Immunology, 1969, **16**, 241.

'Nuclear' Antigens and Antinuclear Antibodies in Mink Sera

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(*Received 5th August* 1968)

Summary. Aleutian disease of mink is transferrable by cell-free extracts and is characterized by hepatitis, vasculitis, nephritis, and hypergammaglobulinaemia. Because of increasing evidence incriminating antigen–antibody complexes in vasculitis disorders, the presence of nuclear antigens and antinuclear antibodies in mink sera was investigated.

Serum pools as well as individual serum specimens were obtained from uninfected mink, mink with experimentally induced Aleutian disease, mink with spontaneous Aleutian Disease, all of genotype Aa as well as from 'normal' mink of similar age from colonies without Aleutian disease.

The serum pool from mink before and after experimentally induced Aleutian disease appeared to contain 'nuclear' antigens detectable by rabbit anti-DNA antibodies in complement fixation and precipitin tests. The protein-free extracts of these serum pools gave strong reactions for deoxypentose in the diphenylamine tests. These serum pools also were shown to contain antinuclear antibodies by the immunofluorescence tests on human leucocyte nuclei and in precipitin tests against single strand calf thymus DNA. Sera from individual mink were similarly shown to contain 'nuclear' antigens and antinuclear antibodies. The incidence and quantity of antigens and antibody detected were much greater in sera from mink after experimentally induced disease than in sera taken from mink before inoculation. The presence of 'nuclear' antigens and antinuclear antibodies did not correlate with the degree of hypergammaglobulinaemia. Sera from 'normal' mink in colonies without overt disease had neither antigens nor antibodies detectable in precipitin tests. Sera from mink with spontaneously acquired Aleutian disease had a high incidence of 'nuclear' antigens and anti-DNA antibodies detectable in precipitin tests.

The 'nuclear' antigens were detectable in Ouchterlony precipitin tests by specific rabbit anti-DNA antibodies. The precipitins formed lines of partial identity with those between the rabbit anti-DNA antibodies and single strand calf thymus DNA. However, the antigens in mink sera were not destroyed by prior incubation with DNAse which had been the case with DNA antigens detected in some human and mouse sera.

The antinuclear antibodies were detected in immunofluorescence tests using specific antibodies to mink γ-globulins, were shown to fix complement with single strand calf thymus DNA, but not with DNA that had been digested with

DNAse, and formed precipitins with single strand calf thymus DNA which showed complete identity with precipitins formed by rabbit anti-DNA antibodies. Evidence for the simultaneous presence of 'nuclear' antigens with antinuclear antibodies in the serum from mink with Aleutian disease was frequently evident. This observation is consistent with the hypothesis for the pathogenetic role of antigen-antibody complexes.

Aleutian disease of mink has certain clinical pathological and serological similarities with disease in New Zealand Black mice and in man with systemic lupus erythematosus. Since Aleutian disease of mink and disease of New Zealand black mice may both be examples of 'slow virus' infections, a similar aetiology should be considered for certain autoimmune diseases of man, e.g. systemic lupus erythematosus.

INTRODUCTION

Antibodies to nuclear constituents have now been described in the sera of man (Friou, 1958; Holman and Deicher, 1959; Alexander, Bremmer and Duthie, 1960; Barnett, Condemi, Leddy and Vaughan, 1964; Seligmann, Connat and Hamard, 1965), mouse (Helyer and Howie, 1961; Holmes and Burnet, 1963; Norins and Holmes, 1965), dog (Lewis, Schwartz and Henry, 1965), chicken (Bardawil, Gallins and Gavalle, 1965) and the rabbit (Barnett and Vaughan, 1966). Of the many nuclear antigens reacting with antinuclear antibodies in serologic tests, only desoxyribonucleic acid (DNA) has been detected in human sera (Tan, Schur, Carr and Kunkel, 1966). Several reports (Graf and Koffler, 1966; Krishman and Kaplan, 1967; Koffler, Schur and Kunkel, 1967) have strongly implicated sequestration of DNA–anti-DNA complexes at the basement membrane as operative in the pathogenesis of the nephritis in systemic lupus erythematosus. This is also thought to be the mechanism for nephritis seen in NZB mice (Mellors and Huany, 1966; Lambert and Dixon, 1968). Aleutian disease of mink is characterized by plasma cell hepatitis, vasculitis, nephritis and hypergammaglobulinaemia (Hartsough and Gorham, 1956; Helmboldt and Jungherr, 1958; Henson, Leader and Gorham, 1961; Porter, Dixon and Larsen, 1965; Williams, Russell and Kenyon, 1966). The disease can be readily transferred from mink to mink by cell-free extracts of tissues, urine, or saliva, as well as by serum or various serum fractions (Henson, Williams and Gorham, 1966). Recently, evidence has been obtained by filtration studies which would suggest that the agent may be less than 5 mμ in size (Buko and Kenyon, 1967). However, size estimates obtained by ionizing irradiation suggest a target size somewhat larger, but still in the class of extremely small viruses (Kenyon and Hutchinson, unpublished data). Although Aleutian mink of the genotype Aa are most susceptible, Aleutian disease can be transmitted to mink of a variety of genotypes and even to a related mustelid, the ferret (Kenyon, Howard and Buko, 1966). Because of increasing recent evidence incriminating antigen–antibody complexes in the pathogenesis of vasculitis (Dixon, Feldman and Vasquez, 1961; Krishman and Kaplan, 1967; Koffler *et al.*, 1967; Barnett, 1968b), the presence of nuclear antigens as well as antinuclear antibodies in mink sera was investigated.

