CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY 39, 81–92 (1986)

Accelerated Autoimmune Disease and Lymphoreticular Neoplasms in F_1 Hybrid PN/NZB and NZB/PN Mice

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This report describes the first studies of inheritance of autoimmunity in inbred Palmerston North (PN) mice, a model of systemic lupus erythematosus (SLE). Mating of PN mice with the nonautoimmune DBA/2 strain produced evidence that PN disease had a recessive mode of inheritance. When PN mice were crossed with autoimmune NZB mice, female offspring from both crosses developed anti-DNA antibodies and died prematurely with vasculitis, renal disease, and lymphomas. In contrast, reciprocal hybrid males had different patterns of mortality: PN/NZB males from the PN female × NZB male mating had moderately prolonged life spans, whereas NZB/PN males from the opposite cross (NZB female × PN male) had prolonged survival to the mean age of 104 weeks. To determine if testicular hormones were solely responsible for increased longevity in hybrid males, PN/NZB and NZB/PN mice were castrated at 2 weeks of age and compared to sham-operated littermate controls. Prepubertal castration did not influence longevity in PN/NZB males, but loss of gonadal hormones significantly reduced life spans in reciprocal NZB/PN males. Female hybrids were not affected by oophorectomy. Because castration changed disease expression only in male hybrids from the NZB female × PN male cross, it was concluded that parentage influenced sensitivity to the protective effects of male hormones. Although surgical sterilization had disparate effects on males, castrated PN/NZB and NZB/PN males consistently outlived oophorectomized females. The lack of clear-cut reversal of disease in males subjected to early castration suggested that nonhormonal, possibly genetic, factors contributed to longevity in both groups of male hybrids.

INTRODUCTION

The availability of a number of inbred strains of mice which spontaneously develop autoimmune disease has facilitated investigations of genetic transmission of systemic lupus erythematosus (SLE). Recently, matings between two separate autoimmune strains have been reported to produce female offspring in which autoimmunity was accelerated compared to that of the parents. When BXSB females with late-onset disease (1) were crossed with New Zealand Black (NZB) (2) males of a second autoimmune strain, female offspring had severe disease with 50% mortality at 48 weeks. Similar acceleration of disease has been reported in F_1 female hybrids produced from matings of NZB females or BXSB females with New Zealand White (NZW), a long-lived strain with late-onset lupus glomerulonephritis (1, 3). These observations were compatible with genetic complementation of SLE in F_1 females, and it has been postulated that the recessive traits which were accentuated were carried on the X chromosomes (1).

0090-1229/86 $1.50

Palmerston North (PN) mice, which were defined as a model of lupus in 1978 (4), spontaneously develop antibodies to DNA and die with glomerulonephritis and arteritis. Mean longevity is 52 weeks in females and 62 weeks in males (5). Because inheritance of autoimmune traits has not been examined previously in PN mice, experiments were undertaken to determine if inheritance of disease was dominant or recessive, and to examine possible genetic complementation in offspring of PN crossed with a second autoimmune strain.

To obtain accurate longevity data and permit valid comparison between parent strains and F_1 offspring, mice from parental strains and hybrids were followed until spontaneous death and examined by complete necropsies. We first crossed PN mice with normal DBA/2 mice and found that inheritance of PN disease was recessive. Reciprocal matings between PN and autoimmune NZB mice were then undertaken in a complementation study. Females from both crosses developed accelerated autoimmune disease. PN/NZB and NZB/PN males outlived females; survival was disproportionately long in male offspring of the NZB female × PN male mating.

Gonadal hormones influence disease in F_1 offspring of NZB mice (6, 7), and it was anticipated that hormones contributed to longevity in the current study. Examination of castrated hybrids from reciprocal crosses of PN and NZB in a separate experiment led to the conclusion that reciprocal matings of PN mice with NZB mice produced male offspring with divergent sensitivity to testicular hormones. Because male castrates consistently outlived female castrates, nonhormonal factors appeared to contribute substantially to increased longevity in hybrid PN/NZB and NZB/PN males.

MATERIALS AND METHODS

Mice. Palmerston North mice were generously contributed in 1974 by Dr. Richard D. Wigley, Palmerston North, New Zealand. New Zealand Black (NZB) mice were obtained from Mr. W. D. Hall, the University of Otago, Dunedin, New Zealand, in 1969. Both colonies are maintained by brother–sister matings. DBA/2 mice were purchased from Jackson Laboratories (Bar Harbor, Maine). Mice were kept in facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care. Experiments were conducted according to the "Guide for the Care and Use of Laboratory Animals" (1972, Institute for Laboratory Animal Resources, National Research Council–National Academy of Science).

