SPONTANEOUS MURINE LUPUS-LIKE SYNDROMES

Clinical and Immunopathological Manifestations in Several Strains*

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New Zealand mice, particularly the (NZB × NZW)F_1 mice (NZB × W) have been extensively used as an experimental model of human systemic lupus erythematosus (SLE)[1] (1–4). Among the murine disease's postulated etiologic factors are retroviruses (5, 6), thymic atrophy or failure (7), anti-thymocyte antibodies (8, 9), immunologic hyperreactivity (10, 11), deficiency in suppressor T cells (12, 13) and other subsets of T cells (14), abnormalities of phagocytic cells (15), and abnormal T-cell cytotoxicity (16). Attempts at genetic analysis have indicated that multiple genes are involved in the expression of the disease in the NZB × W mice (17). Clearly these mice demonstrate many immunologic, virologic, and other abnormalities, and it is extremely difficult to determine which are primary etiologic factors of the murine SLE syndrome and which are secondary or even incidental features.

To help identify the essential elements in murine SLE, we have undertaken a comparison of immunologic, virologic, and genetic features of NZB and NZB × W mice with those of the newly described murine strains, substrains MRL/l and MRL/n, and strain BXSB (18, 19), which also develop a SLE-like disease. If the disorders of these several kinds of mice represent a single disease, one might expect to find the essential etiologic and pathologic factors present in all the mice. The studies reported here indicate that the lupus-like syndromes of NZB × W, MRL/l, and BXSB mice are clinically and immunopathologically quite similar, as alike as those of randomly selected humans with SLE (20). All these mice have B-cell hyperactivity, auto antibodies, circulating immune complexes (IC), abnormalities of Ig and complement, extensive thymic cortical atrophy, and severe IC-type glomerulonephritis with retroviral gp70 glomerular deposits. The major differences among the strains are the amounts and specificities of autoantibodies, age of onset and rapidity of progress of

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[1] *Abbreviations used in this paper:* AMG, aggregated mouse gammaglobulin; ANA, antinuclear antibody; IC, immune complex; PAS, periodic acid shiff; PBS, phosphate buffered saline; RIA, radioimmunoassay; RF, rheumatoid factor; SLE, systemic lupus erythematosus; SRBC, sheep red blood cell.

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disease, incidence by sex, evidence of arteritis, and extent and nature of lymphoid hyperplasia.

Materials and Methods

Mice. NZB (H-2^d) and NZW (H-2^z) mice were originally obtained from the Laboratory Animals Centre, Medical Research Council, Surrey, England and have been maintained by brother-sister matings since 1965 at Scripps Clinic and Research Foundation. Mating of NZB females with NZW males produced the NZB × W hybrids (H-2^d/H-2^z). The inbred strains BXSB (H-2^b), MRL/l, and MRL/n (H-2^k) were obtained from the research colony of Dr. E. D. Murphy of the Jackson Laboratory, Bar Harbor, Maine. BXSB is a recombinant inbred strain derived from a cross between a C57BL/6 female and an SB/Le male. Nearly 100% of the BXSB develop a spontaneous progressive lethal SLE-like disease affecting males much earlier than females. The autoimmune phenotype is transmitted as a dominant trait to F1 hybrids with an accelerating factor in males contributed by the BXSB male parent (19). The MRL substrains were derived mainly from strain LG/J with contributions from AKR/J, C3H/Di, and C57BL/6J. A spontaneous autosomal recessive mutation, *lpr* (lymphoproliferation), producing massive T-cell proliferation and an early onset SLE-like syndrome was first observed at the 12th generation of inbreeding (18). Two inbred substrains sharing ≅ 89% of their genomes were developed: MRL/l (*lpr/lpr*), with massive lymphoproliferation, and MRL/n (+/+), without.

All mice were maintained on 6–10% lipid, 24% protein diet, and water ad lib.

Histology. Mice were sacrificed when moribund and autopsied. Sections of thymuses, spleens, mesenteric and peripheral lymph nodes, hearts, lungs, livers, kidneys, and gonads were fixed in Bouin's fluid and stained with H&E and PAS.

Immunofluorescence Studies. Kidneys from 27 females NZB × W (5–9-mo-old), 13 male BXSB (3–5-mo-old), and 11 male MRS/l (2–5-mo-old) mice were studied for IgG and C3 by direct immunofluorescence (21). Murine retroviral antigens, gp70 and p30, were sought by using goat anti-Rauscher virus gp70 (supplied by J. T. August, Johns Hopkins University, School of Medicine, Baltimore, Maryland) and goat anti-Rauscher p30 antisera, respectively, followed by fluorescein isothiocyanate (FITC) conjugated rabbit anti-goat IgG serum (22). The anti-gp70 serum had virtually no anti-p30 activity when tested by radioimmune assay; however, the anti-p30 serum did contain a low level of reactivity against Rauscher gp70 antigen.

Urinary Protein. 24-h urinary protein was determined by the sulfosalicylic acid precipitation method (23). Values in excess of 2 mg/24 h were considered abnormal.

Serologic Studies. Samples of serum were analyzed electrophoretically on cellulose acetate membranes. Levels of serum IgG and IgM were measured by radial immunodiffusion using rabbit anti-mouse IgG and rabbit anti-mouse IgM. The Coomb's test was used to detect anti-erythrocyte antibodies (24). Anti-nuclear antbody (ANA) titers were determined as described (4) using immunofluorescence and serial fourfold serum dilutions up to 1:192. Anti-ds DNA and anti-ss DNA antibodies were titrated by a modification of the Farr DNA-binding radioimmune assay (25), and the presence of antibodies to Sm, an acidic soluble nuclear protein, was determined as in (26). Presence of IgM rheumatoid factor (RF) was assessed by a solid phase radioimmune assay performed as follows: 100 μl of a 10 μg/ml solution of mouse IgG were used to coat the cells of microtiter plates in a reaction that lasted for 5 h at 24°C. After washing, the wells were further coated with 0.5% solution of bovine serum albumin before 100 μl of a 1:1000 dilution of test serum samples were added. The plates were then incubated overnight at 4°C and washed. Then 1 ng of ^{125}I labeled anti-mouse IgM (affinity purified) was added, and the plates were incubated another 5 h at 4°C. After a final series of washes, the individual wells of the plates were cut out for counting. Each sample was simultaneously tested at a 1:10,000 dilution for IgM concentration in a parallel assay that differed only in that the wells were coated initially with 1 μg/ml anti-mouse IgM instead of mouse IgG. A standard curve was determined by using a purified monoclonal mouse IgM (ABPC-22) in this latter assay. Counts for both RF and IgM levels were referred to this same standard curve. Anti-thymocyte antibodies were assayed by a two-step chromium release test similar to that of Raveche et al. (27), using as targets C57BL/6 thymocytes. Complement levels were determined as described (28). Comparative hemolytic values were established using pooled BALB/c serum as the complement reference.