MATERIALS AND METHODS

Antigens

Calf thymus deoxyribonucleic acid (DNA) shown to be free of protein was obtained

from Dr Alexander Dounce, University of Rochester, School of Medicine and Dentistry and from Worthington Biochemical Corporation, Freehold, New Jersey. Protein-free T_4 phage DNA was obtained from Dr Verne Schumacker, Department of Chemistry, University of Los Angeles. DNA of human liver origin, human liver nucleoprotein, calf thymus histone and DNA of rat liver origin were all generously provided by Dr Dounce. Single strand DNA was prepared by heating at 100° for 10 minutes and then rapidly chilling in an ice bath. Yeast ribonucleic acid (RNA) was obtained from Worthington Biochemical Corporation.

Mink Sera

Sera obtained from mink before infection with Aleutian disease were collected under sterile technique from mink housed at Storrs, Connecticut and Pullman, Washington. Injections of cell-free spleen extract from infected animals were then given to normal uninfected mink of genotype Aa. Ten weeks later all injected animals showed manifest clinical signs of Aleutian disease and serum samples were again obtained. Every precaution was taken to avoid haemolysis and during collection of serum samples all sera were promptly separated from clots. Sera were also obtained from mink of genotype Aa without clinical disease. All serum samples were inactivated at 56° for 30 minutes prior to testing.

Antisera

Antisera capable of detecting DNA were prepared by immunization of rabbits with single strand calf thymus DNA complexed to an equal amount of methylated bovine or rabbit serum albumin and administered in complete Freund's adjuvant (Plescia, Braun and Palczuk, 1964). Human sera from patients with systemic lupus erythematosus and chronic active hepatitis were chosen from their ability to precipitate in agarose gels with single strand and native calf thymus DNA. These three types of antisera capable of reacting with single strand calf thymus DNA had been characterized in previous studies (Barnett, 1968a, b). Both rabbit antisera and human sera with anti-DNA specificities were used for the detection of nuclear antigens in the mink sera studied.

Enzymes

Desoxyribonuclease and ribonuclease for digestion experiments were obtained from Worthington Biochemical Corporation. Digestion was performed at 37° for 1 hour in the presence of magnesium and calcium ions. One microgram (1 μg) of DNAse was used per 2 μg of DNA. Trypsin (obtained from Nutritional Biochemical Corporation) was used for digestions at 37° (Barnett, 1968a).

Serological tests

Quantitative complement fixation was performed by the methods of Wasserman and Levine (1961) was modified by Stollar and Standberg (1966).

Immunofluorescence tests for antinuclear antibodies

All mink sera were screened in a three-layer test for the detection of antinuclear antibodies (Barnett *et al.*, 1964). Three drops of mink serum dilution were applied to human peripheral blood smears and incubated for 30 minutes at room temperature. After washing, two drops of specific rabbit anti-mink γ-globulin were applied at a dilution of 1 : 10. The slides were then incubated for $\frac{1}{2}$ hour and then again washed in buffered saline.

One drop of fluorescein-conjugated goat anti-rabbit γ-globulin (Hyland Laboratories, Lot No. 70–898) were then applied at a dilution of 1 : 20. Re-incubation for ½ hour at room temperature was followed by washing in buffered saline and mounting in glycerol–saline under a glass coverslip. Appropriate controls were included by omitting anti-mink sera, or rabbit anti-mink γ-globulin or fluorescein-labelled goat anti-rabbit γ-globulin.

Precipitin tests

Precipitating antibody to DNA was detected in Ouchterlony systems using 0·4 per cent agarose and phosphate-buffered saline, pH 7·3, as described tby Tan *et al.* (1966). Antisera containing precipitating antibodies to various nuclear antigenic constituents included both rabbit and human sera described above.

Chemical tests for DNA

An equal volume of 20 per cent perchloric acid was added to mink serum and the mixture heated to 70° for 15 minutes. The mixture was then spun at 1800 rev/min for 20 minutes, the supernatant removed, and the material centrifuged again at 1800 rev/min for 45 minutes. A calf thymus DNA preparation was similarly treated with hot perchloric acid. The supernatants were then tested in the diphenylamine tests by the modification of Giles and Myers (1965).

