GENETIC STUDIES OF AUTOIMMUNITY AND RETROVIRUS EXPRESSION IN CROSSES OF NEW ZEALAND BLACK MICE

II. The Viral Envelope Glycoprotein gp70*

By SYAMAL K. DATTA,‡§ PATRICIA J. McCONAHEY,‖ NOGA MANNY,§ ARGYRIOS N. THEOFILOPOULOS,‖ FRANK J. DIXON,‖ AND ROBERT S. SCHWARTZ§

(From the New England Medical Center Hospital, Tufts University School of Medicine, Boston, Massachusetts 02111, and The Scripps Clinic and Research Foundation, La Jolla, California 92037)

The expression of infectious xenotropic viruses by New Zealand Black (NZB)[1] mice is governed by two autosomal dominant genes, Nzv-1 and Nzv-2 (1, 2). The phenotypic expression of these two genes was revealed in the progeny of crosses between NZB and xenotropic virus-negative SWR mice: F_2 and first backcross (F_1 × SWR) animals were either *high-virus* (virus titers similar to those in the NZB parent), *low-virus*, or *virus-negative* (undetectable virus, as in the SWR parent). All F_1 and (F_1 × NZB) backcross progeny had the high-virus phenotype. In a preceding paper (3), we showed that the phenotypes determined by Nzv-1 and Nzv-2 were independeht of the development of autoantibodies and glomerulonephritis. In the present experiments, the viral envelope glycoprotein gp70 was measured in the serum of NZB × SWR crosses and the levels were analyzed in relation to the expression of infectious xenotropic virus, presence of autoantibodies, and development of glomerulonephritis and lymphomas.

Materials and Methods

Mice. NZB and SWR mice were obtained from The Jackson Laboratory, Bar Harbor, Maine and crosses were bred in our laboratory. Reciprocal crosses were made to breed F_1 and backcross progeny. Because the maternal direction of the cross did not affect the results, all data for a given cross were pooled. All crosses were 20–24 mo old when the samples were obtained, except for those mice that were dying of nephritis or lymphomas. In those cases, the youngest animal was 11 mo old.

[1] Abbreviations used in this paper: AMG, aggregated mouse gamma globulin; dDNA, denatured DNA; nDNA, native DNA; F_1, (SWR × NZB)F_1 and (NZB × SWR)F_1, F_1 × SWR, First backcross to SWR, (F_1 × NZB), first backcross to NZB, F_2, (SWR × NZB)F_2; gp, glycoprotein; NZB, New Zealand Black; PAS, periodic acid Schiff.

Retrovirus Assay. A quantitative assay for infectious xenotropic virus was done as described in the preceding paper (3).

Autoantibodies. Antiglobulin (direct Coombs') test and antibody to native (nDNA) and denatured (dDNA) were determined as described in the preceding paper (3).

Immunofluorescence Analysis of Kidney Sections. Immunoglobulin deposits and *retroviral antigen (gp70) deposits* were detected by specific fluorescein conjugated antisera as described in the previous paper (3).

Histopathology. Glomerular lesions in hematoxylin and eosin and periodic acid Schiff (PAS) stained kidney sections were graded according to the method used in (3). Lymphomas were diagnosed and classified by the criteria of Dunn and Deringer (4).

Radioimmunoassay for gp70 in Serum. Sera were analyzed for gp70 by a radioimmunoassay employing iodinated Rauscher gp70 and goat anti-feline leukemia virus. The technique is a modification of the procedure described by Oroszlan et al. (5), Parks and Scolnick (6), and Strand and August (7). All tests were done on coded samples of sera.