TABLE I
Murine SLE Mortality

Mice	Sex	Number	50% Mortality	90% Mortality
NZB	female	22	16*	21
	male	21	17	23
NZB × W	female	19	8.5	12.8
	male	17	15	19
MRL/I	female	17	5.0	7.3
	male	22	5.5	8.6
MRL/n‡	female	14	17	23
	male	14	23	27
BXSB	female	17	15	24
	male	21	5.1	8.0

* Age in months.
‡ E. D. Murphy and J. B. Roths. Personal communication.

IC in serum were detected and quantitated with a modification of the Raji cell radioimmune assay (RIA) (29). We used aggregated mouse γ-globulin (AMG) for the standard curve and ^{125}I-IgG fraction of rabbit anti-mouse IgG for quantitation of Raji cell-bound IgG. Pooled BALB/c serum stored at −70°C was the source of complement in the standard curve. Results are expressed as micrograms equivalents of AMG/ml murine serum.

Serum concentrations of gp70 were determined by a modification of a described RIA (30) that uses goat anti-feline leukemia virus as the primary antibody and ^{125}I gp70 from Rauscher leukemia virus as the labeled antigen. The presence of antibody to AKR gp70 or to Rauscher gp70 was determined by using a radioimmune assay in which the primary binding was to ^{125}I radiolabeled AKR gp70 or Rauscher gp70 followed by precipitation with rabbit anti-mouse IgG.

Cryoglobulin Isolation. Cryoglobulin was separated from serum as described (31). Protein was determined with the Folin method and IgG concentration with radial immunodiffusion. Qualitative analysis of cryoglobulin was performed by double immunodiffusion using antisera against murine Ig isotypes and murine C3. ANA and antibody to DNA and AKR gp70 were determined as in serum.

Elution of Ig from Kidneys. Kidneys from each of the strains (NZB × W, MRL/l and BXSB) were pooled, homogenized, and washed 5 times in phosphate buffered saline (PBS). After the final centrifugation, the precipitates were suspended in 5 ml PBS containing 0.005M Ca^{++} and 0.01 M Mg^{++}, digested with DNAse (32), and eluted with KSCN in PBS (33). Titers of ANA and antibodies to ds DNA, ss DNA, and gp70 at multiple dilutions were determined in the respective sera and kidney eluates. The ratio of IgG in the serum relative to the kidney eluate at the endpoint titer indicated the degree of antibody concentration in the tissue.

Results

Mortality. Table I lists the age in months of the four immunologically abnormal murine strains at the time of 50% and 90% mortalities. The lupus-like syndrome in female and male MRL/l mice and male BXSB mice was considerably earlier in onset and more acute than that of the NZB × W female.

Clinical Picture. A marked, generalized lymphadenopathy was evident in virtually all male and female MRL/l older than 3 mo and in ≅ 10% of the older male BXSB mice. NZ mice did not develop clinically evident lymphadenopathy. In about one-third of MRL/l mice, the lymph nodes shrunk markedly 7–10 days before death.

Heavy proteinuria and associated anasarca was most frequent in the NZB × W females. At 4 mo of age these mice had a mean urinary protein of 6.6 mg/day and terminally 40% had advanced anasarca. MRL/l males and females and BXSB males

between 3 and 6 mo of age had urinary protein values from 2.6 to 3.8 mg/day with an incidence of terminal anasarca approximately one-half that found in the NZB × Ws.

Histologic Observations. At autopsy there were many histopathologic similarities in the mice with relatively early SLE: BXSB male, MRL/l female, MRL/l male, and NZB × W female. The major cause of death in all four of these groups was glomerulonephritis which ranged from an exudative and proliferative acute form in the BXSB male to a largely subacute proliferative form in the MRL/l male and female and a more chronic obliterative form in the NZB × W female (Fig. 1 A, B, and C) (Table II). Only in the BXSB mice were polymorphonuclear leukocytes a significant element in the glomerular lesions. In the MRL/l mice glomerular lesions involved proliferation of both endothelial and mesangial cells with occasional crescent formation and basement membrane thickening. The obliterative lesion in the NZB × W female was accompanied by heavy mesangial and at times intravascular proteinaceous deposits, proliferation of all glomerular cellular elements, and frequent crescent formation.

15–30% of mice in each group had old and/or acute myocardial infarction involving either ventricle and judged extensive enough to be a contributing cause of death (Fig. 1 E). Although coronary arterial disease usually was not evident, there were occasional instances of hyalin thickening of small arteries in or near the infarcts as well as of arteries unrelated to myocardial lesions. Acute polyarteritis most frequently involving renal and coronary arteries occurred in over one-half of female and male MRL/l animals (Fig. 1 D) but in none of the BXSB or NZB × W mice. In spite of the high incidence of coronary arteritis in MRL/l mice, their incidence of myocardial infarction was the same as that of the BXSB and NZB × W suggesting that arteritis, per se, did not predispose significantly to myocardial infarction.

20–25% of old, sick MRL/l mice had swelling of the joints and surrounding tissues of the hind feet and lower legs. These lesions consisted of an acute to chronic inflammatory process in the absence of detectable cutaneous abnormality. There was destruction of articular cartilage, proliferation of synovium, pannus formation, and, at times, joint effusions which *in toto* produce a picture not unlike that of rheumatoid arthritis. The periarticular tissues were involved in acute and/or chronic inflammation with occasional foci of necrosis reminiscent of rheumatoid nodules.

Thymic atrophy, most severe in the cortex but also involving the medulla in most mice, was similar in all groups. The initial lesion appeared to be loss of cortical thymocytes with later degeneration, often cystic, of the medulla. In 5–10% of mice in each group, there appeared to be a medullary or stromal hyperplasia which maintained or even increased the normal size of the thymus in spite of a loss of cortex. This medullary hyperplasia did not correlate consistently with any other clinical or pathologic feature of the mice.