Longevity studies. Background longevity data in parent strains were obtained by daily observation of 20 PN females and 20 PN males (described in reference (5)), 13 NZB females, and 12 NZB males. PN/NZB hybrids were produced by breeding PN females to NZB males; reciprocal NZB/PN hybrids were derived from NZB females mated to PN males. To assess early progression of disease, 10–14 PN/NZB mice and 10 NZB/PN mice of each sex were bled, killed electively, and autopsied at 24 weeks of age. Long-term study of additional groups of 30 PN/NZB females, 28 PN/NZB males, 29 NZB/PN females, and 15 NZB/PN males permitted comparison of longevity and causes of death in reciprocal hybrids of both sexes. To examine effects of prepubertal castration on disease in F_1 mice,

hybrids from reciprocal PN and NZB matings were castrated surgically at 14 days of age. Groups of 11–21 PN/NZB and NZB/PN mice of each sex were compared with equal numbers of sham-operated littermate controls. PN and DBA/2 mice were crossed in reciprocal matings to test F_1 offspring of PN and a nonautoimmune strain. The DBA/2 strain was chosen for this experiment because it shares the H-2^d haplotype with NZB (8) and, like NZB, it has high incidence of expression of RNA tumor virus group-specific antigen (9). The background incidence of positive tests for heterogeneous antinuclear antibodies in DBA/2 is 26% (10), and repeated examinations of kidneys from mature DBA/2 mice in this laboratory have shown no evidence of glomerulonephritis or vasculitis (11). Twenty-two PN/DB females, 25 PN/DB males, 24 DB/PN females, and 25 DB/PN males were studied.

All mice were examined daily for signs of disease; moribund mice were bled and killed. Complete necropsies were performed and tissue sections were processed and examined as described in an earlier publication (12). The diagnosis of amyloid was confirmed using congo red staining of selected tissues with appropriate positive controls. Severity of renal disease was scored by counting numbers of specified abnormalities in 20 glomeruli on a 4-μm cross section of each kidney, using a grading system which has effectively evaluated progressive glomerular lesions in autoimmune NZB/NZW (12) and PN mice (5).

Leukocytes were counted in the conventional manner, and blood films were stained with Wright's stain to permit identification of circulating blast forms in mice with leukemia. Serum samples were stored in sealed capillary tubes at −20°C.

Classification of abnormalities in lymphoid tissue. Hyperplasia of lymphoid tissue is common in autoimmune strains (13), and care was taken in this study not to confuse hyperplastic and intermediate (preneoplastic) changes with lymphoid malignancy. Abnormal changes in lymph nodes and spleen were classified using criteria described by Collins *et al.* (14), Goldenberg *et al.* (15), and Della Porta *et al.* (16).

Two major classifications of lymphoreticular neoplasms were recognized. Lymphoblastic lymphomas (thymic lymphosarcoma, nonthymic lymphosarcoma, and lymphocytic lymphoma) contained a fairly homogeneous population of relatively large neoplastic lymphocytes with scant cytoplasm, large round nuclei, and prominent nucleoli. Large foamy macrophages were often distributed diffusely within the tumors. Capsules of affected lymphoid organs were invaded by neoplastic cells. Composite lymphomas (histiocytic sarcoma, reticulum cell sarcoma types A and B, and mixed lymphoma) were characterized by a heterogeneous population of neoplastic lymphocytes of varying sizes with polygonal shapes, large indented nuclei, and prominent cytoplasm. Mitotic figures were often present and numerous, necrosis was not uncommon, and a nodular pattern of growth and invasion of the capsule of the involved lymphoid organ was always present. Occasionally, spindle-shaped cells were present in varying degrees. Some neoplasms contained areas of granulomatous inflammation, including neutrophils, eosinophils, plasma cells, macrophages, and giant cells.

Anti-DNA antibodies. A modified Farr assay was used to measure binding of

heat-inactivated mouse serum to ^{14}C-labeled *Escherichia coli*-derived DNA (Amersham Corp., Arlington Heights, Ill.). The DNA substrate was diluted in standard sodium citrate solution and passed through a nitrocellulose filter before use to remove single-stranded fragments. Values greater than 20% binding indicated the presence of antibodies to DNA (17, 18).

Statistical analysis. Student's *t* test was performed as described by Snedecor and Cochran (19).

RESULTS

Autoimmunity and lymphomas in 6-month-old PN/NZB and NZB/PN hybrids. Forty-four mice were sacrificed electively and necropsied to assess severity of disease in reciprocal hybrids of both sexes at the age of 24 weeks (Table 1). In the female hybrids, early acceleration of disease was reflected in increased levels and increased numbers of positive tests for anti-DNA antibodies. Proliferative glomerulonephritis was found in 17% of hybrid females; malignant lymphomas were discovered at autopsy in 21% of females.

