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Antigen and Antibody in Aleutian Disease in Mink. I. Precipitation Reaction by Agar-Gel Electrophoresis[1]

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It is generally believed that Aleutian disease (AD) is a slowly progressive viral disease of mink (*Mustela vison*), but this interpretation refers only to its long incubation period and prolonged clinical course (1). AD is one of the several naturally occurring immune complex diseases, and has many features in common with equine infectious anemia (2), lymphocytic choriomeningitis (3) and lactic dehydrogenase infection of mice (4). In each instance, glomerulonephritis is characterized by deposition of γ globulin and complement in the renal glomeruli. Circulating virus-antibody complexes have been detected in AD and some other immune complex diseases (5).

Many attempts have been made to establish a sensitive and reliable serologic method to detect antigen and antibody specific for AD; however, most were unsuccessful. Recently Porter and his co-workers (6) reported an immunofluorescence test capable of detecting viral antigens and antibody in AD-infected mink.

During the present investigations, partially purified and concentrated AD antigen was prepared. An antibody against this antigen was demonstrated in sera from mink with AD by a precipitation reaction using agar-gel electrophoresis. This technique can be utilized to detect the infectious agent or the antibody and is rapid and sensitive.

MATERIALS AND METHODS

Aleutian and standard dark mink mainly 3 to 8 months old were obtained from the AD-free herd of the Ontario Veterinary College. Mink were inoculated intraperitoneally with 1.0 ml of a 10% (w/v) spleen suspension of an AD-infected mink. Sera were collected at intervals and stored at −40°C.

AD antigen was prepared from spleen, liver and kidney from 3-month-old Aleutian mink 11 days after experimental infection. The procedures of

fluorocarbon (Du Pont Chemical, Wilmington, Del., trichloro-trifluoro-ethane $CCl_2F\text{-}CClF_2$) treatment were described elsewhere (7). After fluorocarbon extraction the aqueous phase was further concentrated by ultracentrifugation at 180,000 × G for 90 min. The pellets from 100 g of infected tissue were resuspended in 1.5 ml of phosphate-buffered saline (pH 7.4). If required the pellets were resuspended in glycine-HCl buffer (pH 2.4) and after another ultracentrifugation at the same speed, the antigen was resuspended in phosphate-buffered saline. Tissues from normal mink were treated similarly and used as control antigen.

Agar-gel electrophoreses were carried out in 50 x 75 mm glass plates containing 10 ml of 1.0% to 1.5% ion agar and 0.01% sodium azide in a HR buffer (ionic strength 0.05). Wells were punched with 3.0 mm cutters. Antigen was placed in the wells of the cathode side and the opposite wells were filled with antisera. The precipitin lines were read 30 to 45 min after electrophoresis at 70 volts had begun.

RESULTS

The precipitin lines generally became visible after 20 min of electrophoresis in the 5 mm distances between antigen and antiserum wells, but were more distinct at 30 to 45 min. The results of the precipitation reaction from 24 Aleutian and 4 standard dark mink are shown in Table I. As early as 9 days after experimental infection, precipitin lines were demonstrated. With antisera obtained 9 days after infection the precipitin lines were faint; however, by 11 days and thereafter, there was no difficulty in identifying the precipitin line.

Antigen prepared from tissues of animals 11 days after experimental infection showed strong reaction, whereas antigen, prepared from 13 days after infection, did not form the precipitin line. After these latter antigen preparations were treated with glycine-HCl buffer, precipitation could be demonstrated. The precipitin line was

TABLE I

Precipitation reaction with AD-infected and normal mink sera by agar-gel electrophoresis with the fluorocarbon concentrated antigen

						Days after Infection							
0	4	7	9	11	13	14	28	39	60	90	120	140	184
0/28[a]	0/5	1/8	4/4	5/6	10/10	8/8	9/9	3/3	8/8	6/6	6/6	6/6	6/6

[a] Number of positive sera/number of sera tested.

only visible when antigens prepared from infected mink tissue were reacted with sera from infected mink. No precipitation occurred between AD antigen and normal mink sera, or between antigen prepared from normal mink tissue and infected and normal mink sera (Fig. 1a and 1b). No precipitin line was detected when antigen was added to AD antiserum before agar-gel electrophoresis, whereas if similar materials from normal mink were added to antiserum, antibody activity was not affected, ie., the precipitin line was demonstrated. Precipitating antibody was recovered in immunoglobulins eluted from DEAE-cellulose with 0.0175 M phosphate-buffered solution (pH 6.3). Immunoelectrophoretic analysis indicated that these immunoglobulins were IgG.

After experimental infection, all the tested animals developed hypergammaglobulinemia, positive iodine agglutination test, positive direct Coombs' test, and histopathologic examination also showed typical lesions of AD.

