FURTHER IMPLICATION OF MURINE LEUKEMIA-LIKE VIRUS IN THE DISORDERS OF NZB MICE

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Evidence implicating murine leukemia-like virus in the etiology of the hemolytic, renal, and neoplastic disorders of NZB mice has been presented elsewhere (1–5). Further substantiation of the relation of virus infection to disease is now afforded in the study of antigens associated with murine leukemia virus (MuLV). These antigens include MuLV group-specific or nucleocapsid antigen common to the several strains of murine leukemia and sarcoma viruses and detectable by immunoprecipitation (6); MuLV group antigens with group and type specificities detectable by complement fixation (7–9); G (Gross, G+) antigen, type-specific cell-surface antigen associated with Gross leukemia virus (10, 11); and G (Gross) soluble antigen, a recently discovered type-specific soluble antigen (12–14).

MuLV nucleocapsid antigen has already been identified in extracts of spleen, kidney, and milk of NZB mice by immunoprecipitation (15). NZB mice have also been shown to be a G+ mouse strain by cytotoxic test (15). In preliminary work, MuLV group antigens and host immunoglobulins have been demonstrated by immunofluorescence in the glomerular lesions of NZB mice with renal disease (5).

Methods and Materials

Mice.—The colony of NZB mice was derived from breeding stock provided by Dr. Marianne Bielschowsky in 1964 and has been maintained since then by brother-sister matings. The general methods of care and study of these mice have been described elsewhere (16), including monthly antiglobulin (Coombs) tests, proteinuria tests, and autopsy and histopathology procedures. C57BL/6, AKR, and C58 mice were obtained from our colonies.

Leukemias and Other Neoplasms.—The lymphomas and sarcomas arose spontaneously in NZB mice and in some instances were carried as cell-passage lines within this strain, as indicated elsewhere (1, 4, 17, 18). The sarcomas were osteogenic sarcomas (4, 19) and one example of soft tissue sarcoma. The G+ test cell for the determination of G antibody was the trans-

planted C57BL/6 leukemia E♂G2 (10) originally induced by Passage A Gross virus (22). EL4 cells were the long-term transplanted ascites leukemia converted from a chemically induced leukemia in C57BL mice (23).

Vaccination of NZB Mice.—A vaccine was prepared aseptically, by the method of Friend (20) and used as described elsewhere (4, 5). Formaldehyde was added to cell-free filtrates of 10–20% extracts of aged NZB mouse spleens to obtain a final concentration of 1:500. The mixture was kept at 4°C for 14–21 days, with daily shaking of contents, and then passed through 0.45 μ Millipore filters. Two intraperitoneal injections of vaccine in 0.2 ml amounts were given to baby NZB mice, generally at 3 and 5 wk of age.

MuLV Group Antigens.—The complement fixation (CF) method for detection of murine leukemia virus group antigens is described elsewhere (7, 8). The rat antiserums used in the CF test were obtained from inbred Fischer rats bearing isologous transplanted lymphomas or sarcomas induced respectively by murine leukemia viruses or the Moloney strain of murine sarcoma virus (21). These serums were carefully selected not only for antibody specificity with respect to MuLV group antigens but also for absence of nonspecific reactions with extracts of normal tissues (spleen, thymus, kidney) of various mouse strains and other control tissues.

TABLE I

MuLV Group Antigens in Tissue Extracts of Untreated NZB Mice at Various Ages

NZB Mice		Tissue Extract		
Age	No.	Thymus	Spleen	Kidney
		No. antigen-positive/No. tested		
5 days	5	3/3	1/1	4/4
21–24 days	5	4/5	5/5	5/5
5 months	5	4/5	5/5	5/5
9–18 months	5	2/5	5/5	5/5
Total.....	20	13/18	16/16	19/19

10% extracts of tissues were screened at dilutions of 1:2 and 1:4. Positive 3+ to 4+ reactions at a dilution of 1:2 or 1:4, in the absence of anticomplementary effects and in the absence of positive reactions in negative control extracts, were scored as positive (Tables I and II). Concentrates of tissue extracts were prepared by a modification (47) of the Moloney procedure.

MuLV Group IF Antigens.—The indirect immunofluorescence (IF) method for the location of murine leukemia virus group IF antigens in frozen sections of NZB mouse tissues utilized precipitating antiserum prepared and kindly provided by Geering, Old, and Boyse (6). The pooled antiserum with precipitating activity for MuLV group antigens was obtained from (W/Fu × BN)F_1 rats bearing subcutaneous transplants of the Gross virus induced leukemia W/Fu (C58NT)D. This antiserum did not contain HI polyoma antibody (6) and was shown not to react in immunodiffusion tests with mouse serum in various dilutions or with mouse albumin, α-, β-, and γ-globulins over a wide range of concentrations. Fluorescein-labeled rabbit antibody against rat serum globulins (Microbiological Associates, Bethesda, Md.) was carefully absorbed with lyophilized mouse serum until no longer reactive with mouse serum proteins although continuing to show a single line of precipitation of rat γ-globulins in immunodiffusion reactions with rat serum. Negative control reactions in the indirect immunofluorescence method included the use of normal (nonimmune) W/Fu rat serum and immune (W/Fu × BN)F_1 rat serum completely absorbed with, and no longer reactive with, MuLV group antigens released from Gross virus by ether treatment of infective

W/Fu rat plasma; additional negative controls included frozen sections of kidneys of normal Swiss mice. Direct staining reactions with fluorescent anti-rat globulin were negative. Fluorescein-labeled rabbit antibody against mouse immunoglobulins was used to locate mouse immunoglobulins in sections of NZB mouse kidneys and spleens, as described elsewhere (16).

