic and Research Foundation. NZB \times W F₁ and F₂ mice were bred in our animal colony, and only female mice were used in this study. Their blood samples were collected by orbital sinus puncture, and the sera were stored at -20°C until used.

Detection of Anti-dsDNA and Anti-ssDNA Antibodies. Serum dsDNA- and ssDNA-binding activities were determined by using a modification of the Farr DNA-binding radioimmunoassay (14). dsDNA and ssDNA were labeled with ^{125}I by the method of Commerford (15). ^{125}I -dsDNA was further treated with S1 nuclease (Miles Laboratories, Inc., Elkhart, Ind.) to remove single-stranded regions within dsDNA preparations (16). The purity of the ^{125}I -labeled dsDNA was tested by using murine antibodies specific for ssDNA but not dsDNA. These antibodies precipitated $<5\%$ of the radioactivity. The results are expressed as a percentage of the 20 ng of ^{125}I -DNA precipitated specifically after correction for nonspecific precipitation in pooled sera from five different strains of normal mice. Values of dsDNA-binding activity higher than 2 SD values (7.5%) for the mean level of 8-mo-old NZW mice were considered positive. More than 30% ssDNA-binding activity was regarded as positive. The immunoglobulin class of anti-DNA antibodies was analyzed by sucrose density gradient ultracentrifugation (17). The quantities of anti-DNA antibodies in sera and renal eluates (8) were determined by a solid-phase radioimmunoassay similar to that described for IgG anti-human gammaglobulin antibodies (18).

Radioimmunoassays for Circulating DNA-anti-DNA IC and DNA. The presence of circulating DNA-anti-DNA IC was examined according to a modification (17) of the method of Harbeck et al. (19). In this assay, serum DNA-binding activity was measured before and after treatment of sera with DNase, which liberates anti-DNA antibodies bound to DNA, resulting in the increase of DNA-binding activity.

The amounts of DNA in sera were determined by inhibiting the binding of ^{125}I -ssDNA to anti-DNA antibodies as previously described (17, 20). The specificity of the assay was controlled by DNase treatment of the samples. Because anti-DNA antibodies might interfere with the digestion of DNA by DNase, samples were pretreated for 30 min at room temperature with 0.5 N HCl, which not only dissociates DNA-anti-DNA IC but also abolishes anti-DNA activities. Further, this HCl treatment of DNA increases its binding affinity to anti-DNA antibodies.

Quantitation of Circulating Antibodies to Serum gp70. Because of the great excess of gp70 in sera, free antibodies reactive with serum gp70 were not detectable. Therefore, serum levels of anti-gp70 antibodies were expressed as the concentrations of gp70 bound to anti-gp70 antibodies. To determine the serum concentrations of antibody-bound gp70, 0.05 ml of serum was layered on 5–20% (wt:vol) linear sucrose gradients in 0.01 M phosphate-buffered saline, pH 7.0, and centrifuged at 32,000 rpm for 16 h at 4°C in a Beckman L-75 ultracentrifuge with a SW60 rotor (Beckman Instruments, Inc., Fullerton, Calif.). The positions of IgM, IgG, or gp70 were established by radioactive markers, and the gradients were divided into 14 fractions. Each fraction was radioimmunoassayed for the presence of gp70. Because IgG and gp70 peaked in the 10th and 12th fractions, respectively, gp70 present in the first eight fractions was considered a rapidly sedimenting, heavy form of antibody-bound gp70. From the gp70 content of the first eight fractions, serum levels of heavy antibody-bound gp70 were computed. Values of 3 SD or more above the mean level of 8-mo-old NZW mice were considered positive.

Renal Pathology. Renal tissues of NZB \times W F₂ mice were obtained at autopsy, processed for histologic examination, and stained with hematoxylin and eosin or periodic acid-Schiff. GN was quantitated on a 0–4+ scale based on the intensity and extent of histopathologic changes (21). A grade of 0 was given to kidneys without glomerular lesions. 1+ lesions corresponded to minimal thickening of the mesangium; 2+ lesions contained noticeable increases in both mesangial and glomerular cellularity; 3+ lesions were characterized by the preceding conditions with superimposed inflammatory exudates and capsular adhesions; and in 4+ lesions, the

glomerular architecture was obliterated in >70% of glomeruli, and tubular cast formation was extensive. Grades 3 and 4 GN were considered significant contributors to clinical disease and/or death.