Mortality in PN, NZB, PN/NZB, and NZB/PN mice. Mean longevity in the parent PN and NZB strains is depicted in Fig. 1. In the PN strain, the mean age at death in females did not differ significantly from males. Glomerulonephritis and vasculitis were the major causes of death (Table 2), and the incidence of neoplasms in necropsied PN mice was relatively low (12%). Life spans in NZB mice were not affected by sex. In contrast to the PN strain, NZB mice did not have proliferative glomerulonephritis and inflammatory arteritis was observed in only one animal. Lymphomas were found in 92% of NZB females and 67% of males; the high incidence of malignancy in this series was in accord with earlier reports (20, 21).

Unlike the parent strains, reciprocal hybrids had distinct sex-related differences in mortality. Death was accelerated in PN/NZB females (mean ± SEM life span 42 ± 4 weeks) compared to PN/NZB males (67 weeks ± 5) (Fig. 1). In the reciprocal NZB/PN hybrid, female mice died at the mean age of 44 ± 2 weeks

TABLE 1
ABNORMALITIES IN RECIPROCAL PN/NZB AND NZB/PN MICE SACRIFICED AT 24 WEEKS OF AGE

Group	Sex	*N*	Anti-DNA antibodies[a]	Glomerular lesion score[b]	Lymphomas[c]
PN/NZB	F	14	25 ± 2 (64)[d]	23 ± 2 (21)	14
PN/NZB	M	10	18 ± 1 (20)	17 ± 2 (0)	0
NZB/PN	F	10	33 ± 7 (80)	24 ± 2 (10)	30
NZB/PN	M	10	18 ± 1 (20)	20 ± 2 (0)	0

[a] Mean ± SEM. Parentheses enclose percentage of mice with positive tests for anti-DNA antibodies (DNA binding >20%).

[b] Mean ± SEM. Parentheses enclose percentage of mice with proliferative glomerulonephritis (glomerular lesion score ≧30).

[c] Percentage of mice with lymphomas. Two composite lymphomas occurred in PN/NZB females, and one composite lymphoma and two lymphoblastic lymphomas were found in NZB/PN females.

[d] Compared to PN/NZB males, $P < 0.005$.

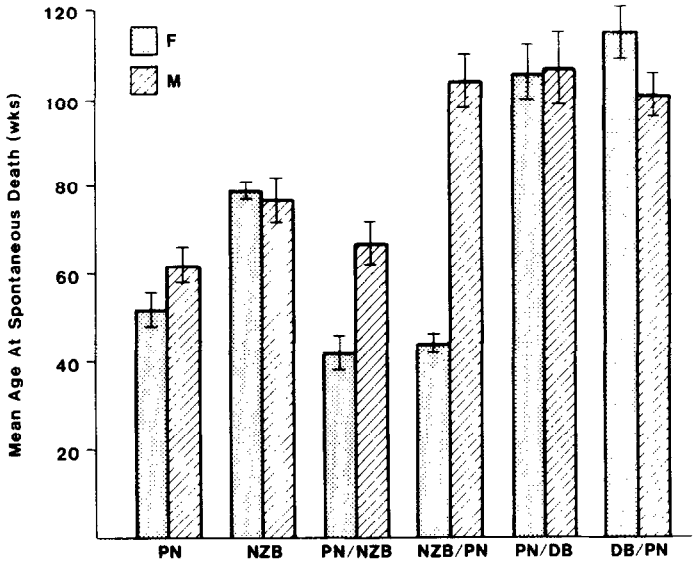

FIG. 1. Mean ages at death (±SEM) in parent PN and NZB strains and PN/NZB and NZB/PN hybrids. Mice lost by autolysis, and deaths from iatrogenic causes and infections were excluded from mortality data. Life spans were not influenced significantly by sex in PN and NZB mice. Mean longevity was clearly decreased in PN/NZB females compared to PN/NZB males ($P < 0.001$), and in NZB/PN females compared to NZB/PN males ($P < 0.001$). A striking difference in life spans was noted between long-lived NZB/PN males and reciprocal PN/NZB males ($P < 0.001$).

and NZB/PN males had extended life spans with mean survival of 104 ± 6 weeks. Although there was remarkable similarity between PN/NZB females and NZB/PN females, mortality in males from the PN female × NZB male cross was completely divergent from long-lived NZB/PN males. Mean longevity in these groups differed significantly at the 0.001 level.

Table 2 lists all causes of death in PN/NZB and NZB/PN hybrids. In PN/NZB mice, occurrences of renal disease/vasculitis and lymphomas were similar in female mice and male mice. The pattern of lymphomas in PN/NZB hybrids resembled the tumor-prone NZB strain. In these animals, lymphomas were divided equally between the lymphoblastic lymphoma and composite lymphoma categories. In contrast, causes of death in reciprocal NZB/PN hybrids were influenced strongly by sex. Accelerated autoimmune renal disease and vasculitis were found in 45% of NZB/PN females, and 17% of mice in this group developed neoplasms. The long-lived NZB/PN male hybrids developed inflammatory lesions associated with autoimmune disease late in life. Sixty-seven percent of NZB/PN males with complete autopsies had neoplasms; 90% of these lesions were composite lymphomas.

