Detection of Antibody to Denatured DNA in NZB/Bl Mice* (33959)

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(Introduced by Carl. M. Pearson)
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Mice of the inbred strain NZB/B1 spontaneously develop several manifestations of autoimmune disease, namely autoimmune hemolytic anemia, thymic germinal centers, and a low incidence of severe renal lesions. The serum of these animals contains antibodies to several normal body components, including red blood cells, nuclear constituents, and kidney components. A recent review appeared describing the disease (1). The present studies examined the serologic abnormalities in the NZB/B1 homozygotes with emphasis on anti-DNA antibodies. In these respects, the sera of NZB/B1 mouse resembles that of humans with systemic lupus erythematosus.

Materials and Methods. The NZB/B1 mice were raised at the Upjohn Company and the colony was maintained by brother–sister matings. The ICR mice were obtained from a colony raised at The Upjohn Company.

Hermatology. Bleeding from the periorbital sinus provided sufficient blood for serologic studies. More than 5% reticulocytosis was considered abnormal. The indirect Coombs procedure was performed essentially as described by Mellors (2) and the papain-treated mouse erythrocytes in this test were used as test antigens in the standard hemagglutinin assay using "Microtiter" equipment (Cooke Engineering Company).

Chemicals. Salmon sperm DNA was denatured by heating a solution of DNA (Worthington Biochemical Corporation) (1 mg/ml) at 100° for 10 min and rapidly cooling in an ice bath. Mouse globulin antisera (rabbit) and antimouse serum (rabbit) were products of Difco Laboratories. Rabbit anti-DNA antiserum was obtained by immunization with heat denatured calf thymus DNA (Worthington Biochemical Corporation) as described by Plescia *et al.* (3). Using the Ouchterlong method as modified by Tan *et al.* (4), this antiserum precipitated with single strand, heat denatured calf thymus DNA in concentrations of from 5 to 500 μg/ml. It did not react with native calf thymus DNA, native calf thymus histone, native or denatured yeast RNA, normal rabbit serum or single strand calf thymus DNA which had previously been treated with DNAse (5).

Anti-DNA antibody. Precipitating antibody to denatured (single stranded) DNA was detected by double diffusion in 0.4% Sekem agarose gel using a slight modification of the technique of Tan *et al.* (4). The wells (5 mm in diameter) were 3 mm apart (edge to edge). Two levels of denatured DNA were used in order to distinguish between low titer and high titer antibody.

Results. In Table I is shown the increasing frequency of antibody to denatured DNA with increasing age. The results are from tests on 200 individual sera. All sera which precipitated when diffused against 10 μg/ml of denatured DNA were also positive with 500 μg/ml of denatured DNA. A serum which precipitated with 10 μg/ml of denatured DNA had an end point titer of 1:4 and an end point titer of 1:8 when diffused against 500 μg/ml of denatured DNA. Sera which precipitated only with 500 μg/ml and not with 10 μg/ml of denatured DNA were only positive when tested undiluted or at a dilution of 1:4. The results indicate that low titer antibody to denatured DNA (detected only with 500 μg/ml of denatured DNA) exist in most NZB mice tested regardless of age. The frequency of this low titer antibody is less in older animals. In sera obtained from NZB mice older than 4 months of age there is a

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TABLE I. Incidence of Antibody to Denatured DNA and to Papain-Treated Erythrocytes and Incidence of Reticulocytosis in NZB/Bl Mice.

Age (months)	NZB/Bl mice (%) with antibody to Denatured DNA (μg) 10	500	Papain-treated erythrocytes	NZB/Bl mice (%) with reticulocytosis
0.5	0	100	—	—
1	0	100	0	28
2	0	100	1	22
3	2	100	2	5
4	3	100	10	0
6	18	90	5	5
7.5	55	100	15	17
8	15	60	30	20
8.5	65	100	—	—
9	20	65	55	45
11	35	75	80	67
12	30	90	76	—
12+	30	70	87	72

higher frequency of high titer antibody detected by diffusion against 10 μg/ml of denatured DNA.

