Spontaneous Autoimmunity in Mice: Antibodies to Nucleoprotein in Strain A/J

Abstract. *An antibody to nucleoprotein, which appears to be an autoantibody, occurs in the γ-globulin fraction of serum from mice of the A/J strain. This antibody combines with nucleoprotein of several species but not with calf thymus DNA. The frequency of its occurrence increases with age and is greater in females. Serums that contain the antibody produce typical lupus erythematosus cells in vitro.*

In search for an experimental model for systemic lupus erythematosus (SLE), we have surveyed a large number of strains of inbred mice for the occurrence of antibodies to nucleoprotein. The purpose of this note is to report that such antibodies develop spontaneously in mice of the A/J strain, in the γ-globulin fraction of the serum. They appear to be autoantibodies and have the capacity to induce the formation of lupus erythematosus (LE) cells. These antibodies appear to be similar to an antibody characteristic of humans with SLE (*1*).

In our initial studies, we made a survey of serums from 17 strains of inbred mice (*2*). All animals were from breeding stocks and were 6 to 12 months old. Five pools of serums, ten mice to a pool, were tested. We searched for γ-globulin which would react with calf thymus nucleoprotein spots, or with mouse or human peripheral blood leukocyte nuclei, using fluorescein isothiocyanate-labeled rabbit antiserum to mouse γ-globulin in indirect fluorescent-antibody techniques. Aside from the fluorescent conjugate, the procedures used in testing for antibodies to nucleoproteins were identical to those described elsewhere (*3*).

Four of the five pools from the A/J strain were reactive in all three tests. Serums which reacted with homologous or heterologous leukocyte nuclei gave a homogeneous pattern of nuclear staining (Fig. 1) indistinguishable from the pattern produced by nucleoprotein antibodies of human origin. Tests for antibodies to nucleoproteins in 16 other mouse strains were negative. These strains were A/HeJ, AKR/J, C3H/HeJ, C57L/J, C58/J, DBA/1J, SWR/J, 129/J, C3HeB/FeJ, C57BR/cd, C57BL/10J, C57BL/6J, RF/J, BALB/cJ, SJL/J, and CBA/J.

The conjugated rabbit antiserum reacted only with mouse γ-globulin during immunoelectrophoresis in agar. For further confirmation that the antibody was present in the γ-globulin fraction, eluates of serum fractions from starch block electrophoresis (barbital buffer, *p*H 8.6) were tested for reactivity with nucleoprotein and leukocyte nuclei. Positive tests were obtained only with eluates which contained γ-globulin as determined by ring tests with specific rabbit antiserum.

Further serological studies were made on undiluted serums obtained from individual breeding-stock males and females aged 6 to 12 months, and from virgin males and females 6 weeks to 5½ months old. Fifty animals from each group were examined. In addition to serological studies with nucleoprotein and leukocytes, the serums were also tested for the presence of γ-globulin which would react with calf thymus DNA spots (protein concentration 0.73 percent) by means of the indirect fluorescent-antibody technique (*4*). Serums of the older animals were each examined once a week for 5 weeks. The younger animals were tested at 3-week intervals up to 5½ months. The serums of 34 percent of the multiparous females were consistently reactive with nucleoprotein and both human and mouse leukocyte nuclei, but were unreactive with DNA. Of the males of similar age 16 percent yielded identical results. Serums from young virgin animals of both sexes were negative in all tests.

In testing for the capacity of this factor to induce the formation of the lupus erythematosus cell, we used a modification of the conventional clot method. Blood was drawn from the tail vein into a capillary tube. The clot was fragmented with a 27-gauge stainless-steel wire, and incubated for 2 hours at 39°C. The tube was then centrifuged, a smear made of the buffy coat, and stained with Wright's stain. Serums of four out of six animals which possessed the antinuclear γ-globulin factor were unequivocally positive in the LE cell test as indicated by the characteristic pale staining and homogeneity of the ingested material (Fig. 2). Typical uningested homogenous material was also seen. The serums of six animals which did not possess the factor failed to induce the formatory of LE cells.