RESULTS

STUDIES ON POOLED SERA

Rabbit antisera capable of fixing complement or precipitating with single strand and native calf thymus DNA (Barnett, 1968a, b) were used to detect antigens in two pools of mink serum. The first pool of pre-Aleutian disease serum was made from sera obtained from individual members of a mink colony known to have a high incidence of spontaneous Aleutian disease. The mink were then inoculated with a cell-free filtrate of spleen tissue from animals with Aleutian disease. Ten weeks later all of the recipient mink had acquired

TABLE 1

ANTIGEN AND ANTIBODY CONTENT OF A POOL OF MINK SERA OBTAINED PRIOR TO ALEUTIAN DISEASE (PRE-AD POOL) AND ANOTHER POOL TAKEN AFTER ALEUTIAN DISEASE (POST-AD POOL) (SEE TEXT)

Pre-AD pool				Post-AD pool			
Antigen content							
C′ f c̄	Ppt c̄	Ppt c̄	Diphenylamine test	C′ f c̄	Ppt c̄	Ppt c̄	Diphenylamine test
Rab 44	Rab 44	Rab 33		Rab 44	Rab 44	Rab 33	
1 : 800*	1 : 10†	1 : 10	92 μg/ml	1 : 800	1 : 10	1 : 10	39 μg/ml
Antibody content							
			ANA-Fluor. 1 : 16‡	XDNA-Ppt§ 1+			
			ANA-Fluor. 1 : 64	XDNA-Ppt 4+			

* 1 : 800 dilution of the mink serum pool gave a strong complement fixation reaction with rabbit anti-DNA serum No. 44.

† A 1 : 10 dilution of the mink serum pool gave a definite precipitin line with rabbit anti-DNA serum No. 44.

‡ The pre-AD pool at a dilution of 1 : 16 gave bright, nuclear fluorescence in the three layer antinuclear antibody test.

§ An undiluted specimen of the pre-AD pool gave a definite precipitate with single strand calf thymus DNA in a concentration of 10–500 μg/ml. 4+ refers to a very strong precipitate.

overt Aleutian disease. At this time, they were again bled and the sera pooled. As shown in Table 1, the sera labelled pre-AD pool and the post-AD pool from animals bled 10 weeks after inoculation with infectious material were investigated in a variety of tests.

At a dilution of 1 : 800, the pre-AD pool fixed complement with rabbit antiserum No. 44. This antiserum had been obtained from a rabbit immunized with single strand calf thymus DNA complexed with methylated rabbit serum albumin (see 'Materials and methods'). Rabbit antiserum No. 44 produced greatest complement fixation with single strand calf thymus DNA, but also fixed complement with large amounts of native calf thymus DNA, and nucleoprotein. A 1 : 800 dilution of the post-AD pool of mink serum fixed complement with rabbit antiserum No. 44. Both the pre- and post-AD pools of mink sera, when used at dilutions of 1 : 10, formed precipitins in agarose gels with rabbit antisera Nos. 44 and 33. Rabbit antiserum No. 33 (also made against single strand calf thymus–DNA complexes with methylated bovine serum albumin) showed a similar specificity to that of antiserum No. 44, but was capable of detecting much smaller amounts of single strand DNA. Diphenylamine tests on the protein-free supernatants of the pre-AD pool indicated optical density readings equivalent to 92 μg/ml of calf thymus DNA. The post-AD pool produced a reaction equivalent to 39 μg of calf thymus DNA. In immunofluorescent studies the pre-AD pool produced nuclear fluorescence with human white blood cell nuclei at dilutions up to 1 : 16, while the titre of the post-AD pool tested simultaneously was 1 : 64. A typical nuclear fluorescence pattern is shown in Fig. 1. This was generally a peripheral staining or shaggy fluorescence pattern (Bickel, Pearson and Barnett, 1967). In agarose gels the pre-AD pool of mink serum produced a weak precipitin line with 500 μg/ml of single strand calf thymus DNA while the post-AD pool gave a thick precipitin line with 500 μg/ml concentration of single strand calf thymus DNA. From these results it appeared that nuclear antigens detectable by two types of rabbit anti-DNA antibodies and containing deoxyribose as measured in the diphenylamine test were present in the same serum pools as antibodies detectable in immunofluorescence tests on white blood cell nuclei or in precipitin tests with single strand calf thymus DNA.

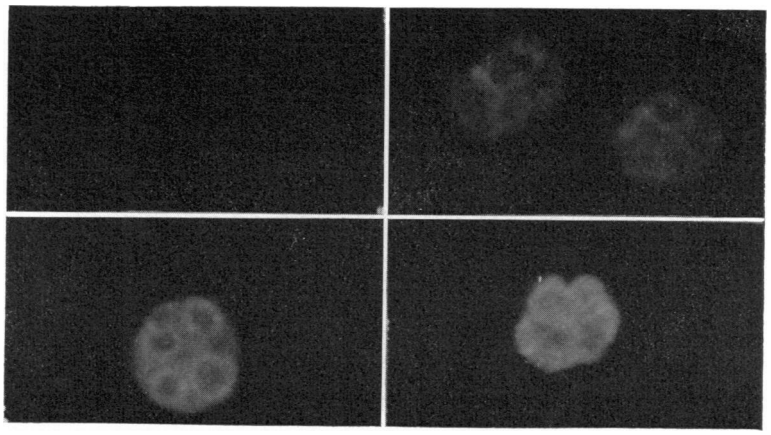

FIG. 1. Indirect immunofluorescence tests obtained with mink sera. × 500. Top left, No immunofluorescence obtained with mink serum number H 1472 obtained before Aleutian disease. Bottom left, 1+peripheral nuclear immunofluorescence obtained with serum H 1472 after Aleutian disease. Top right, 2+nuclear fluorescence obtained with serum H 1468 after Aleutian disease. Bottom right, 4+nuclear fluorescence obtained with serum G 6520 after Aleutian disease.

