## *Brief Definitive Report*

# (NZW × BXSB)$F_1$ HYBRID

## A Model of Acute Lupus and Coronary Vascular Disease with Myocardial Infarction*

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Several kinds of inbred mice serve as experimental models of systemic lupus erythematosus (SLE) and related human disorders. Each of these, including (NZB × NZW)$F_1$ (1), the BXSB (2), and MRL/1 (3), has a genetic background that determines a characteristic pattern of autoimmune responses capable of causing late-life SLE (4, 5). In addition, each strain has a unique SLE-accelerating factor that causes the early death of affected individuals (5). The accelerating factor seems to be a female sex hormone environment in (NZB × NZW)$F_1$ mice (6), an as yet unidentified Y chromosome-linked influence in BXSB mice (7, 8), and a somatic recessive lymphoproliferative gene in MRL/1 mice (3). Thus, each strain offers a different but related perspective on human SLE.

In the course of analyzing the various immunopathologic features of murine SLE, we examined $F_1$ hybrids from crossing several of the SLE strains. As a result, we found that $F_1$ offspring of NZW females and BXSB males (W × B)$F_1$ have an unusual immunologically induced coronary disease. All the males develop an acute SLE quite similar to that of the male BXSB (2, 4, 5) and ~80% have degenerative coronary vascular disease associated with myocardial infarction. The female disease runs a slower course, similar to that in the (NZB × NZW)$F_1$ female (1), and ~30% of the (W × B)$F_1$ females develop the coronary lesion. In contrast, only ~20% of other mice with SLE (4, 9) manifest coronary heart disease.

## Materials and Methods

*Mice.* BXSB mice originated from a single cross of a male SB/Le with a female C57BL/6 (2). This recombinant inbred strain was developed at The Jackson Laboratory, Bar Harbor and has been established in our mouse colony since 1976. NZW mice were obtained from the Laboratory Animals Center, Medical Research Council, Surrey, England in 1965 and have been maintained in our colony at the Scripps Clinic and Research Foundation, La Jolla, Calif. The (W × B)$F_1$ cross results from a single mating of female NZW with male BXSB. Animals used here were weaned at ~ 4 wk of age.

*Castration.* Experimental animals were castrated at 10 d of age. The mice were anesthetized with ether, and testes with attached spermatic cords or ovaries with attached fallopian tubes were removed under a dissecting microscope. Mortality from the surgical procedure was 4%.

*Serologic Assays.* Mice were bled monthly beginning at 2 mo of age. The sera were stored at −70°C. Anti-single-stranded DNA (ssDNA) antibodies were measured by using a modification of the Farr DNA-binding radioimmunoassay (10). Total serum retroviral gp70 and Ig-bound

* Supported in part by grants AI-07007, AG-01743, and CA-27849 from the U. S. Public Health Service and by the Cecil H. And Ida M. Green Endowment Fund. Publication 2398 from the Immunology Departments, Scripps Clinic and Research Foundation, La Jolla, California.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/81/07/0216/06 $1.00
Volume 154 July 1981 216-221

gp70 were quantitatively determined after absorption of sera with staphylococcal protein A (Calbiochem-Behring Corp., American Hoechst Corp., La Jolla, Calif.) as documented in detail in a previous report (11). Values from absorbed serum were subtracted from total serum gp70, and the differences were considered to be Ig-bound gp70, expressed as percent of the total. Antithymocyte antibodies were assayed by a two-step chromium-release test using as targets C57BL/6 thymocytes (4). Serum levels of IgG1, IgG2a, and IgG2b were measured by radial immunodiffusion on agar plates obtained commercially from Meloy Laboratories Inc., Springfield, Va.

*Histopathology.* Blocks of all major organs were obtained at autopsy, and sections stained with periodic acid-Schiff's stain (PAS). Glomerulonephritis was quantitated on a 0 to 4+ scale based on the intensity and extent of histopathologic changes. One-half of each kidney was stored at −70°C, and later cryostat-sectioned at 5 $\mu$ thickness, air dried and stained with fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG and goat-anti-Rauscher murine leukemia virus gp70 sera for direct immunofluorescence. The brightness and extent of glomerular immunofluorescence were graded on a scale of 0 to 4+. Hyperplasia of the lymphoreticular system (spleen and lymph nodes) was graded from 0 (normal) to 4+, with 4+ representing a 400% increase above normal size. An increase in size >1+ (100%) was considered significant. Hepatic sinusoidal lymphocytosis (12) was evaluated on paraffin sections of liver stained with PAS and was considered significant when at least 50% as many mononuclear inflammatory cells as hepatocytes were present within a given area.

