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ALEUTIAN DISEASE OF MINK: THE ANTIBODY RESPONSE OF SAPPHIRE AND PASTEL MINK TO ALEUTIAN DISEASE VIRUS

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The specific antiviral antibody response of sapphire and pastel mink to Pullman strain of ADV has been examined. Sapphire mink inoculated with from 300,000–3 LD_{50} developed high levels of specific antibody and AD. Pastel mink inoculated with parallel doses of ADV also produced antibody but did not develop AD. The low incidence of AD in pastel mink inoculated with Pullman strain of ADV is probably related to factors other than antiviral antibody.

Aleutian disease (AD)[2] is a virus-induced, immune complex disease of mink characterized by glomerulonephritis, arteritis, generalized plasmacytosis, and hypergammaglobulinemia (1–3). Recent studies have shown that mink affected with AD have high titers of antiviral antibody (1, 4–6), but virus is not neutralized (1, 7); infectious immune complexes persist in the blood until the animal dies (8). AD is thought to result from deposition of immune complexes consequent to persistent viremia and a continuing host antiviral antibody response. Viral antigen, antiviral antibody, and complement have been identified in glomerular lesions (1, 3). Because immunosuppression has been shown to prevent immune complex disease without significantly affecting virus replication (9), it seems probable that the humoral immune response is important in the pathogenesis of the disease.

Previous work has shown that the Pullman strain of Aleutian disease virus (ADV) is much more pathogenic for mink of the sapphire color phase than for those of the pastel color phase (2), while the Utah-1 strain of virus is highly pathogenic for both color phases of mink (1). In the present experiments the specific antiviral antibody response of sapphires and pastels to graded amounts of live Pullman ADV was studied to see if quantitative or kinetic differences in antibody response might be correlated with disease susceptibility in the two mink color phases. Our results indicated that mink of both sapphire and pastel color phases can make anti-ADV antibody, and suggested that the resistance of pastel mink to this strain of virus is related to factors other than specific antiviral antibody.

MATERIALS AND METHODS

Animals. Mink, from a single closed herd free of AD, were maintained as previously reported (2). None had received vaccines or inoculations other than those mentioned here.

Preparation of antigen for counterimmunoelectrophoresis (CIEP). For preparation of CIEP antigen, Utah-1 strain of ADV (1) was used. This strain was obtained from Dr. D. Porter as a liver suspension representing the fourth passage in mink (1), and additional passages were made at Rocky Mountain Laboratory in sapphire mink (10). Sapphire males, usually in groups of 5, were inoculated i.p. with 0.5 ml of a 10% splenic suspension containing 7.0 $\log_{10}$ sapphire LD_{50} of seventh passage Utah-1 ADV. Liver, spleen, intestine, and mesenteric lymph node were collected 9 days later and a 30% suspension was prepared from these organs. Antigen was then prepared from this suspension by ultrasonication, Freon extraction, and ultracentrifugation at 50,000 × G for 4 hr (5). Pellets were treated with 0.1 M glycine-HCl, pH 2.8, ultracentrifuged again, resuspended in buffer, and used as the concentrated ADV antigen. Each batch of antigen was serially diluted and evaluated by CIEP in a chessboard titration against a standard pool of positive serums. The antigen dilution giving the sharpest precipitin lines was used. Antigen was kept frozen at −20°C in undiluted form, thawed, and diluted immediately before use. A control antigen was prepared from tissues of normal animals in an identical fashion.

Method of CIEP. CIEP was performed on 50 x 75 mm glass slides coated with 8 ml of 1% agarose (Seakem, Rockland, Maine) in barbital buffer, μ 0.039, pH 8.8. Serial 4-fold dilutions of serums from 1:4 to 1:4096 were made with microdilutors in round bottom microtiter trays (Cooke Engineering, Inc., Alexandria, Va.). Serum dilutions were placed in anodal wells and antigen in cathodal wells. The slides were electrophoresed for 45 min at 4 volts per cm and then viewed under indirect illumination. The titer was recorded as the reciprocal of the highest dilution of serum that formed a definite precipitin line. Results were read blindly by two observers. A standard pool of positive serums, which had an antibody titer of 1024, was included in each group of serums tested.