TABLE 2

ANTIGEN AND ANTIBODY CONTENT OF SERA OBTAINED PRIOR TO (PRE-AD) AND AFTER (POST-AD) EXPERIMENTALLY INDUCED ALEUTIAN DISEASE (TOTAL NUMBER + /NUMBER TESTED)

	Pre-AD						
Antigen content							
C′ f c̄ Rab 44	Ppt c̄ Rab 44	Ppt c̄ Rab 33	Ppt c̄ H.-C.H.*	C′ f c̄ Rab 44	Ppt c̄ Rab 44	Ppt c̄ Rab 33	Ppt c̄ H.-C.H.
15/15	0/10	0/3	9/10	13/15	1/10	8/8	6/10
Antibody content							
				ANA-Fluor.	XDNA-Ppt		
				6/16	0/16		
				ANA-Fluor.	XDNA-Ppt		
				16/16	11/12		

* Human chronic active hepatitis serum.

These data were subsequently expanded by studies of individual animals (Table 2). Because of limitations engendered by the small amounts of sera available in some instances, all test systems could not always be applied. When tested prior to Aleutian disease fifteen animals showed complement fixation with rabbit antiserum No. 44; following onset of Aleutian disease, thirteen of these same animals showed complement fixation with this rabbit antiserum to single strand DNA. Although no precipitin lines in agarose gels were given by mink sera before onset of AD, one of ten tested following onset of manifest Aleutian disease gave lines with rabbit antiserum No. 44 and eight of eight tested gave lines with rabbit antiserum No. 33. When human serum from a case of chronic active hepatitis (Barnett, 1968a) containing antinuclear antibodies was used as antiserum, nine of ten mink sera tested (pre-AD) precipitated, and after Aleutian disease had become established six of ten precipitated with the human serum. It had previously been shown (Barnett, 1968a) that this human serum not only precipitated with DNA-containing antigens, but also precipitated with non-DNA antigens such as histone.

In the immunofluorescence tests, six of the sixteen pre-AD sera gave nuclear fluorescence, while all sixteen of the post-AD sera produced bright nuclear fluorescence. None of the sixteen pre-AD sera showed agarose gel precipitins with single strand calf thymus DNA tested at 500 μg/ml, while eleven of twenty-two post-AD sera available for testing showed precipitins with single strand calf thymus DNA at 500 μg/ml (Table 2).

As shown in Table 3, no correlation was noted between quantity of serum immunoglobulin G as measured by Oudin quantitation (Huntley, 1963) and the amount of antigen detected in the complement fixation tests. The mink sera which produced the brightest nuclear fluorescence after AD had become established tended to show concomitant precipitating antibody to DNA (Table 4).

Selected mink sera were tested by quantitative complement fixation tests against a variety of nuclear antigens. As shown in Fig. 2, serum from AD mink No. 26 in a dilution of 1 : 50 produced 80 per cent complement fixation with 0·015 μg/ml of single strand calf thymus DNA. The quantitative complement fixation curve achieved with a rabbit antibody to single strand DNA (Barnett, 1968a, b) is shown for comparison. Serum from AD mink No. 26 required much more native calf thymus DNA for complement fixation. No complement fixation was detected between AD serum No. 26 in a dilution of 1 : 50 and native DNA of human liver origin, desoxyribonucleoprotein of human liver, single strand T_4 phage DNA, calf thymus histone, nor single strand yeast RNA. Furthermore, no

reaction was detectable with previously reactive single strand calf thymus DNA which had been digested with DNAse.

The above observations were extended with respect to other sera obtained from normal apparently uninfected mink as well as mink with spontaneous presumably naturally

TABLE 3

IMMUNOGLOBULIN G CONTENTS OF SERA FROM MINK BEFORE AND AFTER EXPERIMENTALLY INDUCED ALEUTIAN DISEASE, CORRELATED WITH 'NUCLEAR' ANTIGEN CONTENT