Statistical Analysis. Methods of statistical analysis included the χ^2 test, the linear correlation analysis, and the Wilcoxon two-sample test. Probability values of >5% were considered insignificant.

Results

Mortality with GN. Fig. 1 documents the cumulative mortality of glomerulonephritic NZB, NZW, NZB \times W F₁, and F₂ female mice. NZB \times W F₁ and F₂ mice began dying of renal disease at 5 mo of age, and their 50% mortality points were 8 mo and 11 mo, respectively. However, their parental strains, NZB and NZW mice, developed fatal GN much later in life and did not begin dying until 9 and 13 mo of age, respectively. 50% of NZB mice and <20% of NZW mice died of renal disease by 15 mo of age.

Anti-DNA and the Heavy Form of Antibody-bound gp70 in Sera of NZB, NZW, and NZB \times W F₁, and F₂ Mice. Anti-dsDNA antibodies developed relatively late compared with anti-ssDNA antibodies in all four kinds of mice. NZB \times W F₁ had the highest levels of both anti-dsDNA and anti-ssDNA antibodies, and NZW mice had the lowest during the entire course of experimentation. Amounts of antibodies to both dsDNA and ssDNA in the sera of NZB \times W F₂ mice were similar to those in NZB mice. Representative results of serum dsDNA- and ssDNA-binding activities at 8 mo of age are shown in Table I.

The antibody-bound, heavy form of retroviral gp70 became obvious in the sera of NZB \times W F₁ and F₂ mice at ~4 mo of age. Moreover, the incidence and concentrations increased with the progression of disease. At 8 mo of age, the mean values were highest in NZB \times W F₁ mice (Table I). In fact, 19 of 20 NZB \times W F₁ mice were positive, i.e., exceeded the mean level of 8-mo-old NZW mice by at least 3 SD. Approximately half (33 of 72) of the NZB \times W F₂ mice had significant amounts of anti-gp70-bound gp70 IC in their sera. Only 5 of 20 NZB and none of 20 NZW mice tested developed these gp70 complexes. Although the frequency and quantities of antibody-bound gp70 in sera were very different among these four kinds of mice, their serum concentrations of

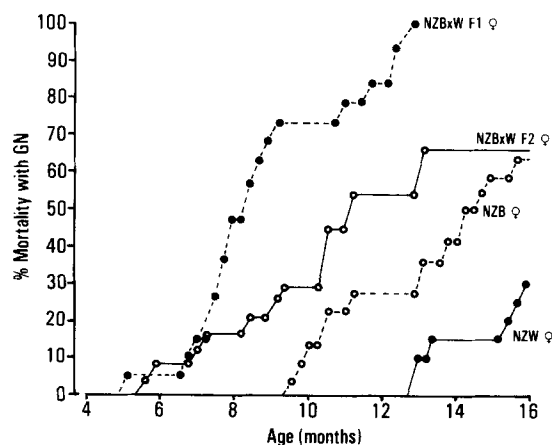


FIG. 1. Cumulative rates of mortality with GN in NZB, NZW, NZB \times W F₁, and F₂ female mice.

TABLE I
Serum Levels of Antibodies to dsDNA and ssDNA and Complexes of gp70 Bound to Anti-gp70 Antibodies in NZB, NZW, NZB × W F₁, and F₂ Female Mice

Mice	Number	Anti-dsDNA*	Anti-ssDNA‡	Anti-gp70 IC§
NZB	20	6.0 ± 3.9	30.7 ± 14.5	2.6 ± 3.0
NZW	20	3.5 ± 2.0	16.1 ± 7.3	1.0 ± 0.8
NZB × W F ₁	20	11.1 ± 6.7	48.4 ± 12.5	15.2 ± 11.4
NZB × W F ₂	72	6.6 ± 10.8	28.1 ± 19.7	5.9 ± 7.9

All values were determined when mice were 8 mo of age.

* Percent binding of 20 ng ¹²⁵I-dsDNA by 10 μl serum.

‡ Percent binding of 20 ng ¹²⁵I-ssDNA by 5 μl serum.

§ Micrograms per milliliter of the heavy form of anti-gp70 complexed gp70 in serum.