G (Gross) Typing Serum.—This serum was prepared by immunization of C57BL/6 female mice with the G+ AKR♀ spontaneous leukemia K36. This serum did not contain HI polyoma antibody. A complete description of the G-typing system was given elsewhere (10).

Determination of G Antibody.—The sera were tested for G antibody by the indirect immunofluorescence test, as modified by Möller for use with suspensions of viable cells. Suspensions of viable E♂G2 leukemia cells were prepared from the spleens of C57BL/6 mice bearing transplants of E♂G2. After two or three washings in Earle's balanced salt solution (EBSS), 1–2 × 10^6 cells were resuspended in 0.05 ml of undiluted serum and were incubated

TABLE II

MuLV Group Antigens in Malignant Lymphomas and Sarcomas of NZB Mice

Lymphoma or tumor		Lymphoma or tumor		Spleen
Classification	No. of independent lines	Extract	Concentrate	Extract
		No. antigen-positive/No. tested		
Lymphoma				
Primary	2	2/2		2/2
Transplantable	4	5/5	1/1	3/3
Sarcoma				
Transplantable	5	1/1	6/6	9/9
Total	11	8/8	7/7	14/14

at room temperature for 20 min. The cells were then washed twice in EBSS and resuspended in 0.05 ml of 1:80 diluted fluorescinated goat globulin *anti*-mouse globulin (Lot No. 12-65, Hyland Lab., Los Angeles, Calif.); this reagent gave a strong single IgG line on immunoelectrophoresis with mouse serum. After incubation for 20 min at room temperature, the cells were washed twice, and approximately 100 cells were examined for fluorescence. Reactions were graded according to the proportion of cells showing characteristic membrane fluorescence: ≦20%: negative; 21–50%: weak +; 51–100%: strong +. A complete description of this method was given elsewhere (24).

Demonstration of G Soluble Antigen Adsorbed on Viable Indicator Cells.—Ascites leukemia EL4 cells served as the indicator cells; EL4 does not carry G or FMR cellular antigens (10). After two or three washings in EBSS, 1–2 × 10^6 EL4 cells were suspended in 0.1 ml of the undiluted plasma, freshly drawn, to be tested for G soluble antigen and incubated for 30 min at room temperature and 30 min at 4°C. After two more washings in 2.0 ml EBSS, the cells were tested for adsorbed G soluble antigen by indirect immunofluorescence with G-typing serum as described above. The plasma of AKR or C58 mice served as positive controls. A detailed description of this method was given elsewhere (12).

RESULTS

MuLV Group Antigens.—The complement-fixation (CF) test for the presence of murine leukemia virus group antigens was performed on 53 tissue extracts (Table I). Spleen and kidneys of untreated NZB mice were positive in all tests

at all ages examined (5 days to 18 months). Thymus was positive in each test at 5 days of age and in the majority of tests performed thereafter. 15 lymphoma and sarcoma extracts and concentrates (Table II) gave positive results

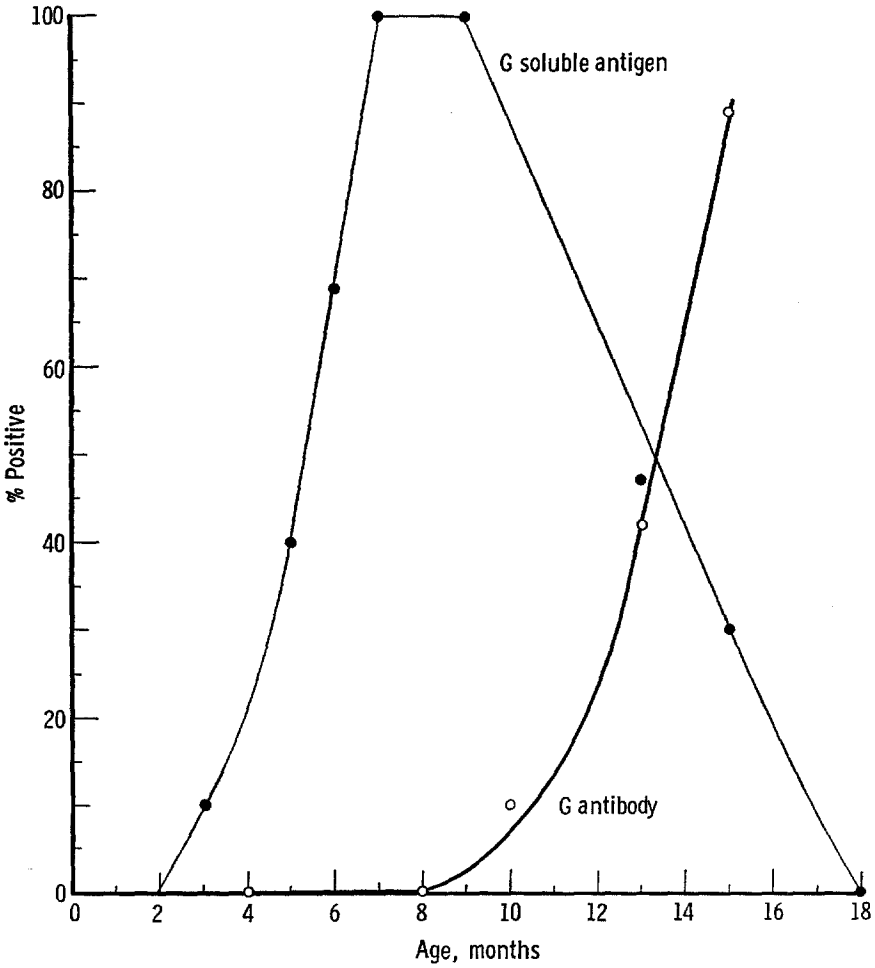

FIG. 1. Tests (% positive) for G soluble antigen (●) and G antibody (○) in plasma and serum of NZB mice at various ages.

in each instance, as did spleen extracts of lymphomatous and tumor-bearing NZB mice. The malignant lymphomas included two primary and four transplantable lymphomas of NZB mice. Among the transplantable sarcomas were four independent cell-passage lines of osteogenic sarcoma and one soft tissue sarcoma, all indigenous to the mouse strain NZB.