Mink No.	Before AD				After AD			
	IgG	AG* (μ)	Ppt† AG 1 : 10 R44	Ppt‡ AG 1 : 10 H.-C.H.	IgG	AF (μ)	Ppt AG 1 : 10 R44	Ppt AG 1 : 10 H.-C.H.
H 1472	3500	0·3	Neg.	+	4000	3·7	Neg.	+
H 1469	1900	2·6	Neg.	+	5100	3·6	Neg.	+
H 1456	1050	0·4	NT	NT	3500	0	NT	NT
H 1452	2200	1·0	Neg.	+	4500	3·0	+	+
H 1457	810	1·6	NT	NT	4500	1·0	NT	NT
G 6521	1900	2·6	Neg.	+	5750	1·3	Neg.	Neg.
G 6515	1700	2·2	Neg.	+	7500	AC	Neg.	Neg.
G 6513	1500	1·5	Neg.	+	4200	3·2	Neg.	+
G 6511	1700	4·7	NT	NT	6500	5·6	NT	NT
H 1476	1150	4·0	Neg.	+	5100	5·8	Neg.	Neg.
H 1468	4000	0·8	NT	NT	4000	0·8	NT	NT
H 1463	4000	14·4	NT	NT	3100	2·0	NT	NT
H 1445	4500	0·6	NT	NT	3500	0	NT	NT
G 6520	1500	3·4	Neg.	+	6500	2·0	Neg.	+
G 6514	2400	AC	Neg.	+	5800	1·4	Neg.	+
G 6512	1500	2·6	Neg.	+	5100	1·6	Neg.	Neg.
Total+/No. tested		15/15	0/10	10/10		13/15	1/10	6/10

NT, Not tested; Neg., negative.

* Antigen equivalent to μg/ml single strand calf thymus DNA detectable before and after AD by complement fixation with rabbit serum No. 33.

† Results of Ouchterlony precipitin tests with 1 : 10 dilutions of mink sera diffused against rabbit antiserum No. 44.

‡ Results of Ouchterlony precipitin tests with 1 : 10 dilutions of mink sera diffused against human antinuclear antibody (chronic active hepatitis serum).

TABLE 4

ANTINUCLEAR ANTIBODIES DETECTED IN IMMUNOFLUORESCENCE TESTS AND PRECIPITATING ANTIBODIES TO DNA AS DETECTABLE IN OUCHTERLONY PRECIPITIN TESTS BEFORE AND AFTER EXPERIMENTALLY INDUCED ALEUTIAN DISEASE

Mink No.	Before AD		After AD	
	ANA	XDNA	ANA	XDNA
H 1472	Neg.	Neg.	1+	Neg.
H 1464	Neg.	Neg.	1+	Neg.
H 1456	Nt	NT	3+	NT
H 1452	+	Neg.	4+	3+
H 1437	1+	Neg.	4+	Neg.
G 6521	1+	Neg.	4+	4+
G 6515	Neg.	Neg.	4+	2+
G 6513	2+	Neg.	3+	2+
G 6511	+	Neg.	3+	3+
H 1476	2+	Neg.	4+	NT
H 1468	+	Neg.	2+	Neg.
H 1463	+	Neg.	4+	1+
H 1445	Neg.	Neg.	2+	Neg.
G 6520	2+	NT	4+	+
G 6514	+	Neg.	4+	2+
G 6512	1+	NT	4+	Neg.
Total+/No. tested	6/16	0/13	6/16	7/14

NT, Not tested; Neg., negative.

acquired Aleutian disease. None of the additional normal mink sera tested showed antigen precipitable by rabbit or human antisera (Table 5). In addition, none of thirteen additional normal mink tested showed precipitins with 10 μg/ml of single strand calf thymus DNA (Table 5). Sera obtained from mink who had spontaneously developed Aleutian disease, but had never received inoculations of infectious material again showed a high incidence of nuclear antigen and antinuclear antibody (Tables 6 and 7).

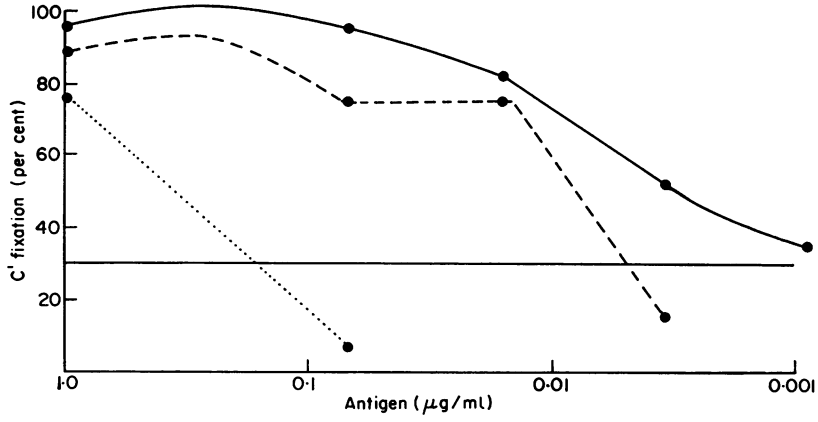

FIG. 2. Per cent complement fixation obtained with rabbit antiserum No. 44 (●— ●) diluted 1 : 400 and various concentrations of single strand calf thymus DNA; AD mink serum No. 26 at a dilution of 1 : 50 and various concentrations of single strand (● - - - ●) and native (● ···· ●) calf thymus DNA. Essentially no complement fixation was obtained with AD mink serum No. 26 at a dilution of 1 : 50 and native DNA obtained from human liver, nuclear protein obtained from human liver, single strand T_4 phage DNA, native calf thymus histone, single strand yeast RNA and single strand calf thymus DNA that had been previously digested with DNAse. AD mink serum No. 26 at dilution 1 : 50 *vs.* native human liver DNA, < 30 per cent; native human DNP, < 30 per cent; T^1 SS DNA, < 30 per cent; native CT histone, < 30 per cent; SS yeast RNA, < 30 per cent; SSCT DNA c̄ DNase, O.