TABLE II
Association between Anti-dsDNA and Anti-ssDNA Antibodies in 8-mo-old NZB × W F₂ Mice

Anti-dsDNA	Anti-ssDNA		Total
	Positive	Negative	
Positive	21	0	21
Negative	20	31	51
Total	41	31	72

$\chi^2 = 20.01$; $P < 0.001$.

total amounts of gp70 (free and complexed) were similar: NZB, 49.0 ± 18.6 μg/ml; NZW, 41.1 ± 11.5 μg/ml; NZB × W F₁, 43.4 ± 18.0 μg/ml; and NZB × W F₂, 41.2 ± 19.4 μg/ml.

Relationship among Anti-dsDNA, Anti-ssDNA, and Anti-gp70-bound gp70 in NZB × W F₂ Mice. To determine whether a correlation existed among anti-dsDNA, anti-ssDNA, and antibody-bound gp70, the sera from 72 individual 8-mo-old NZB × W F₂ mice were analyzed for the three components. There was a notable association between anti-dsDNA and anti-ssDNA antibodies ($\chi^2 = 20.01$, $P < 0.001$; Table II). In fact, when individual values for anti-dsDNA and anti-ssDNA antibodies were compared, the correlation was highly significant ($r = 0.708$, $P < 0.001$).

In contrast, there was a very weak association between the presence of anti-dsDNA antibodies and anti-gp70-bound gp70 complexes ($\chi^2 = 4.47$, $P < 0.05$; Table III). No relationship was observed between anti-ssDNA antibodies and antibody-bound gp70 IC ($\chi^2 = 0.70$, $P > 0.1$; Table IV).

Lack of Association between Anti-DNA Antibodies and Fatal GN in NZB × W F₂ Mice. We next examined which serum components bore a relationship to the development of fatal GN in NZB × W F₂ females. For this purpose, the presence of anti-dsDNA, anti-ssDNA, and antibody-bound gp70 IC in sera of mice up to 8 mo of age was analyzed relative to the development of fatal GN up to 15 mo. Death from GN did not correlate with positivity for either serum dsDNA-binding activity or ssDNA-binding activity measured in 8-mo-old animals (Tables V and VI). Furthermore, mean values of serum dsDNA- and ssDNA-binding activities were essentially

TABLE III
Weak Association between Anti-dsDNA Antibodies and Anti-gp70 IC in
8-mo-old NZB × W F₂ Mice

Anti-gp70 IC	Anti-dsDNA		Total
	Positive	Negative	
Positive	12	14	26
Negative	9	37	46
Total	21	51	72

$\chi^2 = 4.47$; $P < 0.05$.

TABLE IV
Lack of Association between Anti-ssDNA Antibodies and Anti-gp70 IC in
8-mo-old NZB × W F₂ Mice

Anti-gp70 IC	Anti-ssDNA		Total
	Positive	Negative	
Positive	17	9	26
Negative	24	22	46
Total	41	31	72

$\chi^2 = 0.70$; $P > 0.1$.

TABLE V
Lack of Association between Anti-dsDNA Antibodies and Fatal GN in
NZB × W F₂ Mice

Age	Anti-dsDNA				χ^2	P
	Positive*		Negative*			
	Fatal GN	Surviving	Fatal GN	Surviving		
<i>mo</i>						
8	3	18	6	45	0.01	>0.9
10	7	14	10	41	0.89	>0.1
12	8	13	12	39	0.93	>0.1
15	12	9	17	34	2.59	>0.1

* Based on the results of testing 8-mo-old mice.

identical in the group of mice that died from fatal GN and those that survived up to 15 mo (Fig. 2).

The immunoglobulin classes of anti-ssDNA antibodies were also compared in mice with or without fatal GN by determining the ssDNA-binding activity of IgM and IgG peaks in gradient fractions resulting from ultracentrifugation. Again, there were no differences between these two groups of mice (IgM anti-ssDNA; GN group, 10.0 ± 7.6%; no-GN group, 8.8 ± 7.1%; IgG anti-ssDNA; GN group, 24.0 ± 20.9%; no-GN group, 24.1 ± 22.7%).

To seek the possible presence of DNA-anti-DNA IC in the sera of NZB × W F₂ mice with or without GN, serum dsDNA- and ssDNA-binding activities were evaluated before and after treatment of sera with DNase, which liberates anti-DNA

TABLE VI
Lack of Association between Anti-ssDNA Antibodies and Fatal GN in
NZB \times W F₂ Mice

Age	Anti-ssDNA				χ^2	P
	Positive*		Negative*			
	Fatal GN	Surviving	Fatal GN	Surviving		
mo						
8	5	36	4	27	0.03	>0.9
10	9	32	8	23	0.06	>0.9
12	10	31	10	21	0.39	>0.5
15	15	26	14	17	0.48	>0.5

* Based on the results of testing 8-mo-old mice.