The serums used in the CF test (see Methods and Materials) were carefully

selected not only for antibody specificity with respect to MuLV group antigens but also for absence of nonspecific reactions with extracts of normal mouse tissues and other control tissues and with a complete battery of the known conventional murine viral antigens.

G Soluble Antigen.—107 untreated NZB mice at various ages were tested for G soluble antigen in plasma (Fig. 1). Positive reactions occurred in 10% of plasmas at 3 months. Thereafter, G soluble antigen production increased rapidly in line with age, with 50% positive reactions at 5.3 months (Table III) and 100% positive reactions at 7 to 9 months. Beyond the 9th month, G soluble antigen underwent elimination from plasma, with positive reactions reduced to

TABLE III

50% Response Time ($T_{50\%}$) for G Soluble Antigen Production and Elimination and G Antibody Production in Untreated NZB Mice

Response	$T_{50\%}$*	±2 SE	SD	N'
	months	*months*	*months*	
G soluble antigen production	5.3	0.8	1.4	26
G soluble antigen elimination	13.3	1.2	2.9	42
G natural antibody	13.3	0.8	1.4	21

* Estimated by logarithmic-probit graphic procedure and based upon N' determinations in the 6.7–93.3% range of response. Standard error (SE) equals SD $\div \sqrt{\frac{N'}{2}}$.

50% at 13.3 months (Table III) and to 0% at 18 months. The results obtained on individual plasmas are shown in Fig. 2. The reactions were graded by the proportion of cells showing membrane immunofluorescence (see Methods and Materials) as negative (≦20%), weak positive (21–50%), and strong positive (51–100%), corresponding in turn to the time sequence of reactions in G soluble antigen production and to the reverse order in G soluble antigen elimination.

The serums of 39 untreated NZB mice at 3, 7, 9, and 12 months of age were tested for antibody to cell membrane of EL4 indicator cells (see Methods and Materials). All reactions were negative.

G Natural Antibody.—51 untreated NZB mice at various ages were tested for G natural antibody in serum (Fig. 1). Reactions were negative at 4 and 8 months. Positive reactions occurred in 10% of serums at 10 months and increased thereafter in line with age. The 50% positive response time (Table III) was 13.3 months. The results obtained on individual serums are shown in Fig. 3. As discussed under Methods and Materials, reactions were graded by the proportion of cells showing membrane fluorescence, as follows: negative (≦20%), weak positive (21–50%), and strong positive (51–100%).

The curves for G antibody production and G soluble antigen elimination bore

a reciprocal relation to each other (Fig. 1) with crossover at 50% response occurring at 13.3 months.

MuLV Group IF Antigens.—Frozen sections of kidneys of 8 female NZB mice were studied by the indirect immunofluorescence method for the location of murine leukemia virus group IF antigens. Results on two mice were negative at 2 months while trace amounts of leukemia viral antigens were detected