## Results

The (W × B)$F_1$ mouse expressed the usual autoimmune responses and immune complex-induced lesions of SLE, particularly glomerulonephritis, and in addition had a high incidence of severe degenerative coronary arterial disease. The autoimmune responses and resultant SLE glomerulonephritis arose first in the males as shown in Fig. 1. The (W × B)$F_1$ males had a 50% mortality rate at 4.5 mo and 90% at 8 mo. These values reflected a slightly more severe and rapidly progressive disease than that seen in male BXSB parents (50% mortality rate at 6–7 mo and 90% at 9 mo). The $F_1$ females developed SLE later, with 50% cumulative mortality rate at ~11 mo of age. This was quite different from the NZW maternal parent, which lives well into or beyond the second year of life, but resembled the disease of (NZB × NZW)$F_1$ females.

Abnormalities of the lymphoreticular system were more striking in the males than the females. Splenomegaly caused by lymphoid proliferation occurred in 71% of the males as compared with 30% of the females at the time of autopsy. Peripheral lymphadenopathy, involving subcutaneous and mesenteric lymph nodes, was present in 58% of males vs. 16% of females. In addition, there was a high incidence of hepatic sinusoidal lymphocytosis with Kupffer's cell hyperplasia in the male offspring (60%), whereas only 8% of the female offspring showed such changes.

Fig. 2 depicts the serologic abnormalities observed in these $F_1$ mice. The changes included mildly elevated amounts of anti-ssDNA antibodies and gradual increases in proportions of Ig-bound serum gp70 immune complexes. By 4 mo of age, the males showed anti-ssDNA binding and Ig-bound gp70 of 38% and 67%, respectively; whereas females reached comparable levels by 10 mo. 100% of males and 69% of females had detectable antithymocyte antibodies at 4 mo of age. In addition, serum levels of IgG1 were elevated more in the males (6 mg/ml) than in the females (3 mg/ml). However, IgG2a and IgG2b levels were similar in both sexes. The factor that seemed to best parallel and predict the onset of disease was circulating Ig-bound gp70 complexes. Although the total amount of serum gp70 did not change signficantly as the disease progressed, an increase in the proportion of Ig-bound gp70 preceded the onset of glomerulonephritis by ~1–2 mo. Immunofluorescence of the affected kidneys

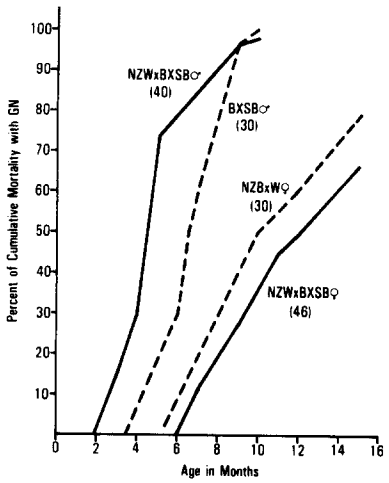


FIG. 1

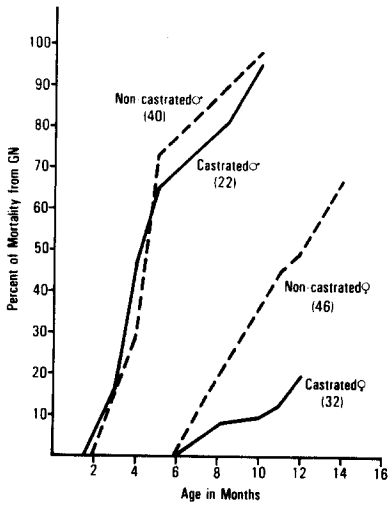


FIG. 3

FIG. 1. Cumulative mortality with glomerulonephritis (GN) of (W × B)$F_1$ males and females, as compared with BXSB males and (NZB × NZW)$F_1$ females. Parentheses indicate numbers of animals in each group.

FIG. 3. Cumulative mortality with glomerulonephritis (GN) of castrated and intact (W × B)$F_1$ males and females. Parentheses indicate numbers of animals in each group.