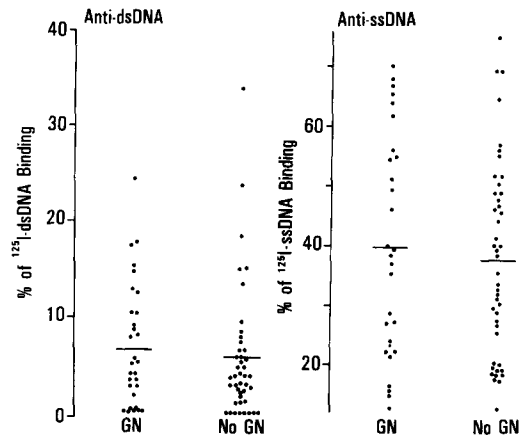


FIG. 2. Serum dsDNA- and ssDNA-binding activities of 8-mo-old NZB \times W F₂ female mice. GN: group of mice that died of fatal GN by 15 mo of age. No GN: group of mice surviving at 15 mo of age. The mean values are indicated by the horizontal line.

antibodies from DNA-anti-DNA IC, resulting in the increase of serum DNA-binding activity (19). However, none of the sera from either group of mice increased significantly in dsDNA- or ssDNA-binding activity after the DNase treatment (data not shown).

Association between Antibody-bound gp70 IC and Fatal GN in NZB \times W F₂ Mice. Unlike anti-DNA antibodies, antibody-complexed gp70 levels tested at 8 mo of age were significantly higher ($7.1 \pm 7.8 \mu\text{g/ml}$) in mice with early fatal GN than in mice that lived up to 15 mo ($2.2 \pm 2.7 \mu\text{g/ml}$; $P < 0.001$) (Fig. 3). Nevertheless, serum levels of total gp70 (free and complexed) were nearly identical in both groups of mice (Fig. 3).

Table VII shows a significant and consistent association between the presence of antibody-bound gp70 IC and the development of fatal GN throughout the test period (8–15 mo of age). In fact, 19 of 26 NZB \times W F₂ mice with anti-gp70 IC by 8 mo of age developed fatal GN by 15 mo, but only 10 of 46 mice without anti-gp70 IC at 8 mo of age died of renal disease. It should be noted that 8 of the 10 mice that developed GN became positive for anti-gp70 IC in a few months before death, i.e., before 12 mo

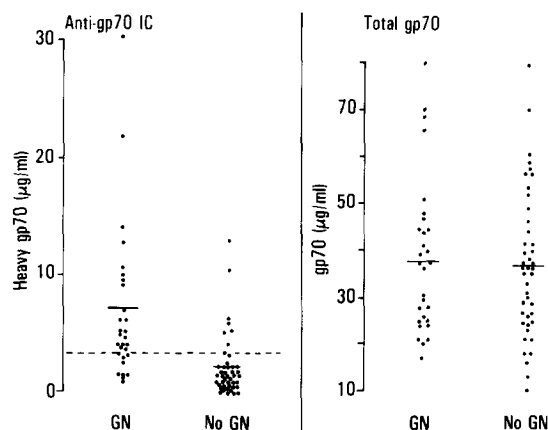


FIG. 3. Serum levels of total gp70 and the heavy form of antibody-complexed gp70 in 8-mo-old NZB \times W F_2 female mice. The mean values are indicated by the horizontal line. The dotted line indicates the mean value of 8-mo-old NZW females plus 3 SD.

TABLE VII
Association between Anti-gp70 IC and Fatal GN in NZB \times W F_2 Mice

Age	Anti-gp70 IC				χ^2	P
	Positive*		Negative*			
	Fatal GN	Surviving	Fatal GN	Surviving		
mo						
8	9	17	0	46	14.72	<0.001
10	14	12	3	43	17.55	<0.001
12	16	10	4	42	19.96	<0.001
15	19	7	10	36	15.66	<0.001

* Based on the results of testing 8-mo-old mice.

of age, whereas only 11 of 36 other mice had significant amounts of anti-gp70 IC by 12 mo.