Inoculation of mink with Pullman strain of ADV. Groups of three sapphires and three pastels, all yearling females, were inoculated i.p. with 0.5 ml of eight serial 10-fold dilutions of tenth passage Pullman ADV (0.03 to 300,000 LD_{50} per mink). The Pullman strain of ADV was obtained from Dr. J. Gorham as a spleen suspension representing the ninth passage in mink and passaged once at Rocky Mountain Laboratory in sapphire mink (2). Blood samples, obtained from a cut toenail, were collected before inoculation and at 2, 4, 6, 7, 11, 15, and 20 weeks after inoculation. All mink that became clinically affected with AD (see Reference 2 for criteria) were allowed to reach a near terminal stage before they were killed and the presence of disease was confirmed at necropsy. Antibody was determined by CIEP and per cent serum γ-globulin by electrophoresis on cellulose acetate (2).

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[2] Abbreviations used in this paper: AD, Aleutian disease; ADV, Aleutian disease virus; CIEP, counterimmunoelectrophoresis; LD_{50}, median lethal dose.

Statistical methods. Statistical comparisons were performed by Dr. M. A. Hamilton who used a standard analysis of variance program on the Montana State University XDS-Sigma 7 computer. In addition, simple correlation coefficients between per cent γ-globulin and $\log_2$ CIEP titer were done on a Hewlett-Packard 9120A calculator (Hewlett-Packard, San Diego, Calif.).

RESULTS

Specificity of antibody detected by CIEP. Specificity of the antibody detected by CIEP was evaluated in several ways. Antibody was not found in serums from 350 normal sapphire and pastel mink (Table I). By contrast, antibody was present in serums from all of 450 mink affected with experimental AD. The reaction in CIEP was the same regardless of color phase of mink or the strain of infecting virus. Anti-ADV antibody was not detected in serums of normal mink inoculated with keyhole limpet hemocyanin or goat erythrocytes (11, 12) or in serums from 27 mink naturally affected with diseases other than AD (Table I). Lastly, serums from persons at Rocky Mountain Laboratory who regularly worked with ADV or infected mink were also free of such antibody. None of the serums tested reacted with control antigen (Table I).

Comparison of antibody response to Pullman ADV in sapphire and pastel mink. All sapphire mink inoculated with 300,000-3 LD_{50} of virus produced antibody, attained greatly elevated levels of serum γ-globulin and became affected with AD. For brevity, data are presented only from virus doses of 300,000, 3,000, 30, and 0.30 LD_{50} per mink in Figure 1, *A-D*. Antibody titers rose to maximum levels of 1024 by 4 to 8 weeks after virus inoculation and remained there until death.

The amount of serum γ-globulin in sapphire mink increased steadily to extremely high levels. In those that received 3,000-3 LD_{50}, these levels did not exceed 20% until 6 to 8 weeks after inoculation. Antiviral antibody was always detectable before serum γ-globulin rose to levels suggestive of AD (2) but the correlation between serum γ-globulin and CIEP titers was good (correlation coefficient r = 0.75). Clinical signs of AD (2) appeared 7 to 11 weeks after inoculation of virus and were independent of dose of virus in sapphire mink receiving from 300,000-3.0 LD_{50} per mink. Gross and microscopic lesions in the kidneys would also be expected to appear between 7 to 11 weeks in sapphire mink (2). Sapphire mink that received 0.3 or 0.03 LD_{50} of ADV did not produce anti-ADV antibody, have elevated levels of serum γ-globulin, or become affected with AD. All were still clinically normal 10 months after inoculation.

In contrast to the anti-ADV antibody and serum γ-globulin response of sapphire mink, the responses of pastel mink were markedly different. All pastel mink that received from 300,000 to 30 LD_{50} and 1 of 3 that received 3 LD_{50} of virus produced anti-ADV antibody, but none of them became affected with AD and all had normal serum γ-globulin levels.

At the time that sapphire mink were attaining maximal titers of 1024 (6 to 8 weeks after virus inoculation), many pastel mink had titers of 256 and 2 that received 3000 LD_{50} of virus had titers of 1024. However, unlike sapphire mink that maintained high antibody levels and developed signs of AD, the pastel mink remained clinically normal. The anti-ADV antibody titers began to decline by 8 weeks after virus inoculation, and although the titers were decreasing at the last observation, 20 weeks after virus inoculation, no pastel mink with antiviral antibody subsequently became seronegative. Pastel mink that received 0.3 or 0.03 LD_{50} of virus did not develop antibody or AD.