The degree of lymph node hyperplasia varied considerably among the different strains resulting in lymph nodes ranging from normal to two or three times normal size in NZB × W females, to 10 to 20 times normal size in BXSB males, and up to 100 times normal size in MRL/l mice. In the largest nodes of about one-third of BXSB males, there was a diffuse loss of lymphocytes with fibrosis of the remaining stroma. In one-third to one-half of MRL/l mice, the larger nodes showed extensive hemorrhage and cystic necrosis that was probably responsible for the clinically evident terminal reduction in lymph node size.

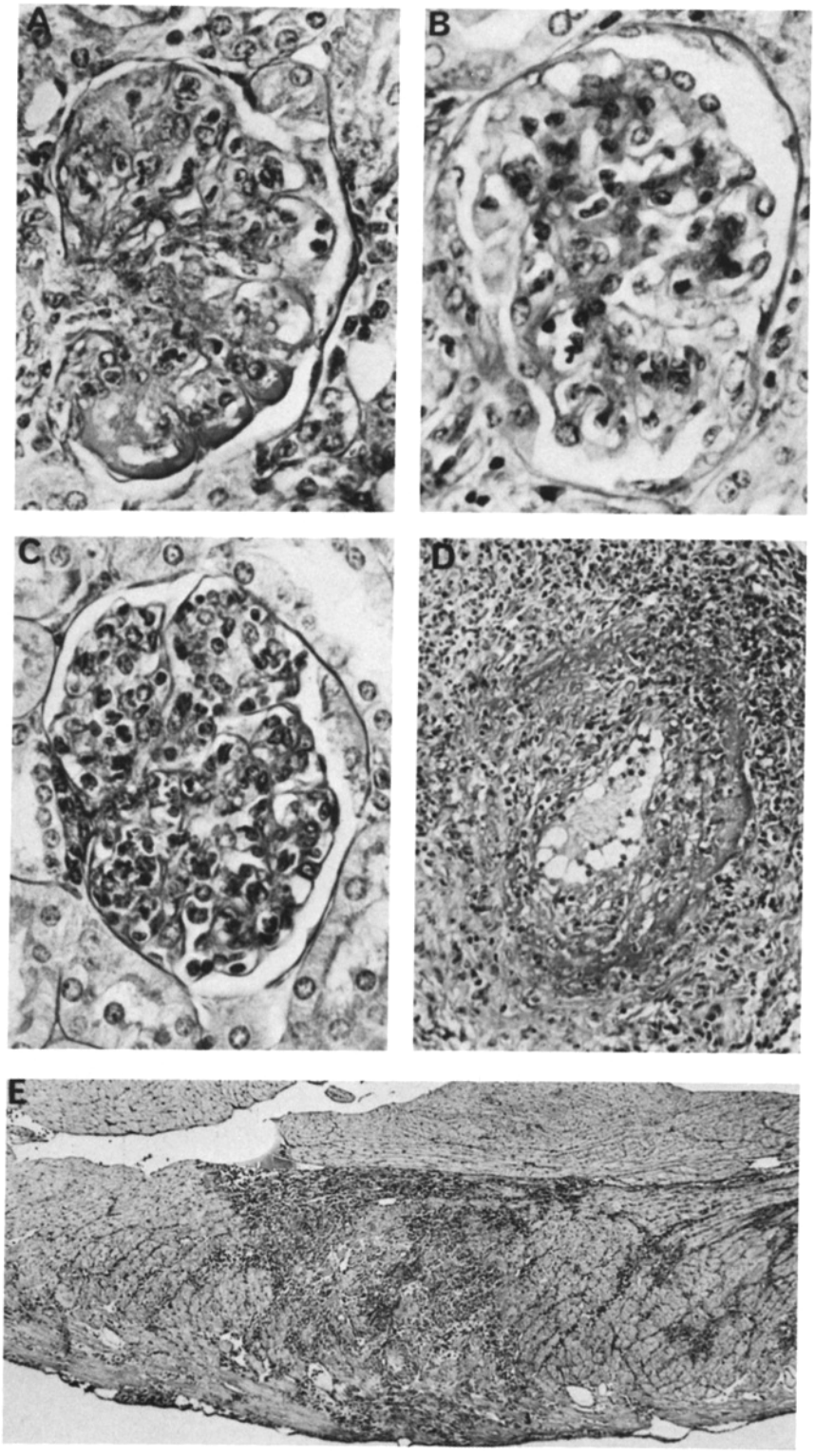
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TABLE II

Murine SLE Histologic Observations

Mice	Sex	Kidneys	Heart	Blood vessels	Thymus	Lymph nodes	Lungs	Miscellaneous
NZB × W (20)*	Female	Chronic Gn‡ (19) severe	Acute (2) and healed (1) infarcts	No vasculitis	Severe cortical atrophy (20) medullary hyperplasia (1)	Normal to slight hyperplasia (19)	Pneumonia (2) lymphoid infiltrate (14)	Lymphoma (1) anasarca (8)
MRL/I (18)	Female	Acute and subacute Gn (18) severe	Acute (3) and healed (3) infarcts	Acute polyarteritis (10)	Severe cortical atrophy (18) medullary hyperplasia (2)	Extreme hyperplasia (17) necrosis and hemorrhage (7)	Pneumonia (2)	Focal hepatic necrosis (2) anasarca (3) arthritis (3)
MRL/I (23)	Male	Subacute Gn severe (21) moderate (2)	Acute (2) and healed (1) infarcts	Acute polyarteritis (13)	Severe cortical atrophy (23) medullary hyperplasia (2)	Extreme hyperplasia (21) necrosis and hemorrhage (8)	Pneumonia (8) lymphoid infiltrate (7) carcinoma (1)	Thyroiditis (1) anasarca (1) arthritis (5)
BXSB (20)	Male	Acute and subactue Gn severe (17) moderate (3)	Acute (4) and healed (1) infarcts	No vasculitis	Severe cortical atrophy (20) medullary hyperplasia (1)	Moderate hyperplasia (19) necrosis ± fibrosis (6)	Pneumonia (6) adenoacanthoma	Cirrhosis (1) anasarca (4)

* Number of animals.

‡ Gn - glomerulonephritis.

In the 81 mice observed throughout life, three tumors developed: a lymphoma in NZB × W female, a carcinoma of the lung in an MRL/l male, and an adenocanthoma of the lung in a BXSB male.