Table VII also reveals that all nine mice that died by 8 mo of age had significant amounts of anti-gp70 IC in their sera, but sera from four of these nine mice were not abnormal in the capacity to bind either dsDNA or ssDNA (Tables V and VI). When we extended testing for antibody-bound gp70 and anti-DNA antibodies until the animals were 15 mo of age, 9 of 29 NZB \times W F_2 mice that died of GN did not develop significant amounts of antibodies to dsDNA or ssDNA, and only 2 did not have significant levels of anti-gp70 IC in their sera. It should be noted that production of anti-DNA antibodies was also negligible in these two mice, although they died of severe GN (grade 4+). The possibility that the presence of free or antibody-bound DNA might affect the detection of antibodies in these nine mice was excluded by the fact that no detectable amounts of free or antibody-bound DNA (<0.5 $\mu\text{g}/\text{ml}$) were found in sera throughout the entire course of their life.

The amounts of anti-DNA antibodies elutable from the kidneys were compared between the groups of mice developing fatal GN with high or low levels of anti-DNA antibodies in sera. Considerable amounts of anti-DNA antibodies were demonstrable

in renal eluates of mice with high serum levels of anti-DNA antibodies (4.12 $\mu\text{g/g}$ of tissue), which were approximately seven times greater than in renal eluates from mice with low serum levels of anti-DNA antibodies (0.67 $\mu\text{g/g}$ of tissue). The quantities of anti-DNA antibodies elutable from the kidneys were 3.2 and 1.4% of total IgG recovered from the kidneys, respectively, and concentrations were ~ 10 times greater than in the serum.

Discussion

We have analyzed the development of fatal GN in the F_2 generation of NZB \times W mice relative to the presence of circulating antibodies to serum retroviral gp70, dsDNA, and ssDNA. The quantity of anti-gp70 antibodies, determined by measuring amounts of the heavy form of antibody-bound gp70 (the complexed form), correlated significantly with the development of fatal GN. In contrast, no significant association was observed among the presence of antibodies to dsDNA or ssDNA and fatal GN. Apparently, circulating IC containing gp70 and anti-gp70 antibodies were indicators of early fatal GN.

The equivalent titer of anti-dsDNA and anti-ssDNA antibodies in 8-mo-old NZB \times W F_2 mice suggests that the appearance of both types of anti-DNA antibodies may be regulated by at least one common control mechanism. Of course, one cannot exclude the fact that the correlation observed between anti-dsDNA and anti-ssDNA antibodies may have resulted from the cross-reactivity of anti-ssDNA antibodies to ^{125}I -dsDNA, although potential contaminants from the single-stranded regions within the ^{125}I -dsDNA preparation were removed by the treatment with single-strand specific S1 nuclease (14, 16). Afterward, the reactivity of ^{125}I -dsDNA to mouse antibodies specific for ssDNA was $< 5\%$. Still, no F_2 mouse had anti-dsDNA antibodies alone, although about half of the anti-ssDNA-positive mice lacked significant anti-dsDNA activities. Apparently, the production of anti-ssDNA antibodies is under multifactorial control unlike that of anti-dsDNA antibodies. In fact, in immunologically normal strains of mice, anti-ssDNA antibodies, but not anti-dsDNA antibodies, are relatively easy to induce as a result of polyclonal activation of B cells by injection of bacterial lipopolysaccharides or infection with certain parasites (20, 22–24). The simultaneous appearance of polyclonal anti-hapten antibodies and anti-ssDNA antibodies in all the SLE-prone mice (25) suggests that the comparatively early appearance of anti-ssDNA antibodies may, at least partly, be related to polyclonal B cell activation.

The production of antibodies to serum retroviral gp70 did not correlate well with that of anti-DNA antibodies. There was no relationship at all between the presence of antibody-bound gp70 and anti-ssDNA antibodies in sera from NZB \times W F_2 mice, although anti-gp70 production was weakly associated with anti-dsDNA antibody formation. Of course, one should interpret these results cautiously, because several factors, e.g., mononuclear phagocytic activity, may influence serum levels of anti-gp70 antibodies complexed with gp70 without affecting the levels of free anti-DNA antibodies. However, anti-DNA and anti-gp70 antibody production were also unrelated in SLE-prone mice treated with prostaglandin E_1 (PGE) or infected with lactic dehydrogenase virus (LDV). PGE treatment greatly reduced serum levels of anti-gp70-complexed gp70 without affecting serum DNA-binding activities (26), and infection with LDV suppressed anti-DNA antibody production without changing serum levels of antibody-bound gp70 IC (S. Izui, L Hang, P.J. McConahey, and F. J.

