DISTRIBUTION OF DNA-REACTIVE SERUM FACTOR IN HEALTHY MINK AND MINK WITH ALEUTIAN DISEASE

E. G. Vasil'eva, V. K. Podgorodnichenko, L. M. Tsyplyakovskaya, A. M. Poverennyi, and V. A. Nasonova*

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A factor reacting with DNA and, in 62% of cases, with dextran sulfate was found in the gamma-globulin fraction of sera from mink with Aleutian disease or with no visible evidence of it. During chromatography of mink sera on a column with Biogel P-200, only the fractions of the first peak, consisting chiefly of a component with sedimentation constant 19S, were able to react with DNA. The presence of the factor and its ability to be neutralized by DNA preparations did not correlate with the hypergammaglobulinemia and were independent of the state of the animals.

KEY WORDS: *Aleutian disease of mink; anti-DNA antibodies.*

Aleutian disease of mink is similar in many respects to systemic lupus erythematosus (SLE), in which the immunologic system of the body is disturbed and, as a result, autoantibodies against various cell components appear [1, 8]. In particular, a spectrum of antibodies against various structures of nucleic acids is present in the sera of patients with SLE. There is reason to suppose that these antibodies participate in the pathogenesis of SLE and that their appearance or disappearance can be used as a diagnostic and prognostic sign of that disease [3]. Ability to react with DNA is a property of the serum of mink affected with Aleutian disease [8].

The distribution of a serum factor reacting with DNA in mink and the relationship between ability of the sera to react with DNA and manifestations of the disease were investigated.

EXPERIMENTAL METHOD

Sera reacting with DNA were detected by the passive hemagglutination test (PHT) with erythrocytes sensitized with DNA that had been denatured in the presence of formaldehyde by the method described earlier [4]. The properties of the sera were then investigated by the antibody neutralization test (ANT) [4]. The methods of obtaining, identifying, and denaturing the DNA were described previously [5].

On the basis of the results of the ANT the sera were placed in 1 of 3 types, using the classification suggested earlier [6].

The fraction of immunoglobulin G was obtained by chromatography on DEAE-cellulose [2] after preliminary salting out of the gamma-globulin with ammonium sulfate in a

*Corresponding Member, Academy of Medical Sciences of the USSR.

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TABLE 1. Results of Comparative Investigation of Sera from "Healthy" and Affected Mink (sample data)

Index	Mink with typical necropsy findings of Aleutian disease					Mink with no visible changes at necropsy					
	№ 1—717	№ 1—778	№ 1—1245	№ 0—421	№ 1—818	№ 1—703	№ 2—1500	№ 0—526	№ 0—488	№ 2—1423	№ 2—1715
Iodine-agglutination test	+++	+++	+++	+++	+++	Neg.	Neg.	Neg.	+	Neg.	Neg.
Concentration of gamma-globulin (in %)	39,0	59,4	37,7	36,6	50,1	9,4	12,6	16,9	20,1	15,5	15,9
Titer in PHT	1/1280	1/640	1/2560	1/2560	1/1280	1/1280	1/640	1/80	1/40	1/80	1/160
Type of serum	II	I	III	I	II	III	III	I	I	II	I

Note. Neg. — result negative.

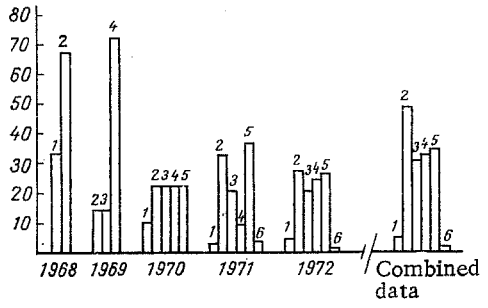

Fig. 1. Passive hemagglutination test with sera of "healthy" and affected mink in different age groups. Ordinate, number of sera with a particular titer as a percentage of total number of sera tested: 1) 1/10; 2) 1/10-1/20; 3) 1/40-1/80; 4) 1/160-1/320; 5) 1/640-1/1280; 6) 1/2560; abscissa, year of birth of mink (sera tested at the same time in 1973). Distribution of mink whose sera were tested by year of birth: 3 in 1968, 7 in 1969, 40 in 1970, 31 in 1971, and 132 in 1972.

concentration of 40%. The mink sera were fractionated on a column (1.8 X 80 cm) with Biogel P-200 (Bio-Rad, USA) in 0.15 M NaCl.