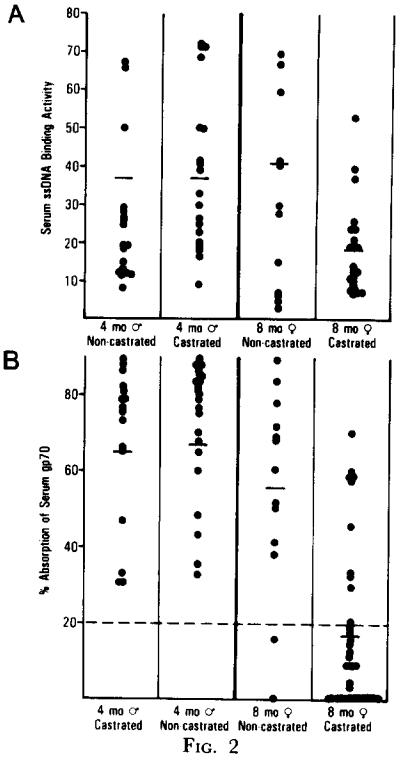


FIG. 2

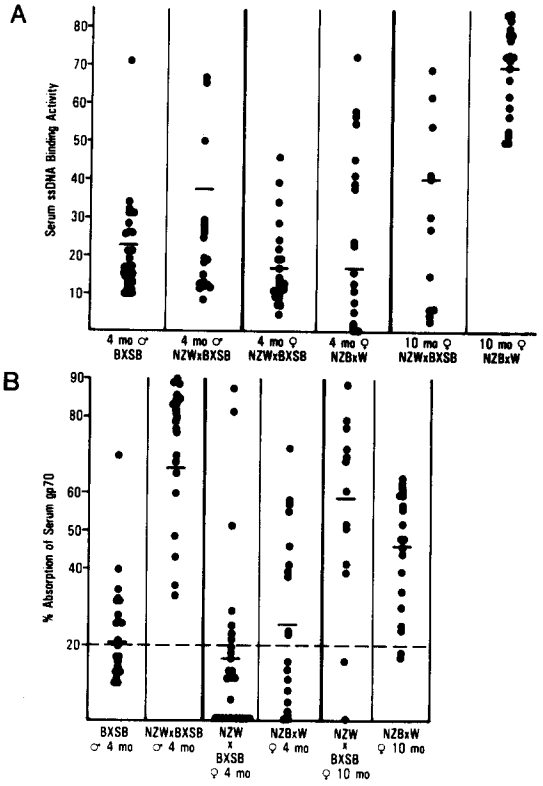


FIG. 4

FIG. 2. A, ssDNA-binding activity of each group of mice. Results are expressed as percent binding of 20 ng $^{125}$I-ssDNA by 10 μl serum. B, absorption of serum gp70 by staphylococcus protein A. Greater than 20% absorption is considered significant for presence of gp70 immune complexes. Bars, mean values.

FIG. 4. A, ssDNA-binding activity in castrated and intact (W × B)$F_1$ males and females at 4 and 8 mo of age. B, Absorption of serum gp70 by staphylococcal protein A in castrated and intact (W × B)$F_1$ males and females at 4 and 8 mo of age. Bars, mean values.