Figures 2 and 3 illustrate typical lymphomas in F_1 mice. Figures 2A and B illustrate lymphoblastic lymphomas, consisting of homogeneous populations of large lymphocytes. The heterogeneous cell populations of composite lymphomas are shown in Figures 3A and B.

Glomerulonephritis, vasculitis, and neoplasms in PN, NZB, PN/NZB, and

TABLE 2
CAUSES OF DEATH IN PN AND NZB MICE AND PN/NZB AND NZB/PN HYBRIDS

Group	Sex	N at start	Causes of death (%)[a]						
			Glomerulonephritis/ vasculitis	Neoplasm	Infection[b]	Cause not determined at necropsy[c]	Autolysis	Iatrogenic	Other
PN	F	20	80	5	10	5	0	0	0
PN	M	20	35	10	30	5	10	5	5
NZB	F	13	0	92	0	8	0	0	0
NZB	M	12	0	67	0	25	0	0	8
PN/NZB	F	30	43	27	0	3	23	0	3
PN/NZB	M	28	25	36	28	4	7	0	0
NZB/PN	F	29	45	17	0	0	31	7	0
NZB/PN	M	15	0	67	0	7	20	7	0

[a] Percentages were calculated by dividing the total number of mice in each group into the number of affected mice.
[b] This category includes PN mice dying with respiratory infections (described in reference (5)) and PN/NZB males with scrotal abscesses induced by fighting.
[c] In nine instances, the cause of death could not be determined at necropsy; five animals in this classification had advanced hyperplasia of lymphoid tissue classified as intermediate (preneoplastic).
[d] One mouse with hydronephrosis, one mouse with coronary thrombosis, and one mouse which bled to death.

NZB/PN mice. In 2 PN, 1 NZB, 7 PN/NZB, and 8 NZB/PN mice advanced glomerulonephritis, inflammatory vasculitis, and lymphomas were found at necropsy in the same animal. In these instances, it was assumed that mice bearing malignancies died of neoplastic disease. Because more than one abnormality often

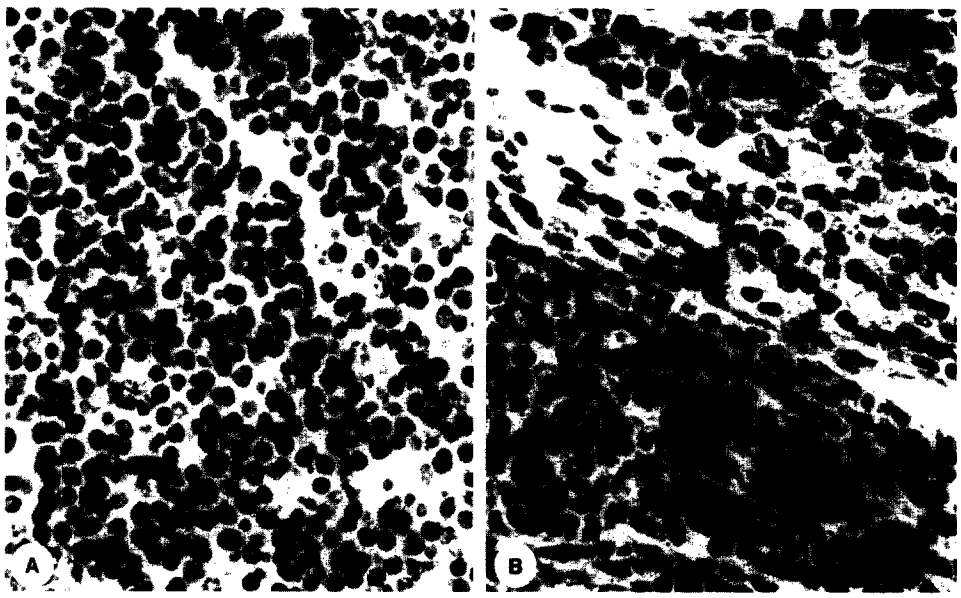

FIG. 2. (A) Typical lymphoblastic lymphoma in the lymph node of a PN/NZB male, consisting of a homogeneous population of large lymphocytes with prominent round nuclei and scant cytoplasm. Mitotic figures are common. Hematoxylin and eosin, 450×. (B) Invasion into the capsule of an involved lymph node by neoplastic lymphocytes in lymphoblastic lymphoma. The tumor is at lower left, and arrows designate invading cells. Hematoxylin and eosin, 450×.