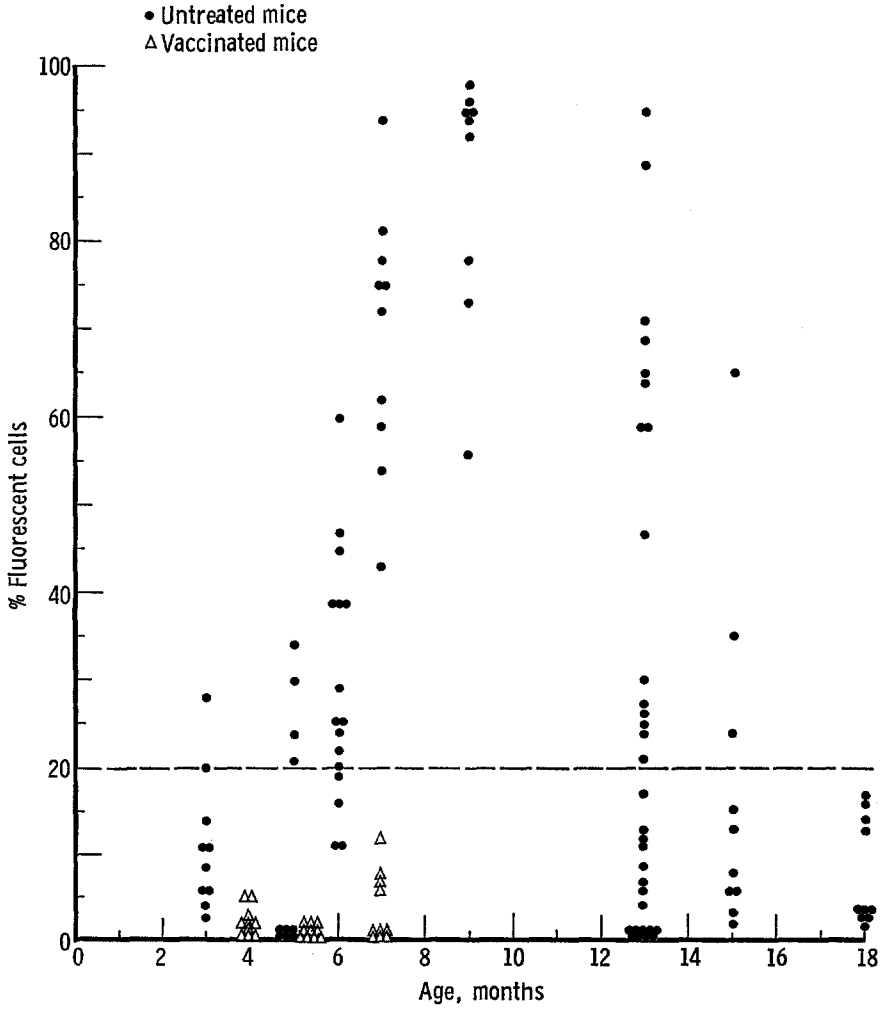

FIG. 2. Immunofluorescence tests for G soluble antigen in the plasma of untreated (●) and vaccinated (△) NZB mice at various ages. Reactions are graded by proportion of cells showing membrane immunofluorescence: negative (≤20%); positive (>20%).

in the glomeruli of one of two mice aged 5 months. Leukemia viral antigens were readily identified in the glomeruli of the remaining 4 NZB mice, aged 10–15 months, with 2–3+ proteinuria and histologically confirmed membranous glomerulonephritis. Leukemia viral antigens were present in the structurally altered glomeruli and mainly deposited focally in the mesangium (Fig. 6), contrasting with a more widespread distribution of host immunoglobulins in the

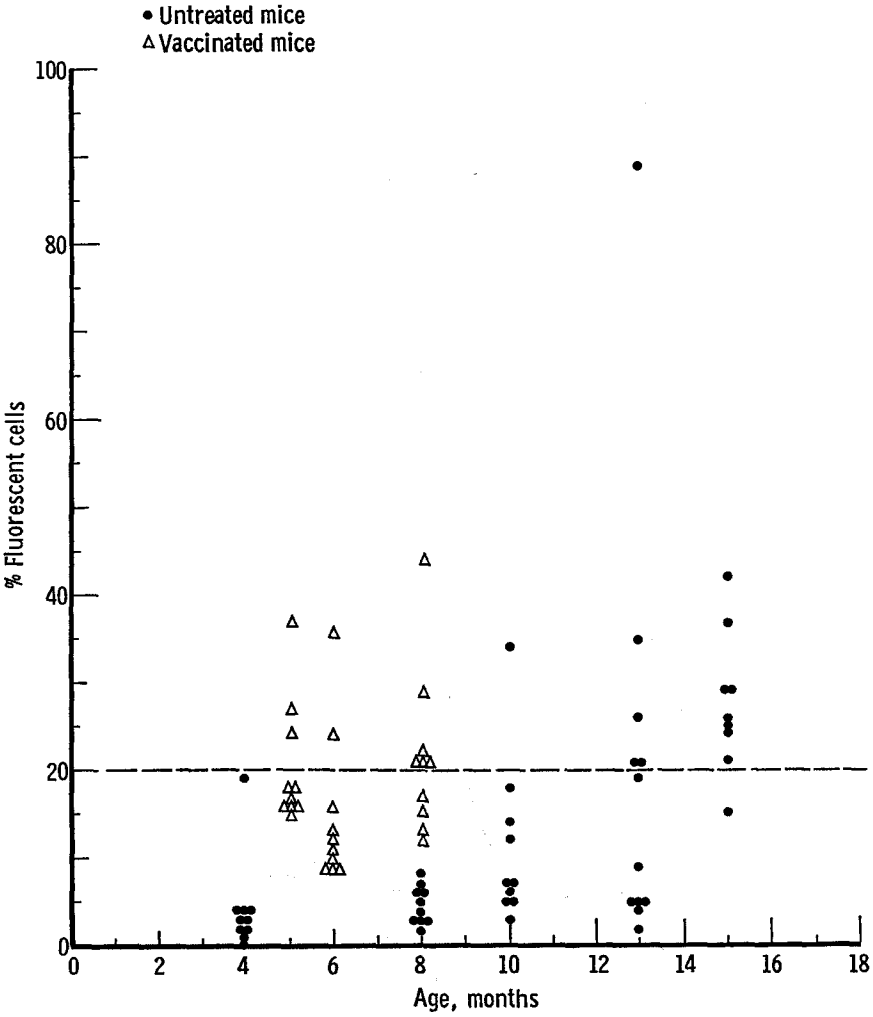

FIG. 3. Immunofluorescence tests for G antibody in the serum of untreated (●) and vaccinated (△) NZB mice at various ages. Reactions are graded by proportion of cells showing membrane immunofluorescence: negative (≤20%); positive (>20%).

glomeruli, in keeping with earlier work (16). Negative control reactions in the immunofluorescence procedure for the detection of leukemia viral antigens included the use of nonimmune serum and immune serum absorbed with MuLV group antigens (Fig. 7) as well as the study of frozen sections of kidneys of Swiss mice aged 2–10 months.