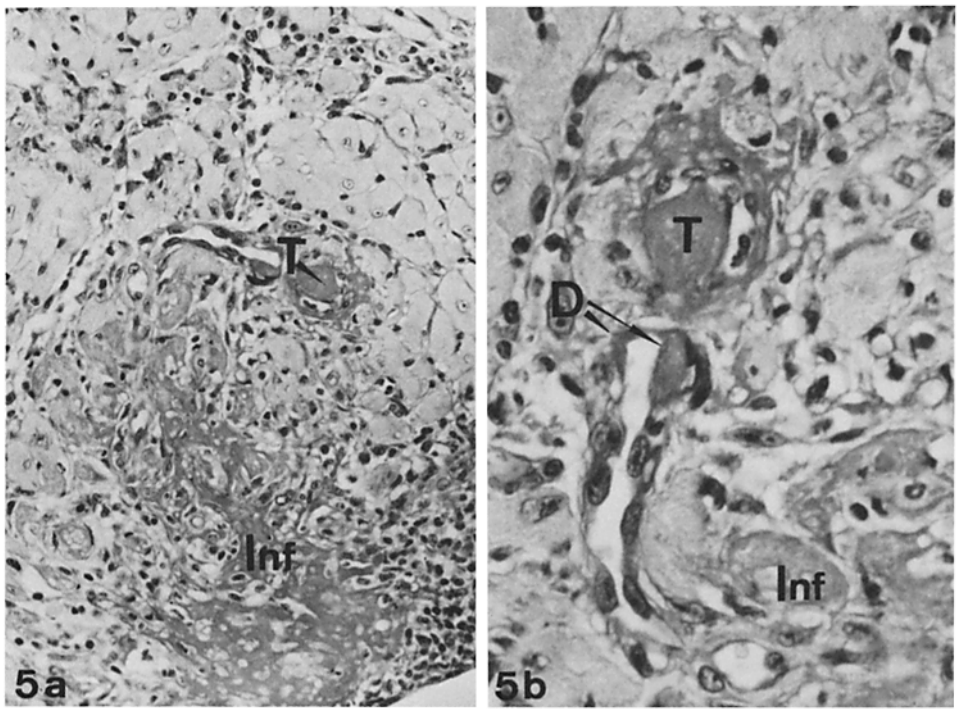


FIG. 5. (a) Representative section of myocardium showing a thrombotic small artery (T) and associated area of infarcted myocardium (Inf). × 160. (b) Higher magnification of involved vessel with deposits (D) in wall and thrombus (T) almost completely occluding lumen. Infarcted myocardium (Inf) is evident at lower right. × 400.

revealed deposits of IgG and gp70 with similar granular staining patterns in most of the nephritic glomeruli.

Castration did not affect the onset or development of disease in males, as indicated by the unchanged mortality rates, severity of glomerulonephritis, or serologic abnormalities (Figs. 3 and 4). The castrated females, on the other hand, survived significantly longer, with a reduced mortality of 10% at 10 mo of age, instead of 40% in the uncastrated group. Decreased levels of anti-ssDNA antibodies (17% in the castrated group vs. 30% in the controls) and Ig-bound gp70 (17% vs. 58%) were observed in the castrated females at 8 mo of age.

The most unexpected finding in this $F_1$ hybrid was the high incidence of degenerative coronary vascular disease; 32 of 37 males and 10 of 31 females had myocaridal infarcts at autopsy (Fig. 5a and b). The majority of these vascular and myocardial lesions were located in the right ventricle, right atrium, and left subendocardium. The vascular changes included deposition of PAS-positive material in the intima and media of affected vessel walls, which is often associated with thrombosis. Significantly, there was minimal or no inflammatory response to the injured blood vessels, although inflammatory cells diffusely infiltrated the areas of myocardial infarcts. The frequency of this coronary and myocardial injury was not significantly affected by castration.

## Discussion

(W × B)$F_1$ mice develop many of the characteristic SLE-like abnormalities found in male BXSB mice and female (NZB × NZW)$F_1$ mice, thereby constituting a single

cross in which one can study two different forms of acute disease. Clearly, the Y-linked accelerator factor of the BXSB strain is expressed fully in the male hybrid, causing an early disease that is resistant to sex hormone manipulation, as has been shown in the BXSB male (7, 8, 13). Quite separately, the noticeably prolonged life span of castrated female hybrids compared with intact females suggests that the females have a sex hormone-related SLE similar in course and character to that seen in the traditional murine SLE model of the (NZB × NZW)$F_1$ females (6). Apparently, in (W × B)$F_1$ females, the latent SLE predisposition of the NZW is complemented by the non Y-related genome of the BXSB as much as it is by the NZB in the (NZB × NZW)$F_1$. Whether there are subtle differences between the disease of the (W × B)$F_1$ mice and that of their parental and related strains remains to be determined.

The most unique aspect of the (W × B)$F_1$ disease was the very high incidence of degenerative coronary vascular disease and myocardial infarction. Similar lesions are found, but less frequently, than in other SLE murine strains (4), and have been shown by immunofluorescence and electron microscope examination to be associated with deposition of immune complexes in affected vessel walls and thrombosis (9). The conspicuous absence of a cellular inflammatory component in the vascular lesion remains unexplained, but the lesions certainly differ from the usual immunologically induced vasculitis. Significantly, similar immune complex-associated coronary disease and myocardial infarction have been documented in humans with SLE (14).

## Summary

Both sexes of the (NZW × BXSB)$F_1$ mice developed an early systemic lupus erythematosus-like disease. In males, the disease resembled that in the BXSB male parent and was not affected by sex hormonal manipulation. In females, the disease duplicated that of (NZB × NZW)$F_1$ females by virtue of a delayed onset and estrogen dependence. Autoantibody production, circulating Ig-bound gp70 immune complexes, and deposition of Ig and gp70 in the affected glomeruli were demonstrated in both males and females. The abnormally high incidence of degenerative coronary vascular disease with myocardial infarction, particularly in these $F_1$ males, provides a useful model for the investigation of a possible immunologic component in coronary vascular disease.

We thank Ms. Cathy Amundson and Ms. Lisa Schemmering for excellent technical assistance.

*Received for publication 23 March 1981.*

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