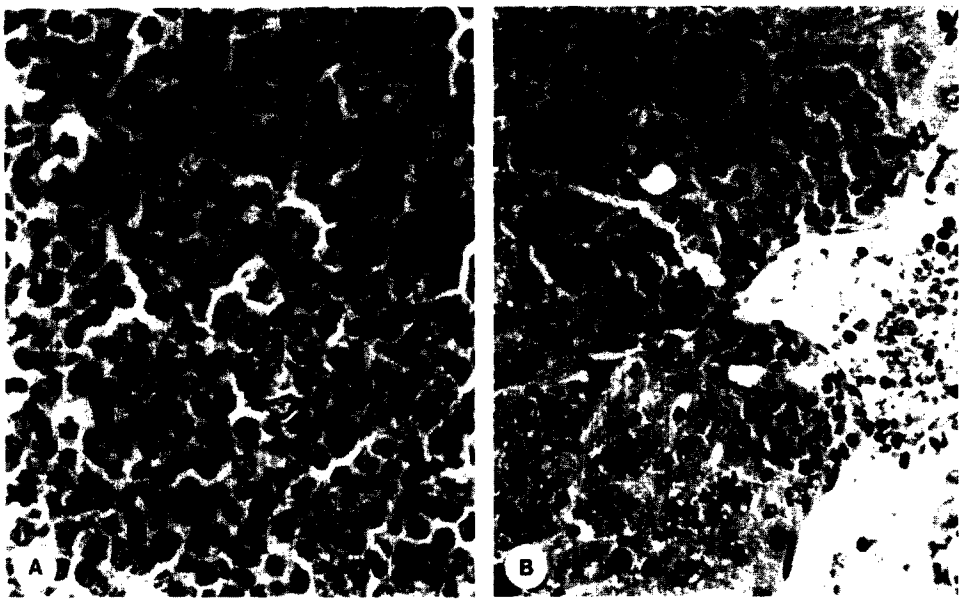

FIG. 3. (A) Composite lymphoma, characterized by a heterogeneous population of polygonally shaped lymphocytes with indented nuclei and generally prominent cytoplasm in the lymph node of a male PN/NZB hybrid. Mitotic figures are numerous. Hematoxylin and eosin, 450×. (B) Metastasis of composite lymphoma to liver. Small dark cell aggregation is composed of neoplastic lymphocytes adjacent to a central vein and hepatocytes. Hematoxylin and eosin, 150×.

existed in the same animal, glomerular lesion scores, vasculitis, and malignant lymphomas were analyzed separately (Table 3). The most advanced changes of glomerulonephritis were found in NZB/PN females; 94% of mice in this group had generalized proliferative glomerulonephritis. Necrotizing arteritis involving

TABLE 3

OCCURRENCE OF PROLIFERATIVE GLOMERULONEPHRITIS, NECROTIZING ARTERITIS, AND LYMPHOMAS IN PN AND NZB MICE AND PN/NZB AND NZB/PN HYBRIDS

Group	Sex	*N* at necropsy	Glomerular lesion score[a]	Vasculitis[b]	Lymphomas[b]
PN	F	20	33 ± 3 (53)	60	5
PN	M	17	25 ± 3 (31)	29	12
NZB	F	13	25 ± 1 (15)	15	85
NZB	M	12	17 ± 2 (0)	0	67
PN/NZB	F	23	35 ± 3[c] (65)	52	27
PN/NZB	M	20	22 ± 3 (20)	55	36
NZB/PN	F	18	44 ± 2[d] (94)	83	28
NZB/PN	M	11	24 ± 2 (45)	45	82

[a] Mean ± SEM. Parentheses enclose the percentage of necropsied mice in each group with proliferative glomerulonephritis (glomerular lesion score ≥30).

[b] Percentage of necropsied mice in each group bearing a specific abnormality.

[c] Compared to PN/NZB males, $P < 0.025$.

[d] Compared to NZB/PN males, $P < 0.001$.

lymphoid organs, ovaries, and kidneys was common in both groups of reciprocal F_1 hybrids. The high incidence of vasculitis (83%) in NZB/PN females, coupled with severe glomerular inflammation, suggested that this group had a predilection for rapidly progressive autoimmune disease.

Vasculitis involving the coronary arteries has been observed in other autoimmune strains of mice and in male hybrid offspring of NZW females × BXSB males (22). In the current study, cardiac lesions were found in 15% of PN/NZB and NZB/PN mice. Four hybrids had myocarditis, three had necrotizing coronary arteritis, and two had degenerative vascular lesions. Multifocal myocardial necrosis and periarterial inflammation each occurred in one mouse.

Anti-DNA antibodies in PN, NZB, PN/NZB, and NZB/PN mice. Figure 4 illustrates anti-DNA antibody levels in terminal serum from parent strains and F_1 hybrids. Antibodies to DNA were common in inbred PN mice; NZB mice, with two exceptions, were anti-DNA antibody negative. In PN/NZB hybrids, positive tests for anti-DNA in terminal sera were found in 47% of females and 55% of males. Severe autoimmune disease in reciprocal NZB/PN females was associated with anti-DNA antibodies in 100% of terminal sera. In long-lived NZB/PN males, anti-DNA were found in 70% of animals at the time of spontaneous death.