Immunofluorescence Studies. Granular deposits of mouse IgG and C3 of variable intensity were present in one or more locales including glomerular capillary walls, mesangia, and tubulointerstitial sites in the three strains of mice (Fig. 2). The patterns of glomerular IgG and C3 deposits in the three strains overlapped sufficiently so that they could not be identified with certainty in most individuals from each strain; however, some general characteristics were evident. In the NZB × W mice, deposits were present largely in the glomerular mesangium at or before 5 mo of age. With progression of disease and widening of the glomerular mesangium, heavy mesangial deposits occurred, generally with accompanying glomerular capillary wall deposits (Fig. 2 A). A predominant or exclusive glomerular capillary wall pattern of deposits was also occasionally seen. Extraglomerular renal deposits of IgG and C3 were present in the peritubular tissue and arterioles and increased in frequency with age.

Striking glomerular deposits of IgG and C3 involving both the mesangium and the glomerular capillary wall of the hypercellular BXSB glomerulus were observed as

FIG. 1. A. Glomerulus of 9-mo-old female NZB × W. Typical of lesions in this strain are mesangial accumulation of amorphous material with proliferation of mesangial cells and hyaline thickening of peripheral capillary walls best seen in lower portion of glomerulus (periodic acid shiff [PAS]-320×). B. Glomerulus of 5-mo-old female MRL/l. Primary lesion is a proliferation of mesangial cells and increased mesangial matrix. A few leukocytes are seen in capillary lumens but peripheral capillary walls are not thickened. (PAS-320×). C. Glomerulus of 5-mo-old male BXSB. Proliferation of mesangial and endothelial cells plus accumulation of polymorphonuclear leukocytes produce a typical proliferative-exudative glomerulonephritis typical of renal disease in this strain. (PAS-320×). D. Coronary artery of 5-mo-old female MRL/l. Entire circumference of this artery is involved in a acute, necrotizing, inflammatory response which has obliterated the normal structure of the still patent vessel. (PAS-125×). E. Right ventricular wall of heart of 5-mo-old male BXSB. Acute myocardial infarction involves entire thickness of right ventricle with normal septal and papillary muscle seen above. This degree of myocardial infarction was present equally in the three types of lupus mice. (PAS-50×).

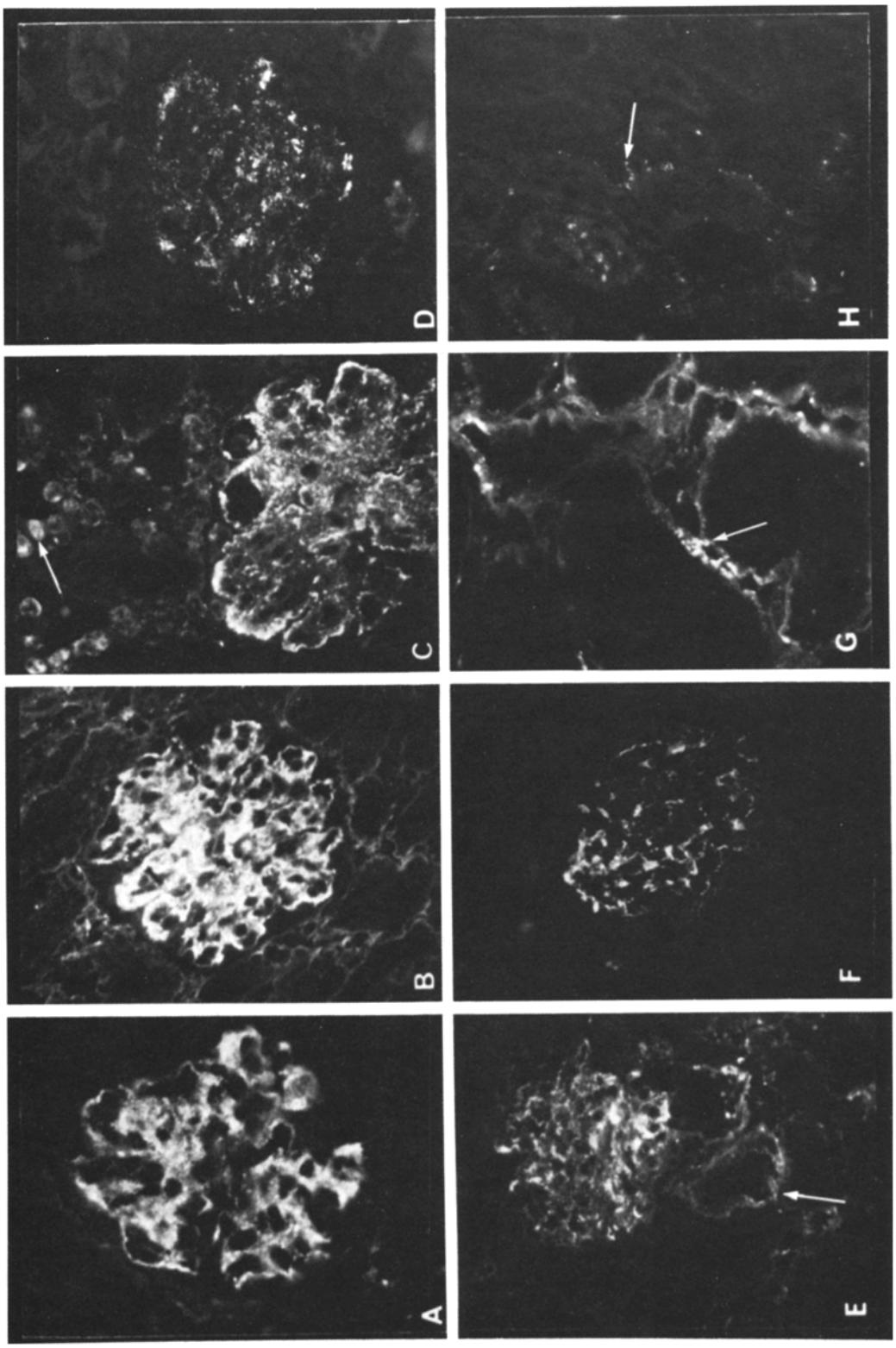
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TABLE III
Murine SLE Serum IgG Levels

Mice	Sex	Age	No. Mice	IgG (mg/ml)*
		mo		
NZB	Female	3	10	14.4 ± 3.6
		6	10	16.0 ± 3.6
		9	21	14.2 ± 7.0
NZB × W	Female	3	15	8.8 ± 2.2
		6	15	15.0 ± 6.3
		9	31	11.0 ± 7.4
MRL/l	Male &	2–3	16	15.6 ± 8.0
	female	4–5	26	26.0 ± 14.7
BXSB	Male	3	22	7.4 ± 4.7
		5	21	19.2 ± 12.0
BALB/c	Male & female	4–6	10	3.5 ± 0.9

* Mean ± SD.

early as 3 mo (Fig. 2 B and C). Extraglomerular deposits similar to those in the NZB × W mice increased in frequency with age (Fig. 2 G).