Dixon, manuscript in preparation). Apparently, the antibody response to DNA antigens is regulated independently from that to serum retroviral gp70 antigen. If so, undoubtedly the gene(s) responsible for the production of anti-gp70 antibodies is segregated from that governing anti-DNA antibody formation in the F₂ generation of NZB × W mice. This possibility is further supported by recent findings in recombinant inbred strains between NZB and ALN mice, that individual traits of an autoimmune phenotype are inherited independently (12).

A significant observation in the present study was that the development of fatal GN in NZB × W F₂ mice correlates notably with the presence of circulating anti-gp70 IC. Generally, antibody-complexed gp70 IC in sera become detectable several months before the mice developed fatal renal disease and increased numerically as the disease worsened. Thus, one could predict the course of renal disease by determining the serum levels of anti-gp70 IC. Because both NZB and NZW strains express the same kind of retroviral gp70 in sera at equally high levels, and only NZB and NZB × W F₁ mice develop antibody-complexed gp70 (8, 26–29), the gene(s) responsible for the formation of circulating anti-gp70 IC should be the gene(s) that determines or regulates the production of anti-gp70 antibodies. Consequently, this could be one of the major genes for GN segregated in the F₂ generation of NZB × W mice.

In contrast, neither anti-dsDNA or anti-ssDNA antibodies are associated with the development of fatal GN in NZB × W F₂ mice. In fact, neither titers nor incidences of these anti-DNA antibodies were significantly different in mice developing early fatal GN and those surviving up to 15 mo of age. Although the qualitative aspects of the anti-DNA-response in murine SLE may be equally important in provoking renal disease, we found no differences in the isotypes of serum anti-DNA antibodies between the short-lived group of mice and the survivors. Of particular interest is the fact that four of nine mice that died of renal disease before 8 mo of age did not have either anti-dsDNA or anti-ssDNA antibody activities detectable in their sera, whereas all nine mice had substantial amounts of anti-gp70 antibodies in complexes. The presence of free DNA might account for the failure to demonstrate anti-DNA antibodies in these mice; however, no detectable amounts of DNA could be demonstrated in these mouse sera, even at the terminal stage of their disease. Furthermore, the fact that none of the sera had rapidly sedimenting (heavy) anti-DNA complexes argues against the presence of DNA-anti-DNA IC in these mice. Although there is some disagreement as to the effectiveness of DNase treatment in the liberation of anti-DNA antibodies from IC (19, 30, 31), none of these sera showed an increased binding to dsDNA or ssDNA after treatment with DNase.

It is noteworthy that significant amounts of anti-DNA antibodies were elutable from the glomerulonephritic kidneys of mice without abnormally high levels of anti-DNA antibodies in sera, although their quantities were much lower than those from mice with high anti-DNA antibodies. This supports the involvement of anti-DNA antibodies in murine lupus nephritis. It should be emphasized that the amounts of anti-DNA antibodies recoverable from kidneys and the amounts of anti-gp70 antibodies in the kidneys estimated from the eluted gp70 (antigen/antibody = 2) together account for less than half the total elutable Ig.

Our observations strongly suggest that circulating anti-gp70 IC are a potential source of renal injury in SLE-prone mice. However, anti-gp70 IC are not the only cause of GN, because 2 of 29 mice that died of severe GN (grade 4+) had neither anti-

DNA antibodies nor anti-gp70 IC in their sera to any significant degree. Certainly, unknown kinds of IC may be involved in the pathogenesis of GN in murine SLE. In fact, unidentified IC-like materials have been found in sera of SLE-prone mice by several kinds of assays (4, 32, 33). Nevertheless, the present study clearly shows that anti-gp70-complexed gp70 is a useful and predictive indicator of murine lupus nephritis.

Summary

NZB \times NZW (NZB \times W) F₁ hybrid mice spontaneously develop a disease most prominently characterized by immune complex glomerulonephritis (GN), which seems to be associated with both antibodies to DNA and to the serum retroviral envelope glycoprotein, gp70. To evaluate the contribution of each of these autoimmune responses to the pathogenesis of the GN, we studied NZB \times W F₂ mice in which the two responses appeared to segregate relatively independently. Use of this model permitted analysis of possible correlations between each response and the GN. The presence of circulating anti-gp70-complexed gp70 correlated significantly with the development of fatal GN and one could predict the course of renal disease by computing the rising serum levels of gp70 complexed with antibodies. In contrast, the presence of free antibodies to either double-stranded or single-stranded DNA was not significantly associated with the development of fatal GN. This association of anti-gp70 antibody production with these animals' early death from GN strongly suggests that the gene(s) governing production of antibodies to serum retroviral gp70 may be one of the major genes responsible for spontaneous renal disease segregated in NZB \times W F₂ generations.