Mortality in PN/DB and DB/PN hybrids. F_1 hybrids from PN female × DBA/2 male and DBA/2 female × PN male crosses were examined to determine if autoimmune PN traits had simple dominant or recessive patterns of inheritance. Longevity in DBA/2 mice in conventional housing has been reported to range from 90 to 102 weeks in females and 101 to 103 weeks in males (23, 24). Life spans in PN/DB and DB/PN mice resembled the normal DBA/2 parent strain, and longevity was not influenced by sex (Fig. 1). Malignant lymphomas were the most common cause of death in these hybrids, appearing in 39% of PN/DB mice and 69% of DB/PN mice. Other hybrids died with sarcomas (8%), ovarian granulosathecal cell tumors, adenocarcinomas, and undifferentiated malignancies. Amyloid infiltration of glomeruli, hepatic vessels, and myocardium was found in 43%

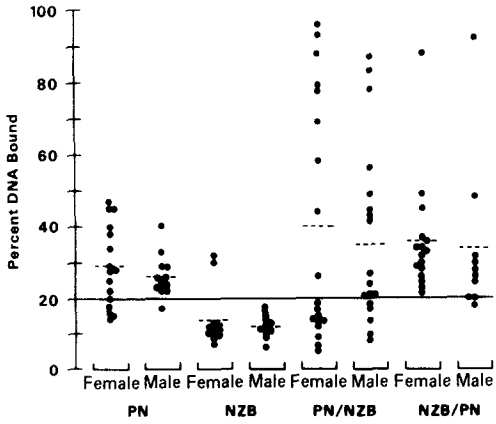

FIG. 4. Anti-DNA antibodies quantified in a Farr assay in terminal sera from parent PN and NZB strains, and reciprocal F_1 hybrid mice. Values greater than 20% binding indicate the presence of abnormal anti-DNA antibodies.

of mice in this group. Proliferative glomerulonephritis and vasculitis were uniformly absent. DNA binding was not elevated in sera from 56 mice tested at 24 weeks of age. Of 55 mice 2 had positive tests at 40 weeks of age, and 9 of 51 mice were positive at spontaneous death. In 5 instances, these elevations were minor (less than 26% binding).

Mortality in castrated PN/NZB and NZB/PN mice. Figure 5 compares mean ages of death in castrated F_1 mice and sham-operated littermate controls. This experiment afforded an opportunity to determine if observations in the first hybridization study would be repeated in separate groups of mice. Examination of intact sham-operated hybrids confirmed that female controls had earlier mortality compared to male controls; however, the sex-determined difference in life spans was statistically significant only in NZB/PN hybrids. The earlier observation that intact NZB/PN males outlived intact PN/NZB males was also confirmed in the castration study, and ages at death in both groups of male controls differed at the 0.025 level.

Detailed longevity studies of castrates and sham-operated controls provided evidence that F_1 hybrids produced by PN × NZB matings were unique models in which both hormones and genetic endowment influenced expression of disease. PN/NZB and NZB/PN females, which were unresponsive to early oophorectomy, resembled the autoimmune NZB/NZW model in which disease in females is not affected by prepubertal castration (25). Unexpectedly, F_1 males responded to castration in a manner related directly to parentage. In PN/NZB males, early removal of testes did not change mortality. In contrast, surgical sterilization ac-

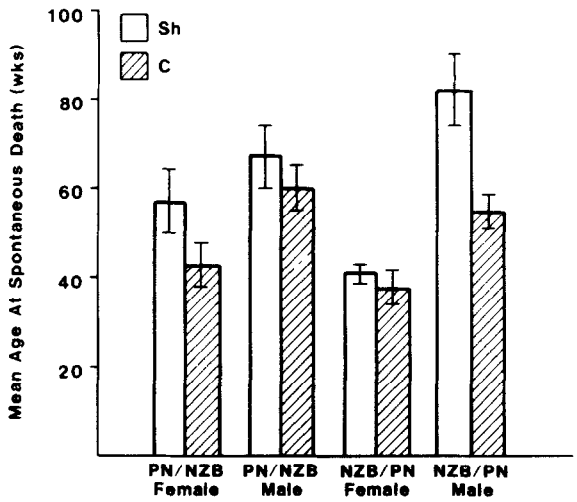

FIG. 5. Mean ages at death in a separate longevity study in which reciprocal F_1 hybrids from PN crossed with NZB were castrated surgically at 14 days of age and compared to sham-operated littermate controls. Open bars represent sham controls and shaded bars represent castrates. Intact control males outlived females in the NZB/PN group ($P < 0.001$). In accord with the earlier study, intact NZB/PN males lived longer compared to intact PN/NZB males ($P < 0.025$). Castration decreased longevity significantly only in NZB/PN males ($P < 0.05$). In both PN/NZB and NZB/PN hybrids, castrated males outlived castrated females ($P < 0.05$ and $P < 0.005$, respectively).

celerated disease in NZB/PN males—controls lived to 82 ± 8 weeks, but longevity was decreased to 58 ± 4 weeks in castrates. In both PN/NZB and NZB/PN hybrids, male castrates had longevity extended significantly compared to corresponding castrated females.