Raji Cell Assay for Circulating Immune Complexes. Raji cells bind complement fixing immune complexes via C3b, C3d, and C1q receptors (8). Immune complexes were determined in mouse serum by using a modification of the Raji cell radioimmune assay described for the detection of immune complexes in human sera (8). The modifications include: (*a*) use of aggregated mouse gamma globulin (AMG) in the standard quantitative curve; (*b*) use of an optimal amount of purified IgG fraction of rabbit anti-mouse IgG which was labeled with ^{125}I (9) as a final reagent for measuring the amount of mouse IgG bound, presumably in complex form, on the Raji cells' complement receptors; (*c*) use of BALB/c fresh serum as the complement source of the assay for constructing the standard curve. Results were expressed in microgram equivalents of AMG/ml murine serum. An upper normal limit of 10 μg AMG eq/ml for immune complexes in serum was established and defined by a mean + 2 SD for a group of 37 control BALB/c taken at 3, 6, and 9 mo.

Results

Serum gp70 in NZB and SWR Mice and Their Crosses (Figs. 1 and 2). The mean levels of serum gp70 in 1 yr old NZB and SWR mice were 46.7 ± 15.5 μg/ml and 5.6 ± 2.3 μg/ml, respectively. Results in the progeny of NZB × SWR crosses are shown in Figs. 1 and 2. Fig. 1 indicates that genes contributed to the progeny by the SWR parent had a pronounced inhibitory effect on the expression of serum gp70. The more closely related the progeny was to SWR (e.g., F_1 × SWR, F_2), the lower the level of serum gp70 tended to be. When a value of 12.5 μg/ml (3 SD above the mean for SWR mice) was used to divide results into "SWR-like" (<12.5 μg/ml) and "NZB-like" (>12.5 μg/ml) levels of serum gp70, there was a significant difference in the incidence of NZB-like gp70 levels between F_1 and (F_1 × SWR) mice ($P < 0.05$) and between F_1 and F_2 mice ($P < 0.05$). The F_1 mice were notable because levels of gp70 were NZB-like in 58% and SWR-like in 42%. This indicates that the expression of serum gp70 probably involves multiple genes, some of which are recessive. The maternal direction of the cross and the coat color or sex of the progeny mice did not influence the serum gp70 levels.

Levels of serum gp70 were independent of the titers of xenotropic virus. All of the F_1 and (F_1 × NZB) mice had, like the NZB parent, high titers of virus (1, 2), yet 42% of the F_1 and 60% of the (F_1 × NZB) animals[2] had <12.5 μg/ml of serum gp70 (Fig. 1). Fig. 2 shows the titers of virus and levels of serum gp70 in all NZB × SWR crosses tested, including the (F_1 × SWR) and F_2 progeny. There