Granular IgG and C3 deposits in the MRL/l strain also increased from 2 to 5 mo (Fig. 2 E). The deposits were present in both the capillary wall and the mesangium, often tending to predominate in the former where the finely granular deposits were in general closely approximated.

Murine retroviral gp70 was detectable with a goat anti-Rauscher gp70 antisera in granular glomerular and tubular basement membrane deposits (Fig. 2 D, F, and H); however, the deposits were often much less striking and distributed differently than the IgG and C3 deposits; some or all glomeruli of mice with impressive IgG accumulations had no gp70. The variability of gp70 straining and its disparity with IgG and C3 were greatest in the MRL/l strain. At best, equivocal staining with p30 antiserum was seen in rate glomeruli of occasional mice in each strain.

Serum Protein Analysis. The allotype of the IgG_{2A} subclass (Ig-1 locus; kindly determined by Dr. Leonard Herzenberg, Stanford, Calif.) for the MRL/l and MRL/n strains is *a*, whereas that of the BXSB strain is *b*. The comparable marker for the NZB and NZW strains is known to be *e* (34). All autoimmune strains had significantly higher IgG concentration that the BALB/c controls (Table III). The highest IgG

FIG. 2. A. Granular deposits of IgG, accompanied by C3 and gp70, were observed within the mesangium and glomerular capillary walls of a 9-mo-old NZB × W female. B. Heavy granular deposits of IgG, accompanied by C3 and small amounts of gp70, were present in the glomerular capillary walls and mesangium of a 6-mo-old BXSB male. C. Diffuse granular deposits of IgG, accompanied by C3, were present in the glomerular capillary walls, and to a lesser extent in the mesangium, of a 5-mo-old BXSB male. Focal nuclear IgG deposits (arrow) were also found. D. gp70 deposits in a glomerulus from the BXSB mouse shown in C are illustrated. E. Diffuse granular IgG deposits were found along the glomerular basement membranes of a 5-mo-old MRL/l male. Granular deposits of IgG (arrows) were also present in the tubular basement membranes. F. gp70 deposits were seen in a glomerulus from a 4-mo-old MRL/l male, predominantly in the glomerular capillary wall and corresponding closely to the IgG and C3 deposition seen in this mouse. G. Focal granular deposits of IgG, accompanied by C3, were observed within the tubular basement membrane and peritubular capillary wall of a 8-mo-old BXSB male. H. gp70 deposits (arrow) were also observed in the tubular basement membrane of the mouse depicted in G. (Original magnifications: A, × 400; B,C,D,E,G,H, × 312; and G, × 500. Staining A,B,C,E,G, anti-mouse IgG; D,F,H, anti-Rauscher gp70).

TABLE IV
Murine SLE ANA

Mice	Sex	Age		
		2–3 mo	5–6 mo	9 mo
NZB	Female	0.4* (37)‡	0.5 (150)	6 (83)
NZB × W	Female	0.2 (180)	13 (208)	43 (23)
MRL/I	Male	14 (45)	192 (29)	§
	Female	89 (33)	182 (22)	§
BXSB	Male	9 (33)	23 (22)	§
BALB/c	Male & female	0.2 (22)	0 (36)	0.2 (11)

* Values shown are geometric mean titers.
‡ Number of animals are included in the parentheses.
§ No survivors.

concentrations were seen in the MRL/l mice which averaged nearly five times control at 2–3 mo and eight times at 4–5 mo.

The most striking feature of the serum protein electrophoresis was the relatively high incidence of monoclonal γ-globulins in the MRL/l mice, particularly in their last 3 wk of life. Overall, 43% of both male and female MRL/l had monoclonal protein with two bands detected in one animal. Monoclonal proteins were found in 23% of BXSB mice and 13% and 17% of NZB and NZB × W females, respectively, although the latter's bands were much less prominent than those seen in the MRL/l. Diffuse hypergammaglobulinemia was most marked in MRL/ls followed by BXSB and then the NZB × W.

Direct Anti-Erythrocyte Antibody Test. Erythrocyte autoantibodies occurred most frequently in the NZB female with incidence of 5% at 3 mo, 32% at 6 mo, and 89% at 9 mo. NZB × W females, though having only 11% incidence at 6 mo, had 78% at 9 mo. In the MRL/l males and females, the incidence of anti-erythrocyte antibodies reached 4% and 11%, respectively, and 18% of the BXSB males were positive at 5–6 mo.

Anti-nuclear Antibodies. ANA levels (Table IV) were highest in MRL/l mice, with the next highest titers in NZB × W females, followed by BXSB males. In all strains tested, a peripheral or rim pattern of nuclear fluorescence was always present at the highest positive serum dilution, whereas the homogeneous pattern was sometimes seen at lower dilutions.

Anti-dsDNA and Anti-ssDNA Antibodies. Anti-dsDNA antibodies developed relatively late in the course of murine SLE with none of the affected strains showing significant levels at 2 mo (Table V). At 4–5 mo all immunologically abnormal strains had significant levels of anti-ds DNA. The highest levels of anti-dsDNA were found in the 4- 5-mo-old MRL/l and the 9-mo-old NZB × W.

Anti-ssDNA antibodies are found at low concentrations in immunologically normal mice (Table V). However, greater increases in these antibodies were found in the NZB, NZB × W, and MRL/l animals at 2 mo and even higher levels observed at 4–5 mo, at which time male BXSB also had abnormal levels.

Sera were also tested for anti-Sm antibodies and, with one exception these were found only in the MRL/l and MRL/n animals of both sexes (26).

Rheumatoid Factor. As seen in Fig. 3, significant elevations of RF were found only

TABLE V

*Murine SLE Serum dsDNA and ssDNA Binding Activity**

Mice	Sex	Anti-dsDNA			Anti-ssDNA		
		2 mo	4–5 mo	9 mo	2 mo	4–5 mo	9 mo
NZB	Male & female	<5	6	10	29	41	54
NZB × W	Female	<5	7	21	34	67	69
MRL/I	Male & female	<5	25	‡	33	81	‡
BXSB	Male	<5	8	‡	10	25	‡
	Female	<5	<5	<5	9	10	10
BALB/c	Male & female	<5	<5	<5	8	10	9

* Expressed as % binding of 20 ng ^{125}I-ssDNA or ^{125}I-dsDNA by 100 μl 1:10 mouse serum.
‡ No survivors.