In 60 sera from ICR mice of various age, only one had high titer anti-DNA antibody, but 7 sera had antibody (low titer) detectable by diffusion against 500 μg/ml of denatured DNA. The finding of occasional animals with antibody to denatured DNA, particularly in low titer, is not unexpected in normal mice since Barnes and Tuffrey (6) have shown that several mouse strains have antinuclear factor in low incidence. In the present study, all of the antibodies to denatured DNA and to erythrocytes detected in sera of NZB/Bl mice were found in that γ-globulin fraction of serum obtained by precipitation in 18% sodium sulfate.

When a positive NZB/Bl serum was treated with 0.1 *M* 2-mercaptoethanol, the precipitation with denatured DNA was abolished indicating that an IgM antibody may be at least partially responsible for the reactivity with denatured DNA.

One serum reacting with 10 μg/ml of denatured DNA did not give a line of precipitation with 10 μg/ml of native salmon sperm DNA but did give precipitation when tested with 500 μg/ml of the native DNA. Reaction to the native (double stranded DNA) was probably not due to contamination with denatured DNA since rabbit antiserum to denatured DNA did not give a precipitin line at this higher concentration of native DNA. Prior treatment of DNA with deoxyribonuclease abolished the reaction with the serum. This indicated that the NZB/Bl sera react with denatured DNA and not with a protein contaminant. A few NZB/Bl mouse sera tested by quantitative complement fixation test gave positive reactions with denatured calf thymus DNA as antigen (7).

Among 100 sera of NZB/Bl mice of various ages, which were tested by double diffusion in agarose, three contained antigen equivalent to 10 μg/ml or more of denatured DNA as detected by rabbit antiserum to denatured DNA. None of the 60 ICR mouse sera tested formed precipitins with the rabbit anti-DNA sera.

In several cases diffusion of sera of NZB/Bl mice in agarose against sera of other NZB/Bl mice resulted in precipitation. A typical plate is depicted in Fig. 1. Serum no. NZB no. 48 formed a precipitin with 500 μg/ml of denatured DNA. This precipitin line gave partial identity with a precipitin formed between NZB serum no. 48 and NZB serum no. 53. Neither NZB serum no. 48 nor 53 formed precipitins when diffused

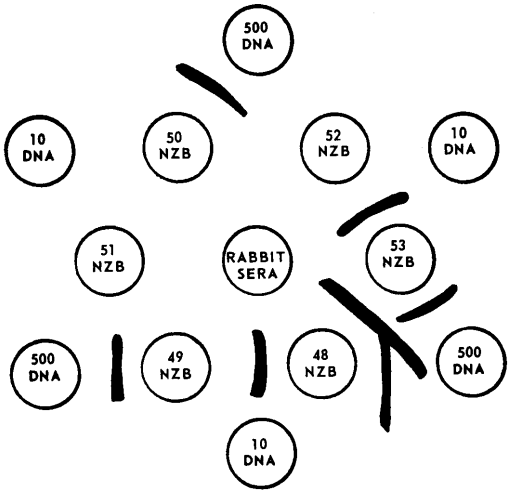

FIG. 1. Aragose gel double diffusion: the DNA was denatured and present in either 500 or 10 μg/ml as indicated; the number in the NZB wells refer to the specific sera tested.

against rabbit anti-DNA sera. Serum NZB no. 53 which precipitates with both NZB no. 48 and 52 also forms a precipitin with 500 μg/ml of single strand DNA. It would thus appear that multiple antibody systems are present in some NZB/B1 sera, some of which giving partial identity with precipitins formed against denatured DNA. The incidence of such antigen–antibody systems are currently under study.