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[2] Serum gp70 could not be determined in some of the (F_1 × NZB) crosses due to insufficient amounts of sera.
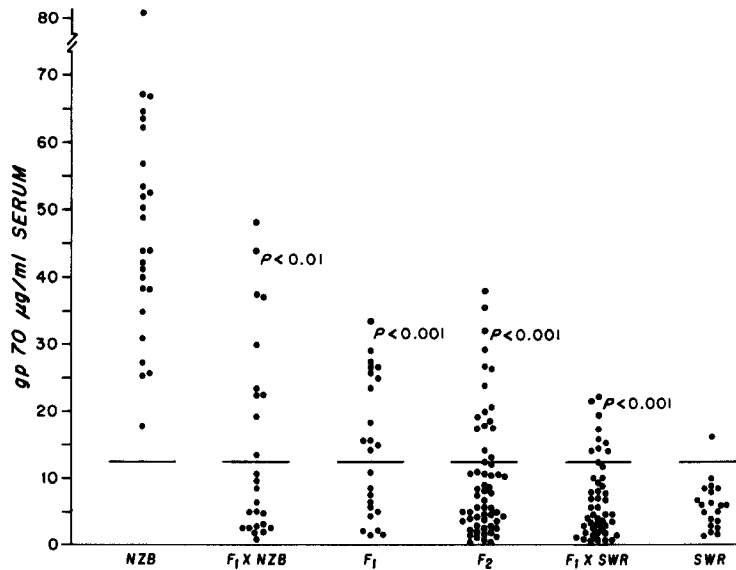
FIG. 1. Serum gp70 levels in NZB and SWR mice and their crosses. Each solid circle represents the level in an individual mouse. Horizontal bars represent 3 SD above the mean for SWR mice. For each cross, the mean of all levels >12.5 μg/ml (solid circles above horizontal bars) was calculated and compared (Student's t test) with those of NZB mice; P values are shown for each of these comparisons.
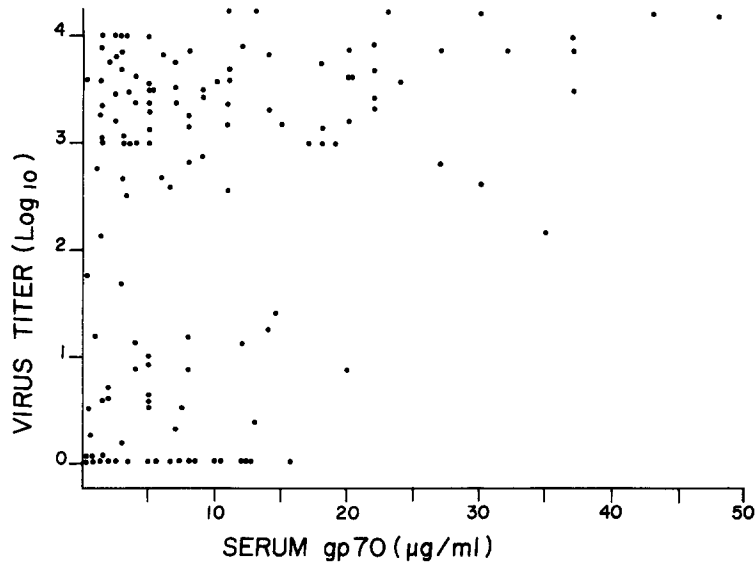

FIG. 2. Serum gp70 in F_1, (F_1 × NZB), F_2 and (F_1 × SWR) mice charted as a function of xenotropic virus titer. Note that 17 of 18 (94%) of the virus-negative mice had serum gp70 levels <12.5 μg/ml; such levels were also found in about $^2/_3$ of mice with high titers of virus (>2.0 $\log_{10}$ focus forming units/10^7 spleen cells). Each closed circle is one animal.

TABLE I

Immunopathologic Lesions Found in the NZB × SWR Crosses in Relation to Levels of Serum gp70

	N	Serum gp70	
		<12.5 µg/ml	>12.5 µg/ml
Anti-DNA >10% binding	53	66.0%	34.0%
Anti-DNA <10% binding	102	71.6%	28.4%
Positive antiglobulin test	15	80.0%	20.0%
Negative antiglobulin test	133	66.2%	33.8%
Immune complexes >10 µg/ml	22	86.4%	13.6%
Immune complexes <10 µg/ml	48	64.6%	35.4%
Nephritis present (PAS stain)	48	72.9%	27.1%
Nephritis absent (PAS stain)	85	62.4%	37.6%
Glomerular capillary gp70(+)	21	47.6%	52.4%
Glomerular capillary gp70(−)	84	73.8%	26.2%
Glomerular capillary Ig(+)	59	64.4%	35.6%
Glomerular capillary Ig(−)	40	77.5%	22.5%
Lymphoma present	37	43.2%	56.8%
Lymphoma absent	63	82.5%	17.5%

The value of 12.5 µg/ml is 3 SD above the mean for SWR mice. Mice were 20–24 mo old when tested, except those with overt disease.

was no correlation between titers of xenotropic virus and levels of serum gp70 in virus-positive mice ($r = 0.34$, $P > 0.10$); 17 out of 18 (94%) of the virus-negative mice had levels of gp70 <12.5 µg/ml. Levels above that value were more frequent in high-virus mice than in low-virus mice ($P < 0.01$), but 65.5% of all high-virus crosses had <12.5 µg/ml gp70.