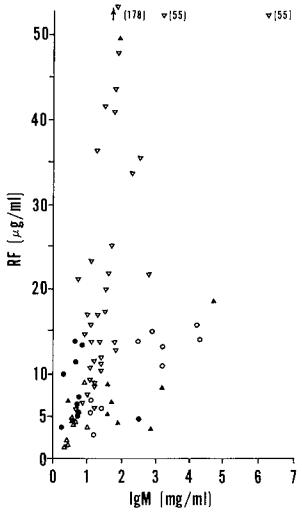

FIG. 3. Rheumatoid factor and serum IgM concentrations. ○, NZB female; ●, normal females CBA/St and C57BL/6 St; △, BXSB male; ▲, NZB × NZW female; ▽, MRL/l male and female.

in about one-half of the MRL/l mice and in a single NZB × W. Normal CBA/St and C57BL/6 St mice had RF concentrations comparable to or higher than the levels found in BXSB males and NZB × W females. The concentrations of RF in NZB mice were within the upper normal range.

Anti-thymocyte antibodies. Male and female NZB and female NZB × W mice have unusually high incidences of positivity ranging from 60 to 78%. Male and female BXSB and NZB × W male mice are from 20 to 40% positive and MRL/l mice of both sexes are 10% positive, all of which are well within the levels found in most immunologically normal mice.

Serum Complement. In all types of mice with a lupus-like syndrome the concentrations of hemolytic complement fell with age (Fig. 4). The progressive fall in serum complement concentrations began with the onset of disease and progressed as the immunologic abnormalities increased.

Circulating IC. The upper normal limit of IC in the sera of 10 μg equivalent AMG/ml was based on the mean plus two standard deviations for a group of 37 control BALB/c mice measured at 3, 6, and 9 mo (Fig. 5). Abnormal levels of IC in

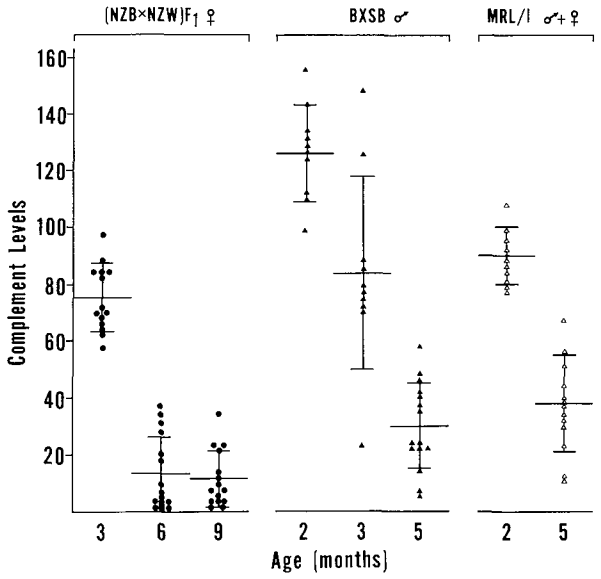

FIG. 4. Hemolytic complement titers. Bars represent mean ± 2 SD.

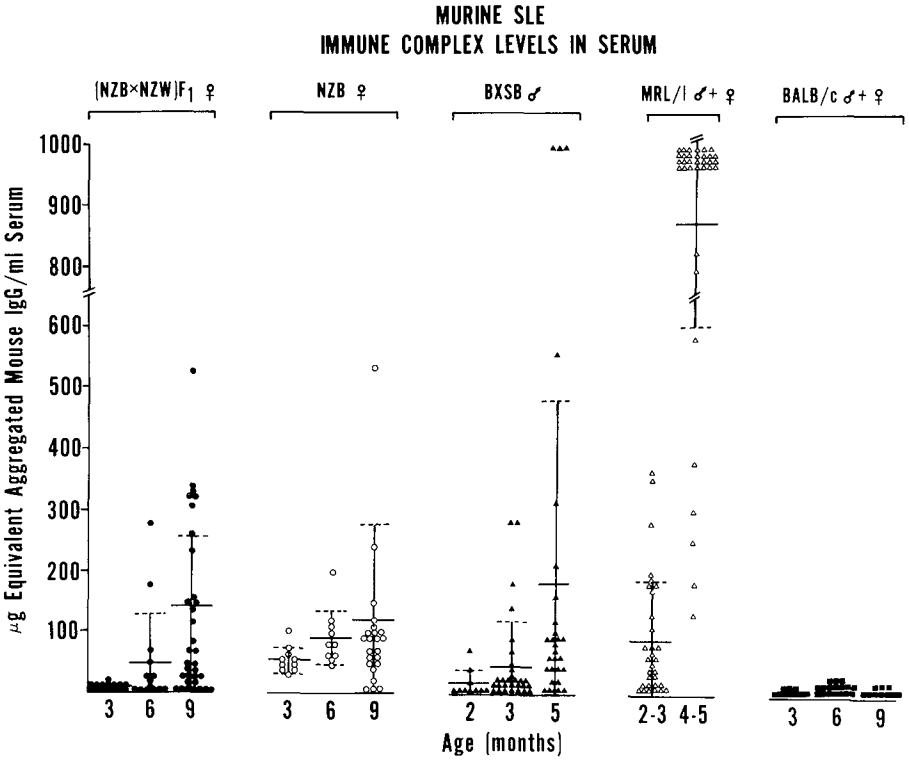

FIG. 5. Immune complex levels in sera of mice with SLE-like syndrome as determined by the Raji cell RIA. Bars represent mean ± 2 SD.

TABLE VI
Serum Retroviral gp70 at 2–4 Mo

Mice	Female	Male
NZB	36* (13)‡	64 (16)
NZB × W	25 (61)	43 (48)
MRL/I	20 (3)	24 (42)
BXSB	8 (33)	17 (46)
NZW	34 (24)	68 (28)
LG J	—	58 (13)
129/J	—	21 (17)
SWR/J	—	11 (5)
C57Bl/6	—	3 (15)
BALB/c	—	2 (12)

* μg gp70/ml.
‡ Number animals tested.