TABLE 5

'NUCLEAR' ANTIGEN CONTENT OF 'NORMAL' MINK SERA AND ANTINUCLEAR ANTIBODY CONTENT OF 'NORMAL' MINK SERA AS DETERMINED IN OUCHTERLONY PRECIPITIN TESTS WITH ANTI-DNA ANTIBODIES OR SINGLE STRAND DNA

Antigen content of 'normal' mink sera			Antibody content of 'normal' mink sera
Ppt c̄ Rab 33	Ppt c̄ Rab 44	Ppt c̄ H.-C.H.	XDNA-Ppt
0/17	0/4	0/4	0/13

Total +/No. tested.

TABLE 6

'NUCLEAR' ANTIGEN CONTENT OF SPONTANEOUSLY DISEASED MINK SERA AND ANTINUCLEAR ANTIBODY CONTENT OF SPONTANEOUSLY DISEASED MINK SERA AS DETERMINED IN OUCHTERLONY PRECIPITIN TESTS WITH ANTI-DNA ANTIBODIES OR SINGLE STRAND DNA

Antigen content spontaneous AD			Antibody content spontaneous AD
Ppt c̄ Rab 33	Ppt c̄ Rab 44	Ppt c̄ H.-C.H.	XDNA-Ppt
5/12	6/9	6/10	3/4

Total +/No. tested.

TABLE 7

NUCLEAR ANTIGEN DETECTABLE IN OUCHTERLONY PRECIPITIN TESTS BY RABBIT ANTIBODY NO. 33 AND ANTI-DNA ANTIBODIES DETECTABLE BY 10 μg/ml SINGLE STRAND CALF THYMUS DNA IN SERA FROM MINK WITH EXPERIMENTAL AND SPONTANEOUS ALEUTIAN DISEASE

	Antigen detected by rabbit No. 33	Antibody precipitate detected by 10 μ SSDNA
Experimental AD, 100 days		
530	+	+
513	+	+
506	+	−
532	+	+
515	+	−
		3/5
Spontaneous AD		
15E28	+	NT
15E32	+	+
15E38	−	−
15E24	+	+
15E21	+	+
		3/4

It should be noted that in three of five sera from mink with experimentally induced Aleutian disease both antigen and antibody were simultaneously detected. In three of four sera from mink with spontaneous Aleutian disease both 'nuclear' antigens and anti-DNA antibodies were also detected. It should also be noted that precipitin bands formed between sera numbers 15E28 and 15E32 as well as between sera 15E32 and 15E38.

The antigen detected in sera from mink with Aleutian disease gave reactions of partial identity with single strand calf thymus DNA in immunodiffusion tests (Fig. 3–5). Serum H 9542 from a mink with Aleutian disease precipitated with serum J-59 from another mink with AD (Fig. 3). This precipitin line gave a reaction of complete identity with the precipitin formed between mink serum J-59AD and rabbit anti-DNA serum No. 33. A similar finding was noted between mink sera 532AD and 15E32AD (Fig. 3). Rabbit anti-DNA serum No. 33 formed precipitate lines with as little as 9 μg/ml of single strand DNA and 50 μg/ml of native DNA (Fig. 4). Precipitins were also formed between the rabbit anti-DNA serum and sera from mink with Aleutian disease, Nos. 506, 530 and 513 (Fig. 4). These three mink sera, in addition, precipitated with single strand (Table 7) and native DNA (Fig. 4). Precipitin lines formed between mink sera and rabbit anti-DNA serum No. 33, usually spurred over adjacent precipitin lines formed between rabbit anti-DNA

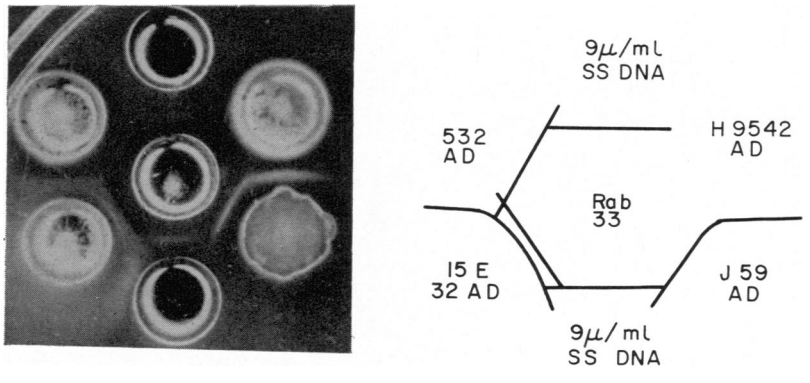

FIG. 3. Ouchterlony immunodiffusion between rabbit anti-DNA serum No. 33 and single strand calf thymus DNA 9 μg/ml as well as various sera from mink with Aleutian disease. See text.