Serum gp70 and Immunopathology in the Crosses of NZB and SWR mice. There were no correlations between levels of serum gp70 and the presence or absence of antibodies to either nDNA or dDNA (Table I and Fig. 3). Mice with NZB-like levels of serum gp70 (>12.5 µg/ml) and antibodies to DNA (>10% binding) had a mean level of gp70 of 25.7 µg/ml ± 8.6, but this value was no different from that of mice with NZB-like levels of gp70 that lacked antibodies to DNA (serum gp70 of 22.2 ± 8.2 µg/ml); moreover, 35 of 53 mice (66%) with serum gp70 levels <12.5 µg/ml had antibodies to DNA. Table I and Fig. 3 also show that elevated levels of gp70 did not correlate with the presence of antibodies to autologous erythrocytes; 12 of 15 mice (80%) with a positive antiglobulin test had <12.5 µg/ml serum gp70.

The presence of histologically identifiable nephritis was independent of levels of serum gp70 ($P < 0.1$) (Table I and Fig. 4); 35 of 48 mice (72.9%) with glomerular lesions of 2+ to 4+ severity had serum gp70 levels <12.5 µg/ml. Although there was a tendency for those mice with deposits of retroviral antigens in glomerular capillaries to have NZB-like levels of serum gp70, in about one-fourth of these cases viral antigens were not detected in the glomeruli (Table I, lines 9 and 10). There was no correlation between serum gp70 and deposits of immunoglobulins in glomerular capillaries (Table I, lines 11 and 12).

Levels of immune complexes in serum, as determined by the Raji cell assay, did not correlate with levels of serum gp70 ($r = .036$, $P < 0.5$) (Fig. 5). By
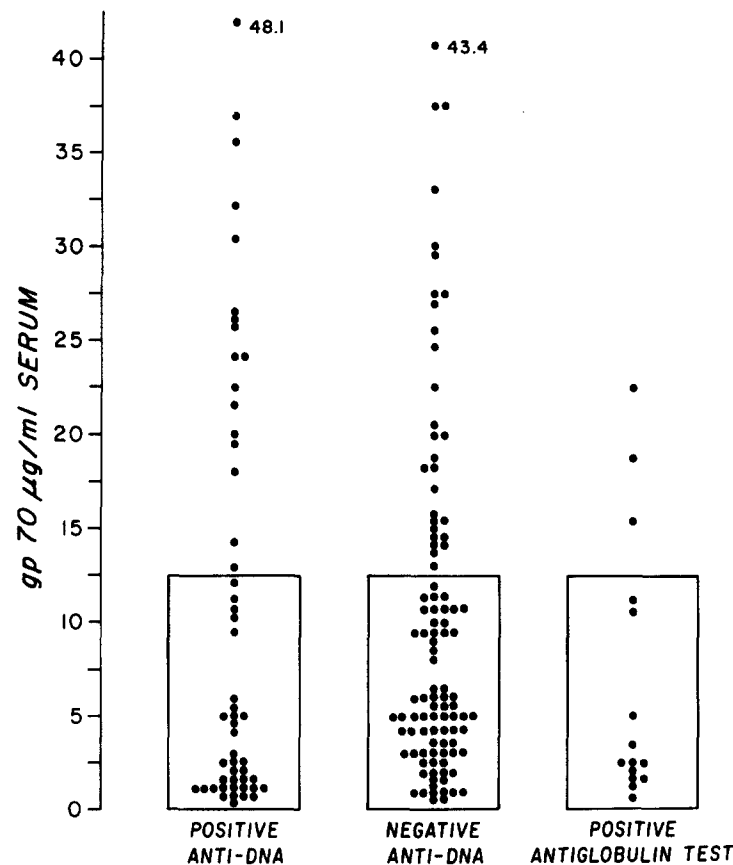
FIG. 3. Serum gp70 levels of the NZB × SWR crosses in relation to presence or absence of anti-DNA antibody (positive = >10% binding to either nDNA and/or dDNA) and positive antiglobulin (direct Coombs'). The value of 10% binding is 2 SD above the mean for SWR mice. Open box separates values for serum gp70 above and below 12.5 μg/ml. Each closed circle is one animal.

contrast, levels of immune complexes did correlate with the presence or absence of nephritis and anti-DNA antibody (Fig. 6), as has been shown in previous work (10). There was a highly significant correlation between increased levels of gp70 (>12.5 μg/ml) and the presence of lymphoma ($P < 0.001$) (Table I, Fig. 4).