NZB × W mice were first observed at 6 mo in ≅ 80% of the mice with a mean of 45 μg. NZB × W mice with obvious disease at the age of 9 mo had mean IC levels of 144μg with ≅ 90% of the animals having values above normal. NZB females showed elevations in circulating IC at 3, 6, and 9 mo with values of 51, 87, and 115 μg equivalent, respectively. BXSB males had barely elevated IC levels at 2 mo, but at 3 mo the mean was 41 μg equivalent with 50% of animals having abnormal levels. At 5 mo the mean was 178 with 84% of the animals showing elevation. The highest IC levels were detected in MRL/l males and females which at 2–3 mo had IC levels of 90 and at 4–5 mo mean values of nearly 900. This measurement of IC was determined to be unrelated to the hyperimmunoglobulin levels in these mice. Further, removal of serum cryoglobulins did not significantly reduce the IC levels.

Cryoglobulins. Control levels of cryoglobulins were determined from observations on normal BALB/c mice in which the mean levels were 134 μg/ml at 3 mo and 206 μg/ml at 9 mo. Comparatively, NZB × W female mice had slightly elevated cryoglobulin levels of ≅ 200 μg/ml at 3 mo and slightly >300 μg/ml at 6 and 9 mo. NZB female mice had larger amounts of cryoglobulin with concentrations of ≅ 350 μg/ml at 3 and 6 mo, and 570 μg/ml at 9 mo. BXSB males had elevated cryoglobulins of 472 μg/ml at 2 mo followed by decreases to 260 μg/ml at 3 mo and 275 μg/ml at 5 mo. The most striking concentrations of cryoglobulins were found in MRL/l mice of both sexes in which values went from 173 μg/ml at 2 mo to 2,180 μg/ml at 5 mo.

IgM and IgG1 were most frequently found in cryoprecipitates of all mice tested; followed by IgG2A. C3 was found in 20% of the cryoglobulins of NZ mice, 50% in those of the MRL/l, but only 11% in those of the BXSB mice. No concentration of anti-AKR, gp70, anti-dsDNA, or anti-thymocyte antibodies were seen in the cryoprecipitates over that seen in paired sera.

Serum Retroviral gp70. Levels of serum retroviral gp70 in mice with lupus-like syndromes and normal mice are listed in Table VI. On the basis of structural studies, the serum gp70 of normal and lupus mice is very similar to the gp70 of a Xenotropic retrovirus uniquely expressed in the NZB mouse (35, 36). Although NZ, MRL/l, and BXSB mice had significant levels of serum gp70, similar levels were observed in several strains of immunologically normal mice.

Antibody Activity in Renal Eluates. The concentrations of ANA activity in IgG eluted from kidneys vs. the concentrations of ANA activity in serum IgG was from 2 to 10

times higher in individual eluates of NZB × W kidneys and 2 to 6 times higher in eluates from both MRL/l and BXSB kidneys. Antibody against dsDNA was considerably more concentrated in NZB × W renal eluates being 25–31 times greater than in serum whereas MRL/l eluates showed only a 1–6 times concentration and BXSB eluates 0–12 times. Antibodies to ssDNA were also more concentrated in renal eluate IgG than in serum IgG with values of 5–13 times for NZB × W and 11–21 times for BXSB eluates. Antibodies to Rauscher gp70 and to AKR gp70 were not found concentrated in renal lg eluates from any of the three SLE strains.

Discussion

All SLE mice shared a number of immunopathologic features that appeared directly related to the progress of their disease. Common to all kinds of affected mice there were several primary serologic abnormalities including: (*a*) elevated serum Ig concentrations with associated monoclonal gammopathy, (*b*) ANA, and (*c*) antibodies to ssDNA and dsDNA. Of these serologic changes, the Ig concentrations which reflect the overall activity of the B cells appeared to correlate best, but by no means perfectly, with the severity of disease in the several strains. The MRL/l and BXSB mice had the most rapidly progressive disease and also had higher Ig concentrations and higher incidence of monoclonality than NZB × W and NZB mice. In addition, NZB, NZB × W, MRL/l, and BXSB mice all show abnormally high spontaneous polyclonal B cell activation.[2] The ANA levels, which were elevated in all mice, were highest in MRL/l and only moderately elevated in BXSB in spite of equally rapid courses of disease in these two strains of mice. The ANA levels paralleled the course of disease within each strain reaching maximum levels at about the time of 50% mortality in each. Anti-ds and -ssDNA were in general elevated and paralleled each other within each strain but did not reflect the severity of disease in the several strains. BXSB and MRL/l mice had modest elevations whereas NZB × W mice had 2–3 times higher levels.

Secondary serologic changes related completely or in part to antigen-antibody interactions were present in all three SLE strains. Levels of circulating IC paralleled the course of disease in each strain reaching maximum levels at about the time of 50% mortality. However, at that time the amounts of IC in the MRL/l mice were approximately four to six times higher than in the NZB × W and BXSB, respectively, in spite of equally acute disease courses in MRL/l and BXSB. Also the levels of IC varied considerably among the individual mice within each strain, and only in the 4–5-mo-old MRL/l group did all individuals have significantly elevated values. Serum hemolytic complement levels reflected the immunologic events in the SLE mice with decreasing values as disease progressed.

Histopathologic and immunopathologic study revealed several lesions common to SLE in all strains as well as unique lesions especially in the MRL/l. The most significant and constant component of the disease in all strains was a severe IC type glomerulonephritis. Consistent with the clinical course of SLE the glomerulonephritis was acute to subacute, proliferative, and exudative in the BXSB male and more subacute, proliferative in the MRL/l, whereas in the NZB × W the disease was

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[2] Izui, S., McConahey, P. F., and Dixon, F. J. Increased spontaneous polyclonal activation of B lymphocytes in mice with genetic autoimmune disease. *J. Immunol.* In press.

subacute to chronic with heavier proteinaceous deposits both in the mesangia and capillaries. In agreement with the presence of circulating IC and presumed IC pathogenesis of the glomerulonephritis, there were moderate to heavy granular glomerular deposits of IgG and C3 in glomeruli of all SLE strains. These were most consistently found in greatest amount in the NZB × W, perhaps because here the immunoproteins had the longest period in which to accumulate. The significance of the murine retroviral gp70 found in some glomeruli of all the SLE strains and most prominent in the NZB × W was uncertain. Its granular glomerular distribution suggested its participation as the antigen in some of the deposited IC, but its distribution often differed significantly from that of the IgG and C3. Quantitative interpretation of immunofluorescence is difficult, however, gp70 was present less regularly and in less intensity than IgG and C3 suggesting that if gp70 were on antigenic component of the glomerular IC, it might be a minor one. This impression is borne out by the lack of concentration of anti-gp70 antibody in the Ig eluted from the SLE kidneys.