Direct Antiglobulin (Coombs) Tests.—Tests for erythrocyte-bound host

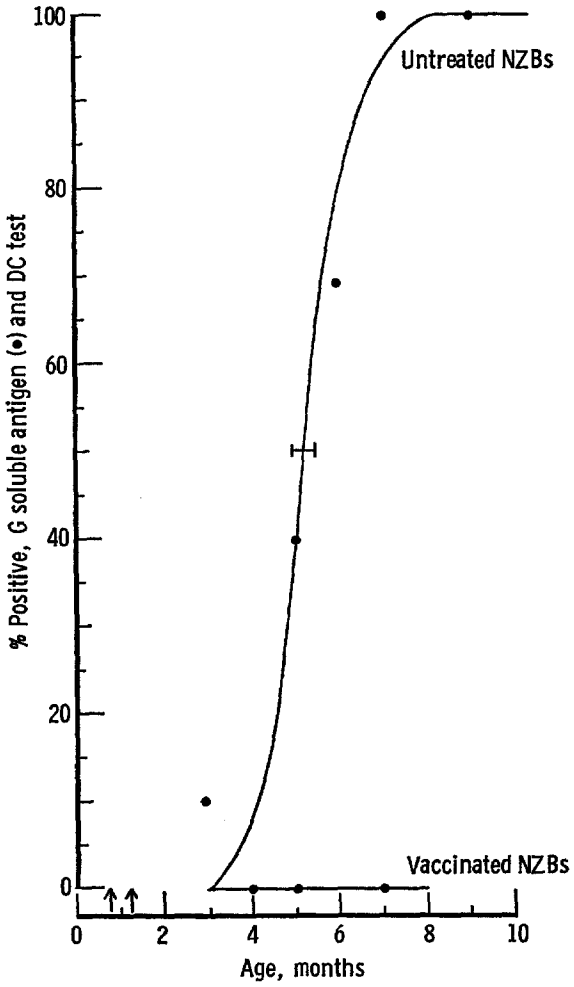

FIG. 4. Direct antiglobulin Coombs (DC) tests, % positive, continuous line, on untreated NZB mice at various ages show positive conversion associated with G soluble antigen (●) production. Comparable tests on vaccinated NZB mice, inoculated at 3rd and 5th wk (arrows), show nonconversion of Coombs tests and nonproduction of G soluble antigen.

globulins (shown to be immunoglobulins) were performed on 145 untreated NZB mice at various ages. The response-time curve (Fig. 4) revealed negative reactions at 2 and 3 months followed thereafter by positive conversion; 50% of direct antiglobulin tests were positive at 5.3 months (Table IV) and 99% were positive at 8 months, remaining so thereafter with rare exception. As shown in Fig. 4, the time-response curve for positive conversion of direct antiglobulin tests corresponded closely to that for G soluble antigen production in untreated NZB mice.

As described elsewhere (4, 5), positive conversion of direct antiglobulin tests was significantly delayed by vaccinating NZB mice at 3 and 5 wk of age with

TABLE IV

50% Response Time ($T_{50\%}$) for Positive Direct Coombs (DC+) Test Conversion and G Antibody Production in Untreated and Vaccinated NZB Mice

NZB Mice		$T_{50\%}$*	±2 SE	SD	N'
Response	Treatment				
		months	*months*	*months*	
DC+	Untreated	5.3	0.2	0.9	145
DC+	Vaccinated	8.7	0.4	1.1	48
G antibody	Untreated	13.3	0.8	1.4	21
G antibody	Vaccinated	7.3	1.4	2.6	30

* See footnote, Table III.

intraperitoneal injections of 0.2 ml formaldehyde-inactivated cell-free filtrate of aged NZB mouse spleen. The 50% response time for positive antiglobulin test conversion was 8.7 months in vaccinated NZB mice (Table IV) as contrasted with 5.3 months in untreated NZB mice. Reciprocally, as brought out in Table IV and Fig. 3, the 50% response time for G antibody production occurred earlier in vaccinated NZB mice, that is, at 7.3 months as contrasted with 13.3 months in untreated NZB mice.

The plasmas of 30 vaccinated NZB mice with negative direct antiglobulin reactions at 4, 5, and 7 months of age were tested at the same time for G soluble antigen. The tests for G soluble antigen were likewise negative in each instance (Figs. 2 and 4), that is, nonconversion in antiglobulin tests was correlated with nonproduction of G soluble antigen in vaccinated NZB mice.

The erythrocytes of normal untreated Swiss mice always served as negative controls in direct antiglobulin tests and gave negative reactions, without exception, in all such tests performed throughout the course of this study.

Proteinuria.—Significant proteinuria as shown by positive reactions greater than 1 plus (25) was a functional manifestation of severe diffuse membranous glomerulonephritis, in turn the major cause of death of NZB mice, especially

females, starting at about 10 months of age (26, 18). Proteinuria tests performed on 568 female NZB mice at various ages indicated that the response-time curve for significant proteinuria (Fig. 5) was sigmoid in shape and approximately