Discussion

gp70 is the major glycoprotein component of the envelope of type C RNA viruses. It is found in tissues and serum of virtually all mice (7, 11, 12). Structural studies of serum gp70 indicate that it is the same in all strains and resembles the gp70 of the NZB xenotropic virus (12). Its presence is independent of the expression of complete retrovirus particles (7, 12), and our results in virus-negative SWR mice, which had low, but consistently detectable amounts of serum gp70, confirm this. In NZB serum, which contains very high levels of gp70 (Fig. 1), there is a 1,000-fold greater concentration of the glycoprotein than
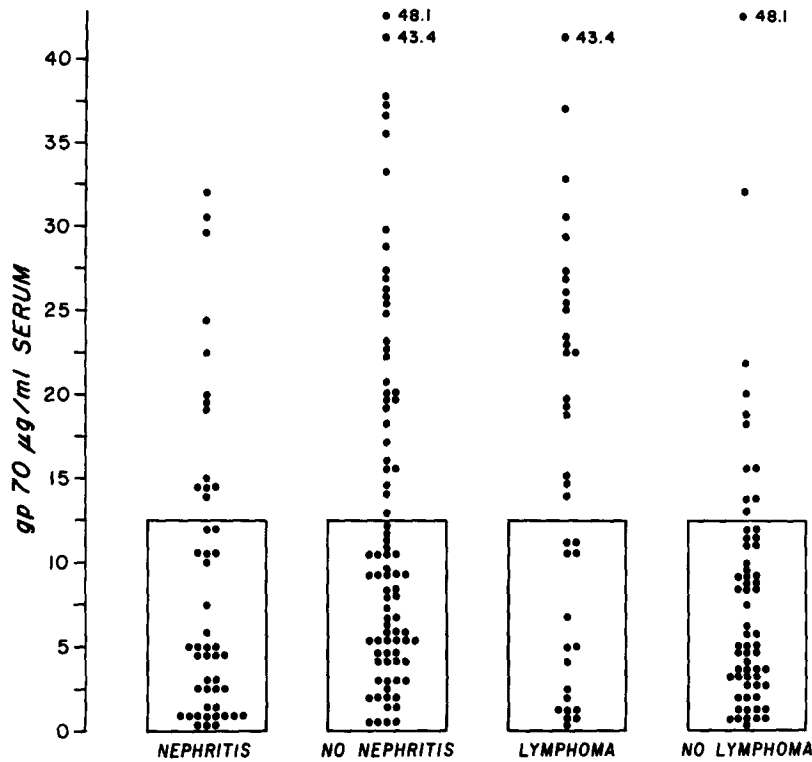
FIG. 4. Serum gp70 levels of the NZB × SWR crosses in relation to histologically identifiable nephritis and lymphomas. Each closed circle is one animal. Open box encloses all values <12.5 µg/ml.
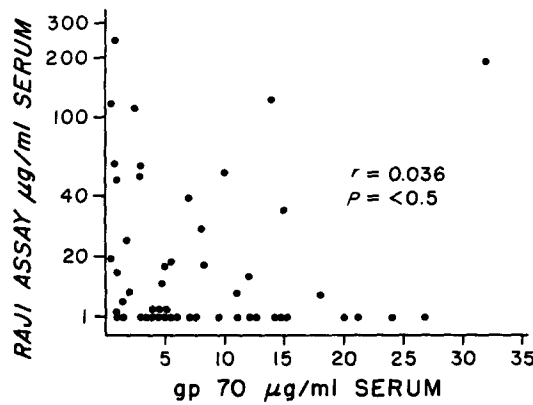