A second consistent part of SLE pathology in all strains was severe thymic atrophy. This involved almost complete loss of cortex and in 90% of instances accompanying medullary atrophy. Whether this thymic atrophy was a primary or secondary event in the SLE was not possible to determine, but it was found before evident disease in kidneys or elsewhere. In addition, its development apparently did not correlate with incidence of circulating anti-thymocyte antibodies. MRL/l mice had a very low incidence of these antibodies, and BXSB male mice had no more than many normal strains. Yet each had significant thymic atrophy by 4–5 mo, as early or earlier than that occurring in NZ mice, which had much higher incidences of antithymocyte antibodies. Further, the high incidence of anti-thymocyte antibodies in some immunologically normal murine strains suggests that these antibodies are unrelated to thymic atrophy.[3]

The occurrence of myocardial infarction in murine SLE has not been noted previously, but infarcts of significant size did occur in 15–30% of mice in all SLE strains. Generally there was no obvious associated coronary vascular disease, except in the MRL/l mice, about one-half of which had an acute polyarteritis, frequently involving the coronaries but unrelated to any increase in myocardial infarction.

The degree and kind of hyperplasia of lymph nodes and spleens varied among the SLE strains much more than did the IgG levels or the levels of autoantibodies. Not only did lymphoid mass differ (1–3 times normal in NZB × W to 10–20 times normal in BXSB and 100 times normal in MRL/l) but the cell type involved also differed. The MRL/l nodes were flooded with small lymphocytes often obliterating normal nodal architecture. The major cell type in the hyperplastic nodes of the MRL/l was a θ positive, Ly-null cell which was not seen in the other strains (to be published). Late changes in the nodes of the BXSB mice included progression of the stromal replacement, however, in the MRL/l there was often extensive cystic and hemorrhagic necrosis. Benign lymphoid infiltration of organs such as lung, kidney, and liver that increased with age was seen to some degree in all SLE strains but was most marked in the NZB × W perhaps because of their longer life.

The differences in onset and severity of SLE in males and females of the several

[3] Eisenberg, R A., Theofilopoulos, A. N., Andrews, B. S, Peters, C. J, Thor, L, and Dixon, F J Natural thymocytotoxic antibodies in autoimmune and normal mice Manuscript in preparation

strains, NZB × W, females first; MRL/l, both sexes about equally, and BXSB, males first; suggest that there is no consistent or mandatory endocrine influence on murine SLE as it is seen in multiple strains. Although a female endocrine enhancement and male endocrine suppresion of SLE in NZB × W mice has been reported (37), preliminary results in BXSB mice indicate no sex hormone-related influence on the disease in this strain (to be published). The BXSB male parent contributes an accelerating factor to male offspring (19).

Several serologic abnormalities including IgM-RF, anti-Sm antibodies, and cryoglobulins were found as part of the SLE-like syndrome in MRL/l mice, but not in BXSB or NZB × W. Although this might in part relate to the extremely high Ig levels in this strain, which might tend to magnify any borderline serologic changes, this is not the entire explanation. MRL/n mice which are closely related to MRL/l but without lymphoproliferation or marked elevation of serum Ig also have anti-Sm antibodies (25) indicating that this immune response was an unique product of the MRL genome. Also, the increased IgM-RF in the MRL/l occurred without much increase in the serum IgM level. IgG RF has also been found in the serum of MRL/l mice (to be published). It is tempting to relate these RFs to the arthritic lesions found in the hind legs and feet of MRL/l mice. Whether these two manifestations are pathogenetically related remains to be determined, but with both serologic and arthritic changes resembling rheumatoid arthritis the MRL/l mouse may be a valuable animal model for the study of this human disease.

Definition of the role of retroviral gp70 in murine lupus is difficult because multiple immunologically related gp70 may be produced in every mouse. The primary serum gp70 of all mice so far tested is similar to that found in the NZB Xenotropic virus (36, 37) and because it is always in excess no serum antibody to it has yet been identifed. Mice also carry the genomes of one or more additional retroviruses the gp70s of which may be expressed but usually in far smaller amounts than the serum gp70. Free antibodies to some of these non serum gp70s have been detected as in the case of antibodies to Rauscher gp70 or to AKR gp70 in mice carrying the AKR viral genome. Although gp70 has been identified in the glomeruli in murine lupus its type is not known. The considerable amounts of serum gp70 found in NZB and NZB × W have been suggested as an etiological factor in the diseases of these mice (5, 6). However, in view of: (*a*) the lack of correlation between serum gp70 levels and the rate of progression of disease in NZ and MRL/l and BXSB mice, (*b*) serum gp70 levels of many immunologically normal murine strains equal to those of mice with SLE, and (*c*) the lack of correlation of serum gp70 levels with autoimmune disease in crosses of NZB and SWR mice (38), it appears that high serum levels of gp70 are not in themselves a cause of murine SLE. If retroviral gp70 proves to be significant in the pathogenesis of murine SLE it is more likely that it will be an abnormal immune response of the host to its gp70 which is the critical factor rather than any given level or unique type of gp70 in the circulation.

Summary

MRL/l and BXSB male mice have a systemic lupus erythematosus (SLE)-like disease similar to but more acute than that occurring in NZB × W mice. The common elements of lymphoid hyperplasia, B-cell hyperactivity, autoantibodies, circulating immune complex (IC), complement consumption, IC glomerulonephritis

with gp70 deposition, and thymic atrophy were found in all three kinds of SLE mice. On the basis of these common elements, SLE seen in these mice can be considered a single disease in the same sense that human SLE is one disease. The differences in the SLE expressed in the different mice are no greater than those found in an unselected series of humans with SLE. However, the significant quantitative and qualitative variations in abnormal immunologic expression suggest that different constellations of factors, genetic and/or pathophysiologic, may operate in the three murine strains and that each constellation is capable of leading, via its particular abnormal immunologic consequences, to the activation of common immunopathologic effector mechanisms that cause quite similar SLE-like syndromes.

From an experimental point of view, the availability of several inbred murine strains of commonplace histocompatibility types that express an SLE-like syndrome makes possible innumerable manipulations which should help to elucidate the nature and cause(s) of this disorder.

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