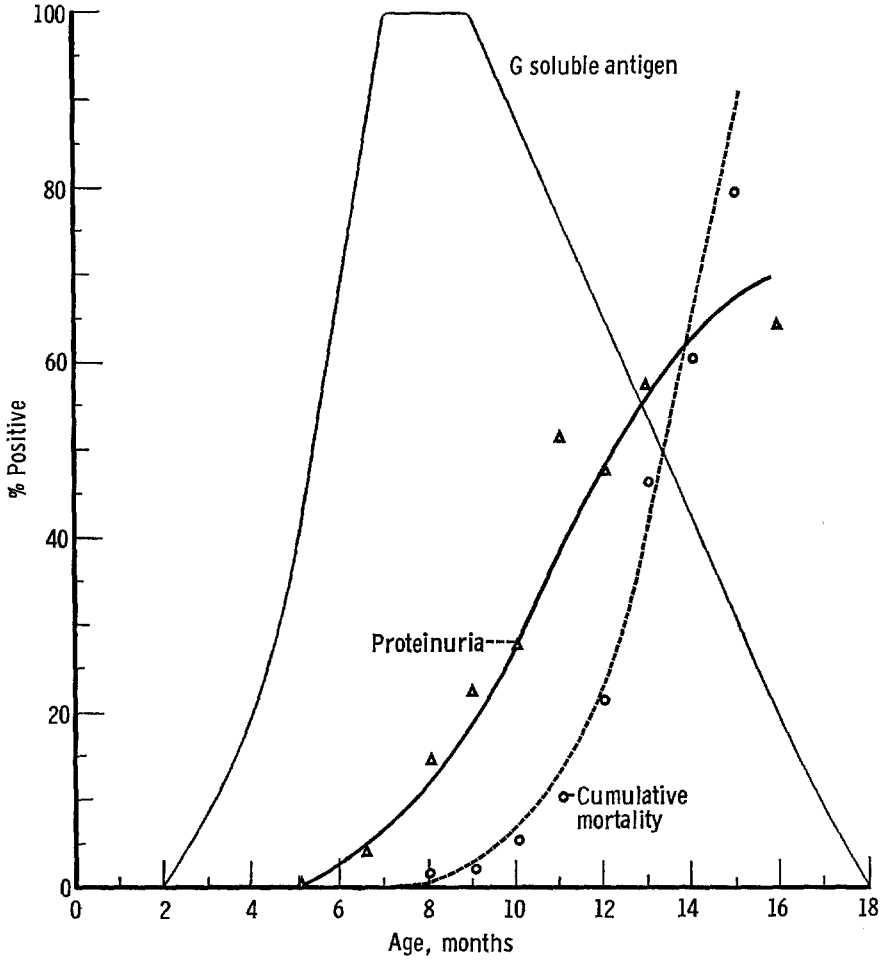

FIG. 5. Tests (% positive) for significant proteinuria (△) and tabulation of cumulative mortality (○), per cent, for untreated female NZB mice at various ages. Curves for G soluble antigen and G antibody formation (broken line) correspond to Fig. 1.

linear in the age range of 9–13 months. At 12.3 months (Table V), 50% of untreated female NZB mice had significant proteinuria.

Cumulative Mortality.—Mortality statistics tabulated on 107 untreated female NZB mice at monthly intervals from weaning to death indicated a 50% response (median survival) time of 13.3 months (Table V). The median survival time for 79 untreated male NZB mice was 15.7 months (Table V).

When plotted as a function of age, the points for cumulative mortality of untreated female NZB mice fell close to the curve for G antibody production (Fig. 5) as did also the 50% response times, 13.3 months, for cumulative mortality (Table V) and G antibody production (Table III).

DISCUSSION

These observations indicate that MuLV group antigens are prevalent in spleen, kidneys and, to a lesser extent, thymus of NZB mice throughout a substantial portion of their life span. The findings are consistent with the electron microscopic demonstration (1, 2, 27–30) of C type, and incomplete, virus-like particles in the corresponding tissues obtained from late embryo stage to aged adult and are in accord with the pattern of vertical transmission of murine

TABLE V

50% Response Time ($T_{50\%}$) for Significant Proteinuria (Positive Reaction Greater than 1+) and Cumulative Mortality in Untreated NZB Mice

Response	$T_{50\%}$*	±2 SE	SD	N'
	months	*months*	*months*	
Significant proteinuria (♀)	12.3	0.5	4.0	468
Cumulative mortality				
♀	13.3	0.3	2.0	583
♂	15.7	0.6	3.1	245

* See footnote, Table III.

leukemia virus from one generation to another (31). Further, the present studies indicate that NZB mice differ from mice of other G (Gross) positive inbred strains, such as the AKR, in the following respects. G (Gross) soluble antigen is *not* detectable in the plasma of NZB mice until 2–3 months of age and, reaching 100% prevalence, undergoes elimination thereafter; whereas this antigen is found in the plasma of other G-positive strains throughout their life span (12–14). Moreover, NZB mice produce G (Gross) natural antibody whereas, apparently owing to immunological tolerance, G antibody has not been found in other G-positive mouse strains (13, 24). The seeming failure of NZB mice to develop lasting tolerance to G antigen may be related to a deficiency in the production of this antigen in the early stages of postnatal life or to the more general inability of this strain to develop or maintain tolerance to some other antigens (32). The paradoxical effect of a vaccine, prepared from formaldehyde-inactivated virus-containing filtrates of older NZB mouse spleens (4, 5, 19), in delaying G soluble antigen production and yet raising G antibody formation may find explanation in an early antigenic priming effect.

Murine leukemia virus infection appears to be widespread and yet, with the exception of mouse strains with a high incidence of leukemia, rarely causes ap-

parent disease until very late in the lifetime of the infected animal (9). Nevertheless by midlife virtually all mice of the NZB strain have autoimmune hemolytic disease with positive Coombs tests (33, 34, 26, 18), the majority have renal glomerular disease (membranous glomerulonephritis) (35, 26, 36, 18), and some develop malignant lymphoma (26, 17, 37, 3, 4) or sarcoma of bone and soft tissue (19).