DISCUSSION

AD of mink is characterized by plasmacytosis, hypergammaglobulinemia, Coombs' positive anemia and glomerulonephritis and is believed to be caused by sustained viral infection (5). The markedly increased γ globulin in AD is the result of overproduction of immunoglobulin rather than a defect in catabolism (8). The nature and specificity of hypergammaglobulinemia observed in AD was obscure until recently.

Some investigators have suggested that plasmacytosis of mink has some characteristic features of autoimmune states. Anti-DNA antibodies were demonstrated in affected AD sera using calf thymus DNA as antigen (9). Anti-gammaglobulin factors (10) and Coombs' positive reaction (11) were reported. However, AD-infected mink serum and glomerular eluate did not contain antibody activity against glomerular basement membrane or nuclear substances, as demonstrated by immunofluorescence studies (12). Furthermore, antibody to Aleutian disease viral antigen(s) was demonstrated at 10 days after infection and by 60 days antibody titer determined by indirect immunofluorescence test was 100,000 (6) The present authors observed that erythrocytes from AD-infected mink carry IgG and complement on their surface and suggested these com-
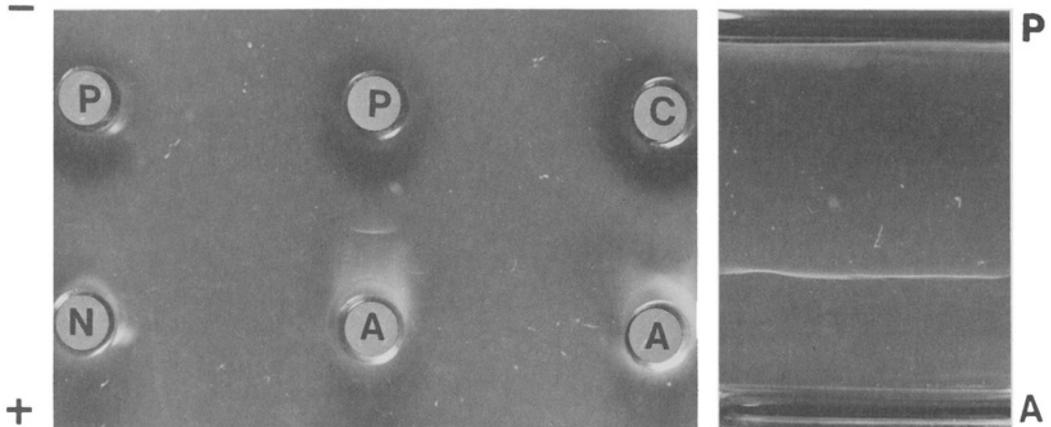

Figure 1, *A* (*left*) and *B* (*right*). Precipitation reaction by agar-gel electrophoresis in Aleutian disease. *P* = AD antigen; *C* = control antigen prepared from normal mink tissue; *A* = AD-infected mink serum; *N* = normal mink serum. (Unstained slides.)

plexes may result from interaction of antibody with AD agent, resulting in fixation of complement. Serum from AD-infected mink was not capable of sensitizing normal mink erythrocytes for the antiglobulin test.

Previous failures to demonstrate antigen and antibody specific for AD would seem to be due to insufficient concentration of AD antigen. In the preparation of AD antigen, date of collecting tissues after infection was critical. After the infected mink produced antibody, the antibody complexed with antigen and caused the loss of serologic reactivity of the antigen. Glycine-HCl buffer treatment of unreactive antigen prepared from tissues of mink obtained 13 days after infection activated the antigens. This activation is probably due to the removal of antibody from the immune complexes in these preparations, thus liberating the antigens and allowing the recovery of antigenic activity. It is not known whether all of the γ globulin produced during AD infection has antibody activity against AD antigen; however, the present results showed that serum from AD-infected mink has antibody activity against AD antigen. Antibody was demonstrated 7 days after infection in the sera of one of the eight mink tested at this period. Nine days after infection, antibody was demonstrated in all sera tested. By the immunofluorescence test, antibody was demonstrated as early as 8 days after infection (6).

The present precipitation test promises to be useful in the diagnosis of AD, being simple, rapid and apparently specific. Further studies are in progress to characterize the physicochemical properties and serologic reactivities of the AD antigen and characterization of the hypergammaglobulinemia from AD-infected mink.

SUMMARY

An antigen in tissue from mink infected with Aleutian disease, detectable by agar-gel electrophoresis, was partially purified by fluorocarbon treatment and concentration by ultracentrifugation from the spleen, kidney and liver of experimentally infected mink. Antibody against this antigen, which seems to be specific for AD, appeared as early as 9 days after experimental infection. Normal animals had no detectable antigen or antibody specific for AD virus.

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