Preliminary examination of necropsy tissues from mice in the castration study showed that early sterilization did not influence occurrence of lymphomas and renal disease/vasculitis. Detailed analyses of histopathology and autoantibodies in castrated mice and controls will form the basis for another report.

DISCUSSION

This report describes the first studies of inheritance of autoimmune disease in the inbred PN mouse model of SLE. Initially, we determined that inheritance of autoimmunity was recessive. We then sought evidence of genetic complementation by breeding PN mice with the well-characterized autoimmune NZB strain. In NZB, disease results from coincidental inheritance of multiple genes, each of which contributes an abnormality such as antibodies to red blood cells, antibodies to single stranded DNA, or antilymphocyte antibodies (26–28).

The design of our experiment, which included lifetime examination of reciprocal hybrids and detailed necropsies, permitted determination of causes of death and accurate diagnosis of neoplasms in most instances. The current report emphasizes the importance of reciprocal PN/NZB and NZB/PN hybrids as new models in which severe autoimmune disease and lymphomas develop in the same animal. Of particular interest was our finding that occurrence and histological types of lymphomas were influenced by parentage and sex of the hybrids. PN females crossed with NZB males produced offspring in which the tumor incidence was 31% and numbers of lymphoblastic lymphomas and composite lymphomas were equal. In contrast, the reciprocal cross produced NZB/PN hybrids in which 67% of males developed neoplasms and the predominant lymphoma type was composite. Lymphoblastic lymphomas are T-cell malignancies (16) and composite lymphomas are composed of transformed lymphocytes and include both B- and T-cell neoplasms (14). Although the current study did not examine factors which might contribute to the preponderance of composite tumors in NZB/PN mice, these animals may be used to gain new insights into genetic and hormonal influences on cell differentiation in lymphoreticular malignancies.

When NZB mice were mated with other autoimmune strains in earlier genetic studies, NZB genes were found to interact with the abnormal genetic backgrounds of BXSB females, MRL/1 females, and NZW males to produce F_1 females with short life spans (50% mortality 36–48 weeks) (2). In the current study, crossing NZB with a second autoimmune strain, PN, had a similar result. PN/NZB and NZB/PN females had early appearance of anti-DNA antibodies and early death at the mean age of 43 weeks. It was concluded that PN resembled other autoimmune strains in that it possessed a complement of genes which interacted with the genetic contribution of NZB to produce autoimmunity with predilection for females.

Because the hybridization studies described in this report could not dissociate genetic effects from the regulatory influences of gonadal hormones, we examined

the influences of prepubertal castration on hybrids from PN and NZB. Castration of both sexes failed to abolish the pattern of early death in females and prolongation of life in males. Our findings, therefore, support complementation of X chromosomal genes from autoimmune parents as a mechanism of accelerated disease in female hybrids. This theory gains support from earlier experiments in which X chromosomal genes were found to regulate immune responses to injected denatured DNA in nonautoimmune DBA/2 and SJL mice (29).

An unexpected finding in this study was the discrepancy between mortality rates of PN/NZB males and NZB/PN males. Mean longevity in PN/NZB males was 67 weeks, but the mean life span in male NZB/PN mice was extended to 104 weeks. Based upon earlier studies in which prepubertal castration of NZB/NZW males resulted in accelerated disease (25), it was anticipated that castration would cause PN/NZB and NZB/PN males to follow the female pattern of early renal disease and premature death. Unexpectedly, castration shortened life spans only in NZB/PN males whereas reciprocal PN/NZB males had no significant change in longevity compared to corresponding intact controls. In this unique situation, in which parentage contributed to long life and sensitivity to castration in hybrid males, we hypothesize that the PN Y chromosome carried a factor influencing longevity. The expression of this factor appeared to be influenced by products of genes on an NZB autosome or on the NZB X chromosome. Because protection was obliterated by castration, it is of interest to speculate that the protective factor was expressed only in the presence of male hormones. In this situation, protection may have been mediated through hormone-sensitive segments of the immune system. Hybrid offspring of PN and NZB matings, therefore, are unique models in which interactions between specific chromosomes and gonadal hormones can be examined for effects on expression of autoimmune disease.

ACKNOWLEDGMENTS

This work was supported by the Medical Research Service of the Veterans Administration and U.S. Public Health Service Grants CA-13297, AM-25543, and AM-28568. Elaine C. Siegfried and Bernadette G. Harris were supported by the Scholarships in Medicine Program, Department of Medicine, University of Missouri, Columbia. Dr. Kier is the recipient of a National Institutes of Health Research Career Development Award DHHS 1R01RR00013. The authors appreciate the expert technical assistance of Barbara Boddy, Mrs. Margaret Ketterer, Julia Burge, and Charles Arnold. Ms. Fortune Campbell prepared the tissue sections.