FIG. 5. Lack of correlation between serum gp70 levels and circulating immune complex levels (Raji assay) in the NZB × SWR crosses. Each closed circle is one animal.

of the viral core protein p30; if all the gp70 and p30 were present as virions, the ratio would be 1:6 (11, 13). Metabolic studies demonstrated that the levels of serum gp70 reflect its rate of production; therefore, a characteristic feature of NZB mice is a very high rate of synthesis of gp70 (11), as well as high-grade expression of xenotropic viruses (1, 14).
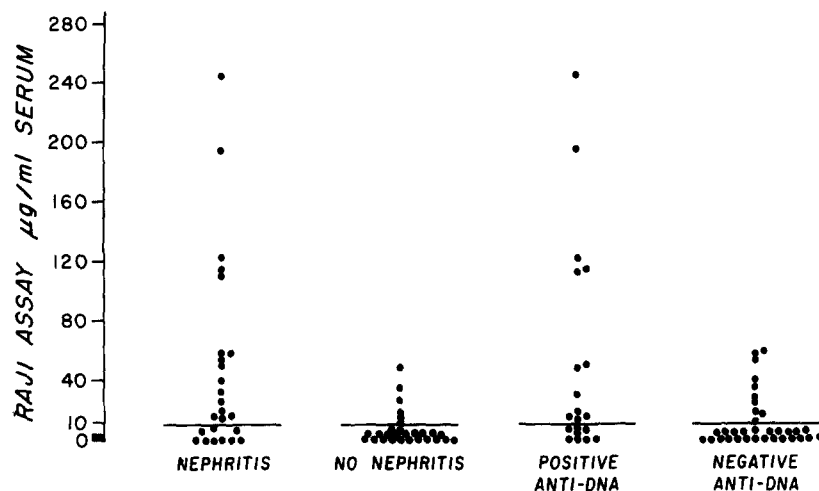
FIG. 6. Correlation between circulating immune complexes (Raji cell assay) with presence of nephritis and anti-DNA antibody in NZB × SWR crosses. Each closed circule is one animal. Horizontal bar denotes upper limit of normal for the Raji cell assay, 10 μg/ml.

Our results indicate that these two features of NZB mice are specified by different mechanisms. NZB mice are homozygous for the dominant alleles of Nzv-1 and Nzv-2 which induce xenotropic virus expression, whereas virus-negative SWR mice have simultaneous homozygosity for the recessive alleles of these loci (1, 2). Therefore, all virus-positive progeny of NZB × SWR crosses, whether high-virus or low-virus, have the dominant allele of the relevant virus-inducing locus from the NZB parent. The progeny of NZB × SWR crosses with high virus titers include all F_1 and (F_1 × NZB) mice, 50% of the (F_1 × SWR) and 75% of the F_2 animals. Titers of xenotropic virus in these mice are the same as those in the NZB parent (1-3). These high-virus crosses must be either homozygous or heterozygous for the dominant allele of Nzv-1 that specifies production of high titers of xenotropic virus (1, 2). Almost ²/₃ of all mice with this allele had low (SWR-like) amounts (<12.5 μg/ml) of the serum glycoprotein (Figs. 1 and 2). By contrast, all of the virologically similar NZB parental mice, which by definition also have the dominant allele of Nzv-1, had high amounts of serum gp70 (Fig. 1). The dominant allele of Nzv-2, which specifies production of low titers of xenotropic virus (1, 2), also segregates independently of the genetic system responsible for high levels of serum gp70 in NZB mice. Progeny with the low-virus phenotype are either heterozygous or homozygous for the dominant allele of Nzv-2 and lack the dominant allele of Nzv-1 (1, 2); only 22% of these animals had NZB-like levels of serum gp70 (>12.5 μg/ml) (Fig. 2). This is further supported by analysis of results in the (F_1 × SWR) backcross: recombination between the high-virus and low gp70 (<12.5 μg/ml) phenotypes was 68%, and 70% of the progeny with the NZB-derived Nzv-2 allele (low-virus phenotype) had SWR-like levels of serum gp70.