DISCUSSION

The most unexpected finding in the present study was the occurrence of high specific anti-ADV antibody in pastel mink inoculated with Pullman strain of ADV. Although sapphire mink had slightly higher titers, it was most revealing to note that many pastels had titers as high as some sapphire mink at 6 to 8 weeks after virus inoculation. Thus, pastel mink are clearly able to form specific antiviral antibody and the fact that they do not develop AD subsequently cannot be related merely to an inability to produce specific antiviral antibody.

Pastel mink develop antibody titers as high as 256 after inoculation with as little as 30 LD_{50} of virus. This strongly implies that ADV must replicate to some extent in pastel mink to produce these high titers. Presence of immunogenic amounts of non-infectious viral antigen in the inoculum cannot explain high antibody titers since killed ADV is a poor immunogen (14). Perhaps kinetics of antibody response in these two color phases of mink reflects differences in persistence of viral infection. Sapphire mink showed no decrease in antibody titer during the period of observation, and it is known these mink have sustained viremia until death (2). Pastel mink had a transient antibody response that reached its peak 6 to 8 weeks after inoculation of virus and then declined. This may be a reflection of the course of viremia in this color phase and, if so,

TABLE I

Screening of Serums for Anti-ADV* Antibody Using Counterimmunoelectrophoresis

	Antigen			
	ADV		Control	
	No. Tested	No. Positive	No. Tested	No. Positive
Normal mink	350	0	10	0
Mink with clinical AD**	450	450	10	0
Mink with other diseases	27	0	27	0
Mink inoculated with KLH† and GE‡	15	0	NT§	NT
Persons exposed to ADV	18	0	NT	NT

*ADV - Aleutian disease virus
**AD - Aleutian disease
†KLH - keyhole limpet hemocyanin
‡GE - goat erythrocytes
§NT - not tested