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References

1. Howie, J. B., and B. J. Helyer. 1968. The immunology and pathology of NZB mice. *Adv. Immunol.* **9**:215.
2. Lambert, P. H., and F. J. Dixon. 1968. Pathogenesis of glomerulonephritis of NZB/W mice. *J. Exp. Med.* **127**:507.
3. Shirai, T., and R. C. Mellors. 1971. Natural thymocytotoxic autoantibody and reactive antigen in New Zealand Black and other mice. *Proc. Natl. Acad. Sci. U. S. A.* **68**:1412.
4. Andrews, B. S., R. A. Eisenberg, A. N. Theofilopoulos, S. Izui, C. B. Wilson, P. J. McConahey, E. D. Murphy, J. B. Roths, and F. J. Dixon. 1978. Spontaneous murine lupus-like syndrome: clinical and immunopathological manifestations in several strains. *J. Exp. Med.* **148**:1198.
5. Yoshiki, T., R. C. Mellors, M. Strand, and J. T. August. 1974. The viral envelope glycoprotein of murine leukemia virus and pathogenesis of immune complex glomerulonephritis of New Zealand mice. *J. Exp. Med.* **140**:1011.
6. Strand, M., and J. T. August. 1976. Oncornavirus envelope glycoprotein in serum of mice. *Virology.* **75**:130.
7. Lerner, R. A., C. B. Wilson, B. C. Del Villano, P. J. McConahey and F. J. Dixon. 1976.

- Endogenous oncornaviral gene expression in adult and fetal mice: quantitative, histologic and physiologic studies of the major viral glycoprotein, gp70. *J. Exp. Med.* **143**:151.
8. Izui, S., P. J. McConahey, A. N. Theofilopoulos, and F. J. Dixon. 1979. Association of circulating retroviral gp70-anti-gp70 immune complexes with murine systemic lupus erythematosus. *J. Exp. Med.* **149**:1099.
 9. Borel, Y., R. M. Lewis, and B. D. Stollar. 1973. Prevention of murine lupus nephritis by carrier dependent induction of immunologic tolerance to denatured DNA. *Science (Wash. D. C.)*. **182**:76.
 10. Parker, L. P., B. H. Hahn, and C. K. Osterland. 1974. Modification of NZB/NZW F₁ autoimmune disease by development of tolerance to DNA. *J. Immunol.* **113**:292
 11. Raveche, E. S., A. D. Steinberg, L. W. Klassen, and J. H. Tjio. 1978. Genetic studies in NZB mice. I. Spontaneous autoantibody production. *J. Exp. Med.* **147**:1487.
 12. Raveche, E. S., E. A. Novotny, C. T. Hansen, J. H. Tjio, and A. D. Steinberg. Genetic studies in NZB mice. V. Recombinant inbred lines demonstrate that separate genes control autoimmune phenotype. *J. Exp. Med.* **153**:1187.
 13. Dixon, F. J., A. N. Theofilopoulos, S. Izui, and P. J. McConahey. 1981. Murine SLE-etiology and pathogenesis. In *Progress in Immunology*. M. Fougereau and J. Dausset, editors. Academic Press, Inc., London. **4**:959.
 14. Izui, S., P. H. Lambert, and P. A. Miescher. 1976. Determination of anti-DNA antibodies by a modified ¹²⁵I-labeled DNA binding test. Elimination of non-specific binding of DNA to non-immunoglobulin basic proteins by using an anionic detergent. *Clin. Exp. Immunol.* **26**:425.
 15. Commerford, S. L. 1971. Iodination of nucleic acids *in vitro*. *Biochemistry*. **10**:1933.
 16. Shishido, K., and T. Ando. 1972. Estimation of the double-helical content in various single-stranded nucleic acids by treatment with a single-strand-specific nuclease. *Biochem. Biophys. Acta.* **287**:477.
 17. Izui, S., and R. A. Eisenberg. 1980. Circulating anti-DNA-rheumatoid factor complexes in MRL/1 mice. *Clin. Immunol. Immunopathol.* **15**:536.
 18. Izui, S., D. C. Morrison, B. Curry, and F. J. Dixon. 1980. Effect of lipid A-associated protein and lipid A on the expression of lipopolysaccharide activity. I. Immunological activity. *Immunology*. **40**:473.
 19. Harbeck, R. J., E. J. Bardana, P. F. Kohler, and R. I. Carr. 1973. DNA: anti-DNA complexes. Their detection in systemic lupus erythematosus sera. *J. Clin. Invest.* **52**:789.
 20. Izui, S., N. Zaldivar, I. Scher, and P. H. Lambert. 1977. Mechanism for induction of anti-DNA antibodies by bacterial lipopolysaccharides in mice. I. Anti-DNA induction by LPS without significant release of DNA in circulating blood. *J. Immunol.* **119**:2151.
 21. Pirani, C. L., and L. Salinas-Madriral. 1968. Evaluation of percutaneous renal biopsy. In *Pathology Annual*. S. C. Sommers, editor. New York Appleton-Century-Crofts, Inc. 249.
 22. Izui, S., T. Kobayakawa, M. J. Zryd, J. Louis, and P. H. Lambert. 1977. Mechanism for induction of anti-DNA antibodies by bacterial lipopolysaccharides in mice. II. Correlation between anti-DNA induction and polyclonal antibody formation by various polyclonal B lymphocyte activators. *J. Immunol.* **119**:2157.
 23. Izui, S., R. A. Eisenberg, and F. J. Dixon. 1981. Subclass-restricted IgG polyclonal antibody production in mice injected with lipid A-rich lipopolysaccharides. *J. Exp. Med.* **153**:324.
 24. Kobayakawa, T., J. Louis, S. Izui, and P. H. Lambert. 1979. Autoimmune response to DNA, red blood cells and thymocytes antigens in association with polyclonal antibody synthesis during experimental African trypanosomiasis. *J. Immunol.* **122**:296.
 25. Izui, S., P. J. McConahey, and F. J. Dixon. 1978. Increased spontaneous polyclonal activation of B lymphocytes in mice with spontaneous autoimmune disease. *J. Immunol.* **121**:2213.
 26. Izui, S., V. E. Kelley, P. J. McConahey, and F. J. Dixon. 1980. Selective suppression of