Our results suggest that levels of serum gp70 are controlled by a complex system of genes, some of which are probably recessive (in contrast to Nzv-1 and Nzv-2, which are dominant). The main evidence for this is in the results of the

F_1: about 60% of these mice had levels of serum gp70 >12.5 $\mu g/ml$; the remainder had lower amounts (Fig. 1). Results of peptide map analyses of serum and tissue gp70 (12) and studies of G_{IX}, a type-specific determinant on gp70 that is expressed on thymocytes (15), also indicate that gp70 is specified by multiple unlinked genes. Our data do not exclude the possibility that environmental factors influence the expression of NZB-like gp70 levels in the crosses. This will be determined by intercrossing the progeny with high or low levels of gp70 with each other.

gp70 has been detected in the nephritic lesions of NZB and (NZB × NZW)F_1 mice (13); however, whether high serum levels of this glycoprotein are required for the development of autoimmune disease in NZB mice and their crosses has not been previously tested. In the preceding paper we showed that typical immune deposit nephritis could develop in the virus-negative progeny of NZB × SWR mice and that in many progeny, regardless of their virological phenotype, the nephritic lesions did not contain detectable viral antigens, including gp70, as determined by an immunofluorescent technique (3). The present results extend these findings by demonstrating that mice with serum gp70 levels the same as normal SWR mice (<12.5 $\mu g/ml$) could develop nephritis. This was observed in 35 of 48 progeny of NZB × SWR crosses. Moreover, 32 of 85 progeny with serum gp70 levels as high as those in the NZB parent failed to develop nephritis, and in 22 of 84 mice with high serum gp70 levels, there was no identifiable gp70 in their glomeruli. Of the 17 mice with histologic evidence of nephritis and high levels of serum gp70, glomerular capillary deposits of gp70 could not be identified in 12. Indeed, the only abnormalities that correlated with nephritis were the presence of circulating immune complexes and antibodies to DNA, which in turn did not correlate with serum gp70 levels. Therefore, the deposition of gp70 in nephritic kidneys of NZB × NZW mice, when it does occur (13), may be a secondary event.

Although there was a correlation between lymphomas and serum gp70, we do not know if this is a cause and effect relationship. The incidence of lymphoma was significantly higher in F_1 and (F_1 × NZB) mice as compared to F_2 and (F_1 × SWR) mice, irrespective of their xenotropic virus titers (3). Other studies have suggested that the level of serum gp70 does not always correlate with the development of neoplasms (11).

Although we have presented evidence that increased levels of serum gp70, high-grade expression of xenotropic virus, and development of autoimmune disease can be dissociated in crosses of NZB × SWR mice, the concordance of these striking abnormalities in NZB mice may not be fortuitous. Conceivably, these findings are "markers" of a genetic abnormality that involves at least three independently segregating, complex genetic systems. In this regard, we note that the expression of gp70, like that of xenotropic viruses, is a feature of differentiating cells (11, 12, 15). A defect of gene regulation might explain why NZB mice have the abnormalities relevant to the glycoprotein and the virus, and why their B cells continue to synthesize autoantibodies when transferred to normal animals (16).

Summary

The retroviral envelope glycoprotein, gp70 was measured in the serum of New

Zealand Black (NZB) and SWR mice and the progeny of their crosses. The serum gp70 values segregated to "NZB-like" and "SWR-like" levels in these mice. A complex mechanism determined the inheritance of NZB-like serum gp70 levels. We found that the factors determining the expression of this retroviral protein were independent of the genes (*Nzv-1* and *Nzv-2*) that determined the expression of infectious xenotropic virus. Autoimmune disease, including immune deposit nephritis could be dissociated from the degree of expression of serum gp70. By contrast, presence of circulating immune complexes and anti-DNA antibody did correlate with the development of nephritis in these crosses. A significant correlation was found between high grade expression of serum gp70 and the presence of lymphomas in these mice.

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