The sensitivity of the factor reacting with DNA to 2-mercaptoethanol was tested as follows: 0.2 ml of the serum was treated with 2.05 ml of 0.15 M NaCl and 0.25 ml of a 1 M solution of 2-mercaptoethanol. After incubation for 3 h at room temperature the mixture was dialyzed against 0.15 M NaCl and used in the PHT.

The concentration of gamma-globulin in the blood serum was determined by electrophoresis on paper.

EXPERIMENTAL RESULTS

Altogether 213 sera from minks with no visible evidence of the disease ("healthy") and mink of different ages with the disease were tested. In 95% of cases the sera of both the affected and healthy mink had the property of agglutinating sensitized erythrocytes in titers of 1:10 or more (Fig. 1). The factor reacting with DNA was discovered in animals of different ages. When mink sera in a positive iodine-agglutination test, with a raised content of gamma-globulin and a typical pathological picture of the disease, were compared with sera from mink in which the features were absent, by comparison in the ANT, no difference was found. In both groups sera of three types were found: those capable of neutralization I) by native DNA, II) by DNA denatured by boiling, and III) by DNA denatured by boiling in the presence of formaldehyde (Table 1).

To study the role of the charge on the reaction between DNA and the sera, the

ability of dextran sulfate and DEAE-dextran (mol. wt. 2 X 10^6, Pharmacia, Sweden) to inhibit the reaction between the mink sera and DNA was studied. DEAE-dextran did not react with the test sera. Dextran sulfate, in 62% of cases, inhibited the reaction between the sera and DNA, but in somewhat larger amounts (0.06-6 μg) than were required to inhibit the reaction with DNA denatured in the presence of formaldehyde (0.015-0.12 μg). The sera reacting with dextran sulfate were distributed among all three types.

Much of the observed agglutination of DNA-sensitized erythrocytes by the mink sera could thus be attributed to nonspecific (due to charge) interaction with DNA. To establish the nature of the factor reacting with DNA the mink sera were fractionated with ammonium sulfate. Since all the activity remained in the gamma-globulin fraction after this fraction had been salted out with ammonium sulfate at 40% saturation, it was concluded that the factor is an immunoglobulin. The immunoglobulin G fraction isolated from the mink sera was unable to react with DNA. On chromatography of the sera on a column with Biogel P-200, only the fractions of the first peak were able to react with DNA. Since the principal component of this peak has a sedimentation constant of 19S [2], the results suggest that the factor is a macroglobulin. This hypothesis was confirmed in experiments with 0.1 M 2-mercaptoethanol. After incubation with 2-mercaptoethanol the titer of the mink sera fell from 1/1280-1/640 to 1/160-1/40, whereas the anti-DNA antisera were unable to react with DNA.

A factor interacting with DNA has been found in the sera of various animals, healthy human subjects, and patients [6]. The mechanisms of origin of this factor may differ. Aleutian disease of mink is known to be a virus disease and the object of viral aggression is the plasma cells [1, 9]. Since the initial proliferation of plasma cells follows the lines of multiclonal hyperplasia [10], some of the transformed plasma cells may perhaps synthesize abnormal gamma-globulins, which react with DNA and with various polyanions. It is less easy to explain the widespread distribution of the factor reacting with DNA among "healthy" mink. One possibility is that these mink are in fact carriers of virus infection. The raised level of anti-DNA antibodies in the mink could perhaps reflect a general increase in the autoantibody content. This is suggested, in particular, by the fairly widespread distribution of antigammaglobulin factor in "healthy" and diseased mink [11].

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