- retroviral gp70-anti-gp70 immune complex formation by prostaglandin E₁ in murine systemic lupus erythematosus. *J. Exp. Med.* **152**:1645.
27. Elder, J. H., F. C. Jensen, M. L. Bryant, and R. A. Lerner. 1977. Polymorphism of the major envelope glycoprotein (gp70) of murine C-type viruses: virioin associated and differential antigens encoded by a multi-gene family. *Nature (Lond.)* **267**:23.
 28. Izui, S., J. H. Elder, P. J. McConeahey, and F. J. Dixon. 1981. Identification of retroviral gp70 and anti-gp70 antibodies involved in circulating immune complexes in NZB × NZW mice. *J. Exp. Med.* **153**:1151.
 29. Nakai, Y., N. Maruyama, K. Ohta, H. Yoshida, S. Hirose, and T. Shirai. 1980. Genetic studies of autoimmunity in New Zealand mice: association of circulating retroviral gp70 immune complex with proteinuria. *Immunol. Lett.* **2**:53.
 30. Feltkamp, T. E. W. 1975. The significance of the determination of anti-DNA and DNA-anti-DNA complexes. *Scand. J. Rheum.* **11** Suppl.:33.
 31. Izui, S., P. H. Lambert, and P. A. Miescher. 1977. Failure to detect circulating DNA-anti-DNA complexes by four radioimmunological methods in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **30**:384.
 32. Casali, P., A. Bossus, N. A. Carpentier, and P. H. Lambert. 1977. Solid-phase enzyme immunoassay or radioimmunoassay for the detection of immune complexes based on their recognition by conglutinin: conglutinin-binding test. A comparative study with ¹²⁵I-labeled Clq binding and Raji cell RIA tests. *Clin. Exp. Immunol.* **29**:242.
 33. Pereira, A. B., A. N. Theofilopoulos, and F. J. Dixon. 1980. Detection and partial characterization of circulating immune complexes using solid phase anti-C3. *J. Immunol.* **125**:763.