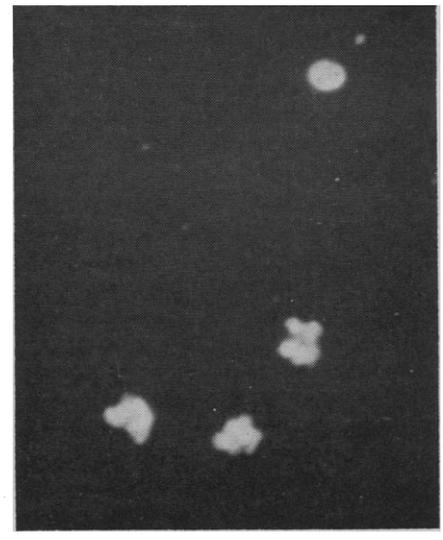

Fig. 1. Immunofluorescent preparation of human peripheral blood leukocytes acted upon by A/J mouse serum.

Our findings appear to be consistent with the occurrence of antibodies to nucleoprotein which occur in systemic lupus erythematosus of man. Familial differences in incidence of this disease have been reported (*5*). The detection of the γ-globulin factor in only one of the strains tested suggests that its occurrence could be genetically determined in mice. The influence of sex has been observed as

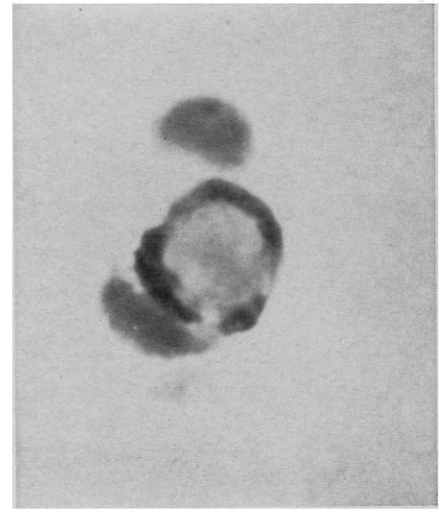

Fig. 2. Lupus erythematous cell produced by an 11-month-old mouse of the A/J strain.

a predisposing factor in humans who develop SLE and antibodies to nucleoproteins (*5*). Our preliminary data indicate that there may be similar sex differences in the occurrence of this autoantibody in A/J mice. We have not found histological changes in these mice resembling those of SLE in humans, but these studies are still incomplete.

Further serological studies have not yet revealed serum γ-globulin that will combine with heterologous DNA such as that found in patients who are acutely ill with systemic lupus erythematosus (*4*).

We have described here the occurrence of a spontaneous autoimmune-like process in A/J mice. The origin of this phenomenon is unknown. Since the A/J mouse is a widely known, commercially produced strain, not noted for any tendency toward short survival, it is evident that early mortality from a lupus-like disease does not occur in a large proportion of the population. If multiple genes are involved in susceptibility to early development of clinically manifest disease, it is possible that lethal genes have been removed from the population by selection of healthy breeding stock. This impression is supported by our preliminary histopathologic studies. It may be possible that further immunologic stimulation with appropriate antigenic material, or hybridization with the NZB strain of Bielschowsky (*6*) or other mouse strains may produce a more fully manifest disease (*7*).

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7. We have recently found antinuclear γ-globulin in serum from some 6- to 12-month-old mice of the C57BL/6J strain. Our studies do not completely exclude the occurrence of small amounts of antibody to nucleoprotein in individual mice of the negative strains listed in this report, since only pooled serum has so far been tested.
8. This investigation was supported by grants T1 AM 5254, A-4750, and 5T1A1 162 from the National Institutes of Health, U.S. Public Health Service.