The present study makes it clear that positive conversion of Coombs tests in untreated NZB mice corresponds closely in time to the production of G soluble antigen and that nonconversion of Coombs tests in vaccinated NZB mice is correlated with the nonproduction of G soluble antigen and the earlier production of G antibody. Elsewhere it has been shown that accelerated Coombs conversion (4, 5, 19) can be induced by injecting weanling NZB mice with passage lines of solid tumors (sarcomas) indigenous to the strain and containing C type particles and MuLV group antigens. These collective findings suggest that there is a direct relation between leukemia-like virus infection and autoimmune hemolytic disease of NZB mice. An explanation for such a relation may be found in the following considerations. The surfaces of erythroid and other cells may be altered antigenically by budding of virus particles (1, 2, 27–30), by presence of G antigen or by some other expression of leukemia virus genome. Cell surfaces thus modified antigenically may raise antibody to the virus-specified determinant and, similar yet not identical with self, may also abrogate self-tolerance, as suggested by some other experimental systems (38). Alternatively, cell surfaces may be changed antigenically by adsorption of G soluble antigen, as occurs with EL4 indicator cells in the method for detecting this antigen (12). Nevertheless, the fact that antibodies eluted from Coombs-positive erythrocytes of NZB mice show specificity for normal, presumably unmodified, mouse erythrocytes irrespective of strain (39) appears to argue against this latter interpretation, as does also the persistence of positive Coombs tests in older NZB mice at the time of elimination of G soluble antigen. Another possibility is that leukemia-like virus infection alters the biological behavior and function of lymphoid cells. The prevalence of malignant lymphoma in aged NZB mice (4, 17, 26, 37), the early induction of thymic lymphosarcoma in the majority of NZB mice appropriately treated with the immunosuppressive drug azathioprine (40, R. C. Mellors, unpublished observations), and the identification of C type virus-like particles (1, 2, 4) and MuLV group antigens in each lymphoma studied, whether early or late primary or passage line, attest to the association of leukemia-like virus with abnormal lymphoid cells. Conceivably, autoimmune proliferation of lymphoid cells may be induced by virus infection and may be a step in the direction of lymphoid neoplasia; histopathologically, autoimmune and lymphomatous proliferations of lymphoid cells in NZB mice are often difficult to distinguish from each other (17).

Renal glomerular disease (membranous glomerulonephritis) of NZB mice is

almost certainly immunologically induced and is the main cause of death, especially of females starting at about 10 months of age (35, 26, 36, 16, 18). The present studies indicate that proteinuria, a functional manifestation of the glomerular lesions, becomes increasingly prevalent as G soluble antigen is eliminated from the plasma of female NZB mice and that cumulative mortality occurs in phase with free G antibody production. Further, MuLV group IF antigens and host immunoglobulins are localized in the glomerular lesions of NZB mice with renal disease. These collective findings suggest that leukemia-like virus is implicated in the etiology of glomerulonephritis of NZB mice. It is also reasonable to suggest that, with waning of tolerance and mounting of an immune response to G antigen, G antibody is formed and in turn circulating G soluble antigen-antibody complexes; the latter, if deposited in the glomeruli, may contribute to the development of glomerulonephritis. As noted by electron microscopic study (1), murine leukemia virus-like particles are present occasionally in the urinary space and the tubular lumens of the glomeruli of NZB mice and frequently in the basal infoldings of the renal convoluted tubules. The present study is the first to implicate a virus-specified cell-surface and soluble antigen in the immunopathogenesis of renal glomerular disease. The several immunological mechanisms for the induction of experimental glomerulonephritis in other systems are discussed elsewhere (41, 42). Recent findings in lymphocytic choriomeningitis (LCM) of neonatally infected mice indicate that the deposition of LCM virus, antibody, and complement in the glomeruli (43) may be one of the causes of the glomerulonephritis which occurs late in the course of this infection (44).

Approximately 5% of adult NZB mice have positive lupus erythematosus (LE) cell tests (34, 35) and a larger proportion develop anti-nuclear antibodies (45). NZB/W F_1 hybrid mice have a high incidence of positive LE cell tests accompanied by an early onset of fatal renal disease (35). DNA and anti-DNA antibodies, presumably deposited as nuclear antigen-antibody complexes, are implicated in the pathogenesis of glomerulonephritis in NZB/W hybrid mice (46). While the relation of anti-nuclear antibody formation to leukemia-like virus infection is presently unknown, although under investigation, it would be surprising not to find some common factors in the etiology and the pathogenesis of renal glomerular disease of NZB inbred and hybrid mice. This seems all the more likely in as much as hemolytic, renal, and lymphomatous disorders similar to those arising in NZB mice were induced in 10–20% of Swiss mice by neonatal inoculation of cell-free filtrates of freshly prepared homogenates of transplantable lymphomas and enlarged spleens of 10 to 20 month old NZB mice (2–5). Nevertheless, the leukemia-like virus of NZB mice has not yet been isolated in tissue culture despite rather extensive efforts with newer techniques such as those used successfully in related systems (7, 8, 47).

A remaining consideration is that of contaminating viruses. While the colony

of NZB mice used in this study is free of reovirus 3, lymphocytic choriomeningitis, and several other defined murine viruses, there is serological evidence of contamination of the colony with polyoma and GD VII virus, as previously reported (2, 4). It may be necessary to exclude polyoma virus as a factor in the accelerated Coombs conversion (4, 5, 19) induced in NZB mice with passage lines of isologous sarcomas, should these tumors carry polyoma.

SUMMARY

Further evidence implicating murine leukemia-like virus in the disorders of NZB mice was afforded by a study of antigens associated with murine leukemia virus (MuLV). MuLV group antigens were prevalent in extracts of spleen, kidney, and, to a lesser extent, thymus throughout a substantial portion of the life span of NZB mice as well as in extracts of lymphomas and sarcomas indigenous to the strain.