REFERENCES

1. Theofilopoulos, A. N., and Dixon, F. J., *Immunol. Rev.* **55**, 179, 1981.
2. Howie, J. B., and Helyer, B. J., *In* "Advances in Immunology" (F. J. Dixon and H. G. Kunkel, Eds.), Vol. 9, p. 215, Academic Press, New York, 1968.
3. Kelley, V. E., and Winkelstein, A., *Clin. Immunol. Immunopathol.* **16**, 142, 1980.
4. Walker, S. E., Gray, R. H., Fulton, M., and Wigley, R. D., *J. Lab. Clin. Med.* **92**, 932, 1978.
5. Walker, S. E., and Schnitzer, B., *Arthritis Rheum.* **23**, 539, 1980.
6. Roubinian, J. R., Talal, N., Greenspan, J. S., Goodman, J. R., and Siiteri, P. K., *J. Exp. Med.* **147**, 1568, 1978.
7. Raveche, E. S., Steinberg, A. D., Klassen, L. W., and Tjio, J. H., *J. Exp. Med.* **147**, 1487, 1978.
8. Klein, J., "Biology of the Mouse Histocompatibility—2 Complex." Springer-Verlag, New York, 1975.
9. Diwan, B. A., Meier, H., and Huebner, R. J., *J. Natl. Cancer Inst.* **51**, 1965, 1973.

10. Barnes, R. D., and Tuffrey, M., *Nature (London)* **214**, 1136, 1967.
11. Hoffman, R. W., Alspaugh, M. A., Waggie, K. S., Durham, J. B., and Walker, S. E., *Arthritis Rheum.* **27**, 157, 1984.
12. Walker, S. E., and Bole, G. G., Jr., *J. Lab. Clin. Med.* **82**, 619, 1973.
13. Andrews, E. S., Eisenberg, R. A., Theofilopoulos, A. N., Izui, S., Wilson, C. B., McConahey, P. J., Murphy, E. D., Roths, J. B., and Dixon, F. J., *J. Exp. Med.* **148**, 1198, 1978.
14. Collins, R. D., Leech, J. H., Waldron, J. A., Flexner, J. M., and Glick, A. D., *In* "Manual of Immunology" (N. R. Rose and H. Friedman, Eds.), 2nd ed., pp. 718–733, Amer. Soc. for Microbiol., Washington, D.C., 1980.
15. Goldenberg, G. J., Paraskevas, F., and Israels, L. G., *Semin. Arthritis Rheum.* **1**, 174, 1971.
16. Della Porta, G., Chieco-Bianchi, L., and Pennelli, N., *In* "Pathology of Tumours in Laboratory Animals" (V. S. Turusov, Ed.), Publication No. 23, Vol. 2, pp. 527–536, International Agency for Research on Cancer, Lyon, 1979.
17. Walker, S. E., and Bole, G. G., Jr., *Arthritis Rheum.* **18**, 265, 1975.
18. Walker, S. E., Solsky, M., and Schnitzer, B., *Arthritis Rheum.* **25**, 1291, 1982.
19. Snedecor, G. W., and Cochran, W. G., "Statistical Methods," 6th ed., Iowa State Univ. Press, Ames, 1967.
20. East, J., de Sousa, M. A. B., Prosser, R. R., and Jaquet, H., *Clin. Exp. Immunol.* **2**, 427, 1967.
21. Yumoto, T., Yoshida, Y., Yoshida, H., Ando, K., and Matsui, K., *Acta Pathol. Japon.* **30**, 171, 1980.
22. Berden, J. H. M., Hang, L., McConahey, P. J., and Dixon F. J., *J. Immunol.* **130**, 1699, 1983.
23. Storer, J. B., *J. Gerontol.* **21**, 404, 1966.
24. Goodrick, C. L., *J. Gerontol.* **30**, 257, 1975.
25. Roubinian, J. R., Papoian, R., and Talal, N., *J. Immunol.* **118**, 1524, 1977.
26. Knight, J. G., and Adams, D. D., *J. Clin. Lab. Immunol.* **5**, 165, 1981.
27. Raveche, E. S., Novotny, E. A., Hansen, C. T., Tjio, J. H., and Steinberg, A. D., *J. Exp. Med.* **153**, 1187, 1981.
28. Bocchieri, M. H., Cooke, A., Smith, J. B., Weigert, M., and Riblet, R. J., *Eur. J. Immunol.* **12**, 349, 1982.
29. Mozes, E., and Fuchs, S., *Nature (London)* **249**, 167, 1974.

Received August 12, 1985; accepted October 4, 1985