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Brucella-Agglutinating Antibodies: Relation of Mercaptoethanol Stability to Complement Fixation

Abstract. Brucella-*agglutinating antibodies from selected bovine blood serums and milk samples did not fix complement, and after treatment with 2-mercaptoethanol they lost agglutinating power. After infection of calves with* Brucella abortus, *strain 19, agglutinins for* Brucella *that were inactivated by mercaptoethanol appeared earlier than those stable to mercaptoethanol. Under the conditions of these experiments, the appearance and development of complement-fixing capacity coincided closely with the mercaptoethanol stability of the agglutinins for* Brucella.

Rapidly sedimenting 12*S* to 19*S* antibodies which agglutinate *Brucella abortus* antigen have been reported in the serum (*1*) and milk (*2*) of certain individual cattle. These have been differentiated from the common 7*S* agglutinins on the basis of ultracentrifugation, isoelectric point, heat lability, and loss of activity upon treatment with 2-mercaptoethanol (ME).

After parenteral injection of cattle with living *Brucella abortus*, strain 19, a class of agglutinating antibodies that is inactivated by mercaptoethanol (ME-susceptible) and by heat has been observed to appear earlier and persist longer than a class that is resistant to these treatments (*3*). Complement-fixing antibodies for *Brucella* have been reported to appear later and recede earlier than agglutinating antibodies after inoculation of cattle with *Brucella abortus,* strain 19, vaccine (*4*).

This report describes experiments concerning the relation between complement fixation and the susceptibility of *Brucella*-agglutinating antibodies in bovine serum and milk to treatment with mercaptoethanol.

Bovine serums were tested by the USDA 48-hour standard tube agglutination test (*5*) accompanied by a duplicate tube test containing 0.05*M* mercaptoethanol in each serum-antigen dilution from 1:25 to at least one tube dilution greater than the end titer of the tube agglutination test. Bovine milk samples, with and without prior treatment with 0.1*M* mercaptoethanol for 18 hours, were tested by the USDA serial dilution *Brucella* ring test (*6*).

In the complement-fixation test, 0.2-ml portions of appropriately standardized reagents were used. The fixation period was 60 minutes at 37°C. Three units of complement were used in the test proper, but to avoid possible compounding of anticomplementary effects, one-half amounts were used in both serum and antigen controls. The procedure was further modified by adding 0.01 ml of fresh normal bovine serum to each test and to the antigen control (*7*).

For the first experiment antibody preparations were isolated from bovine serums and wheys in which *Brucella* agglutinins were susceptible to inactivation by mercaptoethanol and also by heat (*3*). The preparations were isolated by adsorption on *Brucella* antigen followed by elution with water acidified to *p*H 3.5 with acetic acid and, after further purification (*2*), added to serums and milk without antibodies to *Brucella* to obtain adjusted titers of 1:100 for the two tests. In contrast, these preparations had complement-fixation titers of less than 1:4. Exposure of the adjusted preparations to mercaptoethanol decreased their serum agglutination test and milk ring test titers to less than 1:25.

In the second experiment, *Brucella*-agglutinating serums from five cows and milk from three cows were examined with the results presented in Table 1. Agglutination titers varying from 1:3200 to 1:50 were observed for these serums and milk samples. Similar titers were observed for serums 1 and 2 and milk 8 by the complement-fixation test and also after exposure to mercaptoethanol in the tube agglutination test. In contrast, for serums 3, 4, and 5 and milk samples 6 and 7, reactions at 1:4 and greater were not observed with the complement-fixation test and, after exposure to mercaptoethanol, agglutination titers decreased to less than 1:25 for the tube agglutination and ring tests.

In the third experiment a 5-month-old calf was injected subcutaneously with vaccine containing approximately 80×10^8 living organisms of *Brucella abortus*, strain 19. Serum, obtained from blood collected at intervals as indicated in Table 2, was examined by the tube agglutination test, with and without exposure to mercaptoethanol, and also by the complement-fixation test. *Brucella*-agglutinating antibodies were first observed with the tube agglutination test in serum collected on day 3 after injection, at a dilution of 1:25 or greater. Antibodies with agglutination activity in the presence of