G (Gross) soluble antigen, type-specific antigen, was first detected in plasma of untreated NZB mice at 3 months of age. G soluble antigen production increased thereafter in line with age, with 50% of reactions becoming positive at 5.3 months and 100% at 7 to 9 months.

From months 3 to 9, the time-response curve for positive conversion of direct antiglobulin (Coombs) tests in untreated NZB mice corresponded closely to that for G soluble antigen production.

Beyond the 9th month, G soluble antigen underwent elimination from the plasma of NZB mice, with positive reactions reduced to 50% at 13.3 months and to 0% at 18 months. G natural antibody was first detected in the serum of NZB mice at about 10 months of age and increased thereafter in line with age. The curves for G antibody production and G soluble antigen elimination bore a reciprocal relation to each other with crossover at 50% response occurring at 13.3 months.

Significant proteinuria, a functional manifestation of membranous glomerulonephritis, became increasingly prevalent in female NZB mice as G soluble antigen was eliminated from plasma. Cumulative mortality of female NZB mice, mainly attributable to renal glomerular disease, increased in phase with G antibody production. MuLV group antigens were identified in the glomerular lesions by the immunofluorescence method.

Positive conversion of direct antiglobulin tests was significantly delayed by vaccinating baby NZB mice with formaldehyde-inactivated cell-free filtrates of older NZB mouse spleens. The plasmas of vaccinated NZB mice with negative direct antiglobulin reactions at 4 to 7 months were likewise negative when tested for G soluble antigen. The 50% response time for G antibody production in the vaccinated NZB mice occurred at 7.3 months, that is, 6 months earlier than in untreated NZB mice.

The collective findings implicate murine leukemia-like virus in the etiology of

autoimmune hemolytic disease and membranous glomerulonephritis, as well as malignant lymphoma, of NZB mice and suggest that virus-specified cell-surface and soluble antigen is a factor in the immunopathogenesis of the renal disease and possibly also the autoimmune hemolytic disease.

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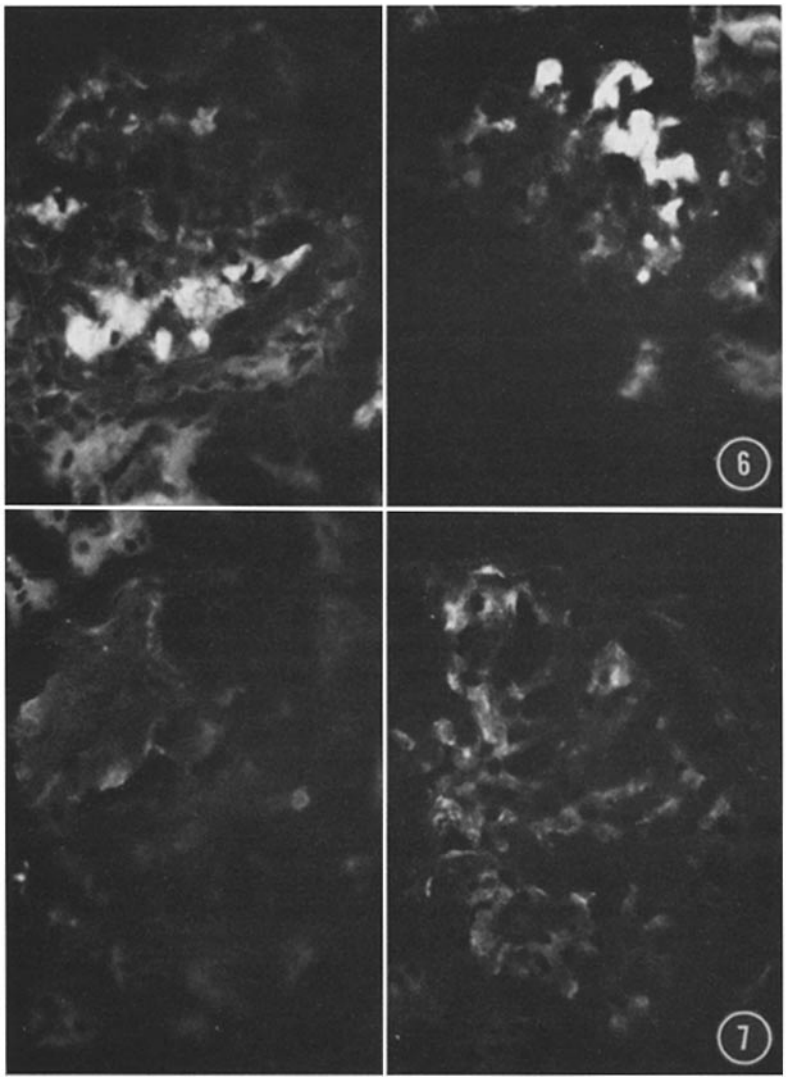

FIG. 6. Immunofluorescence photomicrographs of frozen sections of kidney of 13 month old NZB mouse with 3+ proteinuria and membranous glomerulonephritis. Murine leukemia viral antigens (white areas) located eccentrically in glomerulus (left and right), mainly in mesangium. Indirect procedure using MuLV group rat antiserum followed by fluorescent anti-rat globulin, neither of which reagents react with mouse serum proteins (see Methods and Materials). × 400.

FIG. 7. Negative control reactions for Fig. 6. Frozen sections of same kidney reacted with MuLV group rat antiserum absorbed with MuLV group antigens (left) and reacted with homologous normal rat serum (right), followed in each instance by fluorescent anti-rat globulin. Glomeruli are barely visible; there is no specific immunofluorescence. × 400.