No. 33 and single strand or native DNA (Figs. 4 and 5). Two precipitin bands are seen between serum No. 513 and single strand DNA (Fig. 4), one of which appears to give identity with the precipitin band formed between rabbit antibody No. 33 and Aleutian disease serum No. 513. This may suggest that mink serum No. 513 is capable of detecting some antigen in the rabbit anti-DNA serum which cross-reacts with single strand and native DNA. Mink serum AD 513 was the only one that gave us such an unusual precipitant pattern. Utilizing other techniques we were able to obtain no evidence that rabbit anti-DNA serum No. 33 contained DNA. In Fig. 5, serum from mink J-59 B-AD taken

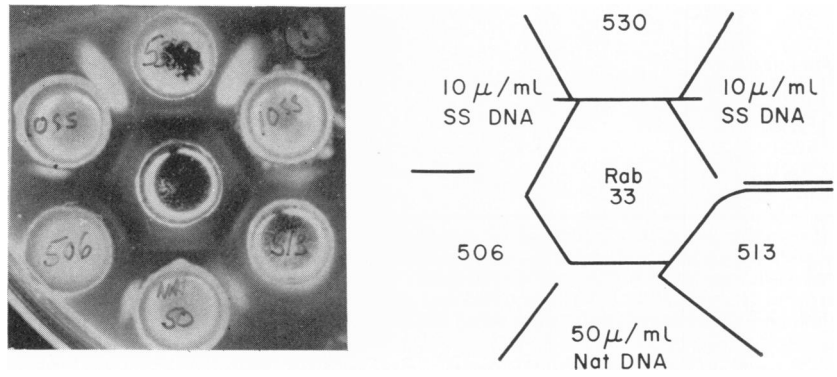

FIG. 4. Ouchterlony immunodiffusion obtained with rabbit anti-DNA serum No. 33 and 10 μg/ml single strand calf thymus DNA, 50 μg/ml of native calf thymus DNA and sera from three mink with experimentally induced Aleutian disease.

before disease gives no precipitin band with rabbit antibody No. 33 while serum J-59 AD taken after disease gives a definite precipitin band. Serum J-59 B-AD (before Aleutian disease) was shown to contain 2200 mg/100 ml immunoglobulin G and serum J-59 AD was shown to contain 2370 mg/100 ml immunoglobulin G. It, therefore, seems unlikely that the immunoglobulin content alone is responsible for the precipitin bands seen. The antigen in mink sera detected by rabbit or human antiserum was not destroyed by incubation with DNAse, RNAse or trypsin. Moreover, sequential exposure to trypsin and DNAse

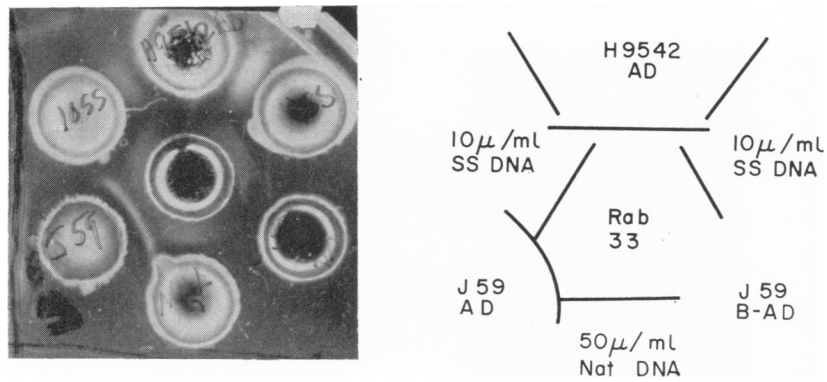

FIG. 5. Ouchterlony immunodiffusion with rabbit antiserum No. 33 and 10 μg/ml of single strand calf thymus DNA, 50 μg/ml of native calf thymus DNA and sera from mink No. J-59 before Aleutian disease (J-59B-AD) and after Aleutian disease (J-59-AD) and against serum from mink H 9542 AD with Aleutian disease.

did not destroy the antigen in mink serum No. 15E34. Absorption of rabbit antiserum No. 33 with mink N 525 or AD 15E28 serum did not reduce its ability to precipitate with single strand calf thymus DNA. However, absorption of rabit antiserum No. 33 with normal mink serum No. 525 or AD serum 15E28AD reduced its capacity to precipitate with native calf thymus DNA; before absorption it was able to detect 62·5 μg/ml of native calf thymus DNA, but after 250 μg/ml of native calf thymus DNA was the lowest concentration it precipitated. These preliminary absorption experiments suggest that some of the anti-DNA antibody in rabbit antiserum No. 33 was removed by absorption with mink sera.