There was no correlation between the presence of antibodies to denatured DNA, antibodies to red cells, or the presence of splenomegaly. Antibody to denatured DNA is found in mice with both high and low titers of antibodies to papain-treated mouse red blood cells. The spleen/body weight index appeared to correlate with the degree of reticulocytosis. Antibody to red blood cells does not appear until about 4 months of age, while low titer antibody to denatured DNA can be detected as early as 2 weeks of age (Table I). These observations further support the interpretation that the anti-DNA and antired cell antibodies are relatively independent immunologic abnormalities.

Discussion. The NZB/B1 mice in common with humans with systemic lupus erythematosus and mink with Aleutian disease (8) have been demonstrated to have "nuclear" antigens and antinuclear antibodies. Lambert and Dixon (9) showed that the glomerulonephritis in NZB/W hybrid mice is closely related to the production of anti-DNA antibodies. They have demonstrated the deposition of DNA antigen–antibody complexes in the kidneys of NZB/W mice. The relationship of the antigens and antibodies to the pathogenesis of disease in NAB/B1 mice is at present unknown. The reports of viral infection of the NZB/B1 mice and the detection of virus particles in 1-month-old mice (10–12) may point to the virus or to the infected host tissue as the source of circulating "nuclear antigen" detected in the NZB/B1 mice as well as in the NZB/W mice. The source of "nuclear" antigens associated with antibodies to denatured DNA found in sera from mink with Aleutian disease may be similar (13). In man, certain of the hemolytic anemias have been associated with viral infections as possible etiologic agents (14). The possible relationships between viruses and autoimmune diseases have been receiving increasing investigative and editorial attention (15, 16). In contrast to the earlier report of Lambert and Dixon (9) where DNA antigens and anti-DNA antibodies are associated with glomerulonephritis in NZB/W hybrid mice, the present study found "nuclear" antigens and antibodies to denatured DNA in NZB/B1 mice with hemolytic anemia but only minimal glomerulonephritis (1, 2).

Summary. Antibody to denatured (single stranded) DNA was detected in the serum of a majority of NZB/B1 mice. The frequency of this antibody in high titer increased in mice beyond 4 months of age. Three of 100 animals tested contained 10 μg/ml or more of single stranded DNA antigen in their sera. Additional precipitin systems were detected when sera from NZB/B1 mice were diffused against each other in agarose gel. The relationship of the antigens and antibodies demonstrated here to the pathogenesis of hemolytic disease in NZB/B1 mice is unknown. These observations should be considered in discussions of possible virus etiology and in pathogenetic mechanisms involving

similar antigen–antibody systems in glomerulonephritis.

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Effect of Chondroitin Sulfate A and Flavonoids on Hypervitaminosis D in Rats* (33960)

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Excess intake of vitamin D has been reported to have a number of adverse effects in several mammalian species. Among these effects are the toxicity response of some subjects to a relatively small excess intake of vitamin D 1), placental transfer and sensitization of the fetus to vitamin D (2), the phenomenon of calciphylaxis (3), involvement of vitamin D in cardiovascular injury (4), and interrelations of a high vitamin D intake with high lipid intake in the induction of atherosclerosis (5). These diverse effects of high vitamin D intake on the mammalian body point to a need for further studies to determine blood and tissue changes in hypervitaminosis D. The elucidation of blood parameters sensitive to vitamin D might enable detection of toxic conditions and institution of therapy before irreversible tissue changes occur. Also further work on treatments to prevent or alleviate vitamin D toxicity is needed. Two classes of substances warrant investigation. Chondroitin sulfate A (CSA) was reported to inhibit calcification (6), and flavonoids to protect animals against certain nutritional stresses (7).

In view of the above evidence the purpose of the present investigation was (i) to determine whether the erythrocyte sedimentation rate (ESR) and hematocrit are sensitive to excess vitamin D intake, (ii) to determine relations between hypervitaminosis D and blood lipid levels at normal lipid intakes, and (iii) to determine whether CSA or flavonoids (rutin and hesperidin) exert any protective effect against hypervitaminosis D.

Materials and Methods. One hundred twelve female Wistar rats, 5–6 weeks old averaging 164 g in weight, were used as experimental animals. The basal diet consisted of

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