DISCUSSION

Antigens related to DNA or nuclear materials were detectable in sera from mink with spontaneous or experimental Aleutian disease. Using complement fixation or precipitin tests with human antibody, similar antigens were detectable in sera from mink prior to overt Aleutian disease. Moreover, the diphenylamine test detected desoxyribose-containing material in serum pools from mink both before and after overt Aleutian disease. Immunofluorescence tests and precipitin reactions detected antinuclear antibodies including anti-DNA antibody prior to overt disease. In general these antibodies appeared to increase in titre after Aleutian disease had become established. Some degree of hypergammaglobulinaemia was consistently found in sera from mink with AD. It appeared that the brightness and number of test leucocytes showing nuclear fluorescence could be correlated with the presence of precipitating antibody to DNA in sera from mink after experimental Aleutian disease. Similar nuclear fluorescence patterns are frequently associated with anti-DNA antibodies in human sera (Bickel *et al.*, 1967). Normal mink sera did not show detectable amounts of antigen or antibody by the less sensitive precipitin tests.

The nature of the 'nuclear' antigen detectable in the sera of some of the mink studied here is not yet clear. It was shown to resist DNAse, RNAse and trypsin as well as the sequential use of trypsin and DNAse. When studied in Ouchterlony analysis the 'nuclear' antigen detected in mink serum gave partial identity with calf thymus DNA in precipitin tests utilizing rabbit anti-DNA serum. Preliminary experiments suggest that the antigen detectable by rabbit anti-DNA serum is destroyed by digestion with pronase. The recent report by Porter and Larson of marked reduction in infectivity of Aleutian disease serum after precipitation of the γ-globulins using anti-mink γ-globulin antisera (Porter and Larsen, 1967) indicates that the infective agent is bound to γ-globulin in serum. Such a viral–antibody complex might interfere or in some way block the ordinary reactions of such digestive agents as DNAse or trypsin. Of course, no evidence is presented here to suggest that antigens detected are in any way related to the infectious material responsible for Aleutian disease. The 'nuclear' antigen detected may be related to non-infectious material released from infected cells or may be released from the nuclei of mink cells undergoing destruction as part of the inflammatory process.

Of considerable interest was the finding that certain mink sera formed precipitin lines with other mink sera giving in turn reactions of identity with the precipitins formed by rabbit antiDNA serum. This finding suggested that certain antibodies in mink sera possessed specificities identical with rabbit anti-DNA sera.

The apparent simultaneous detection of 'nuclear' antigens and antinuclear antibodies (Table 7) in the same mink sera are consistent with current hypotheses that antigen–antibody complexes may have pathogenic significance in relation to vasculities and

TABLE 8

ABNORMALITIES NOTED IN NEW ZEALAND BLACK MICE, ALEUTIAN DISEASE OF MINK AND IN SYSTEMIC LUPUS ERYTHEMATOSUS OF MAN

	NZB mice	Aleutian disease mink	SLE man
↑ Ig globulins	+	+++	+
Vasculitis	+	+	+
Nephritis	++	++	++
Arthritis	−	−	+
Coombs	+	?	+
ANA	+	+	+
Nuclear antigens	+	+	+
Infectious	Probably	+	?

glomerulitis (Dixon *et al.*, 1961; Krishman and Kaplan, 1967; Koffler *et al.*, 1967; Barnett, 1968b).

Aleutian disease of mink is characterized by a plasma cell hepatitis, vasculitis, nephritis and hyperglobulinaemia (Table 8) which shows some similarities to analogous findings in New Zealand Black mice and in humans with systemic lupus erythematosus. Mink with Aleutian disease have been reported to have Coomb's positive haemolytic anaemia (Saison, Karstad and Pridham, 1966), but we have been unable to demonstrate this finding during previous studies. It is of interest that antinuclear antibodies detectable by immunofluorescence tests on human leucocyte nuclei have been found in all three situations. The parallelism falters somewhat when the nuclear antigens present in mink sera are compared to those already studied in human or mouse sera. The latter antigens were frequently shown to be digestable by DNAse (Forsen and Barnett, unpublished data) and produced complete identity with single strand calf thymus DNA (Forsen and Barnett, unpublished data). Ultrafiltrates have been shown to transmit disease in mice (Mellors and Huany, 1966) and mink (Henson *et al.*, 1966). It has been suggested that Aleutian disease and the disease of New Zealand Black mice may be examples of 'slow virus' diseases (Abinanti, 1967) in genetically susceptible strains. The pathological and serological similarities between systemic lupus erythematosus and these diseases of mice and mink deserve careful attention.

ACKNOWLEDGMENT

The technical assistance of Margaret A. Alspaugh is gratefully acknowledged.

This work was supported in part by United States Public Health Service Grant No. AM-10,000-01, United States Public Health Service Training Grant No. AM-05068-13 and by Grant No. AI-07901-01 from the United States Public Health Service.

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