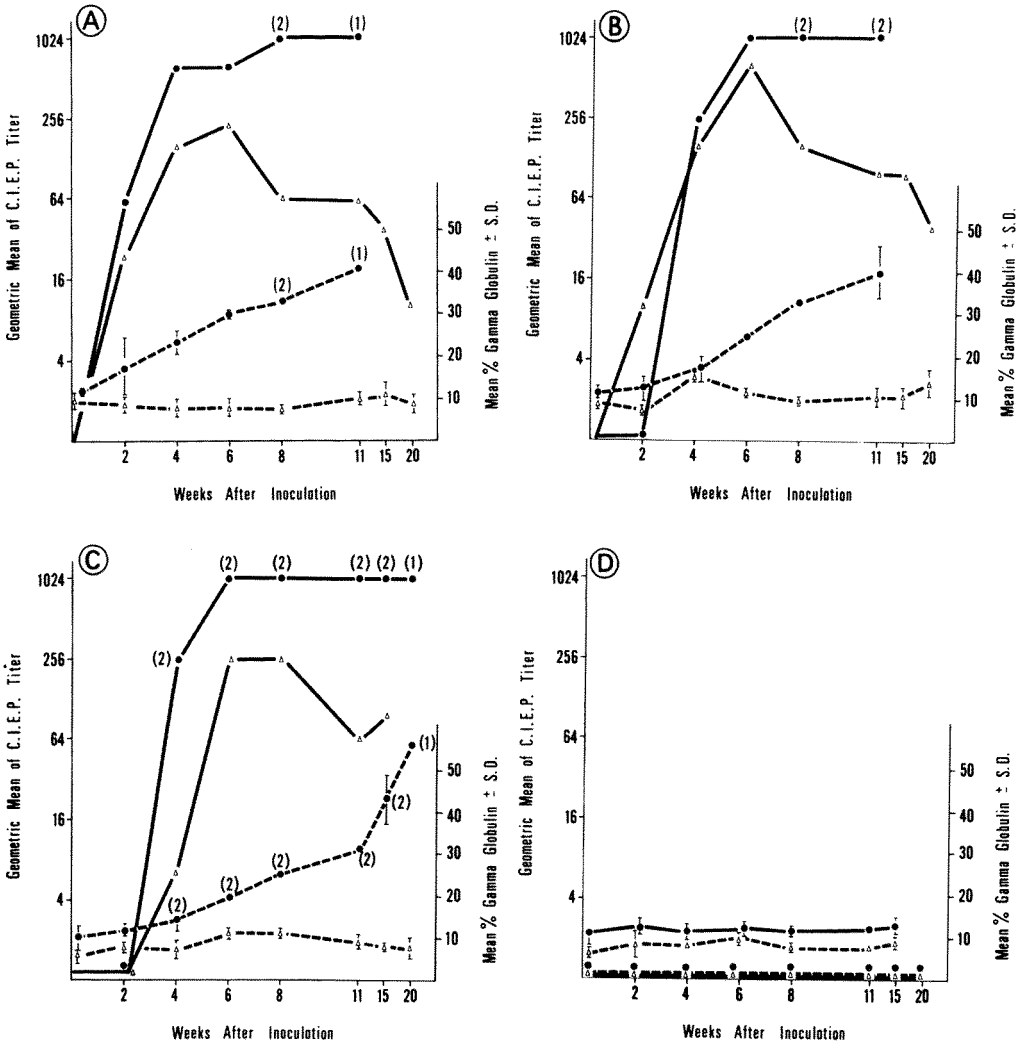

Figure 1. Antibody and serum γ-globulin response of groups of three sapphire and pastel mink to Aleutian disease virus (ADV). Antibody is expressed as group geometric mean of counterimmunoelectrophoresis titers (CIEP titer). Serum γ-globulin is expressed as group mean % γ-globulin ± standard deviation. Time is expressed as weeks following inoculation with ADV. ● represents sapphire mink; Δ, pastel mink; ——, CIEP titer; - - -, % serum γ-globulin; numbers in parentheses represent number of mink remaining in group. Although serial 10-fold dilutions of ADV from 300,000 to 0.03 LD_{50} were performed, serial 100-fold dilutions are depicted in this figure. *A* represents groups of mink receiving 300,000 LD_{50}; *B*, 3,000 LD_{50}; *C*, 30 LD_{50}; *D*, 0.3 LD_{50}.

would explain why pastel mink, despite ample antibody, did not become affected with AD. This suggestion is supported by data from this laboratory (Hadlow, in preparation). The mechanism by which viral persistence might induce the exaggerated antibody response seen in sapphire mink is unknown (1-3).

The Utah-1 strain of ADV appears to be equally pathogenic for sapphires and pastels (Hadlow, unpublished data) and this fact illustrates that pastels are not wholly resistant to the induction of disease. Although paramount in immune complex pathogenesis, specific antiviral antibody does not seem to be involved in virus control or elimination. Neutralizing antibody has not been found in pastels or sapphires (1, 7). Study of basic immune mechanisms has shown that variations in responsiveness to heterologous antigens occur between sapphires and pastels (11, 12, 15) and perhaps such differences are related to the unique ability of pastels to control the infection. Factors other than the immune response may also be involved.

The observation that sapphire mink, but not most pastel mink, develop disease following infection with Pullman strain of ADV suggests the importance of host genetics in the control of ADV infection (2). However, recent observations indicate that genetic control of ADV infection is complex and not simply due to a single gene (16). There are numerous examples in other species in which the genetic composition of the host influences the outcome of virus infection (17-20). In fact, other viruses besides the Pullman strain of ADV preferentially affect one color phase of mink (16, 21, 22). Thus, there does exist wide precedence for modulation of virus infection through host genetic composition in many species, including mink.

The greatly elevated levels of serum γ-globulin that occur in mink affected with AD have been considered to represent specific antiviral antibody (3). However, in the study reported here, titers of antibody in sapphire mink reached maximal levels when serum γ-globulin values were about 20%. The titers remained relatively constant while levels of γ-globulin rose progressively to values of 40 to 50%. This suggests that γ-globulin other than specific AD virus antibody is generated in mink affected with the disease.

Our results have practical implications for control of the disease in commercial mink herds. Detection of naturally infected mink is now usually done by use of a simple field test that demonstrates elevated serum γ-globulin (23). As is evident from our findings, some mink, especially of the less

susceptible color phases, infected with ADV of low virulence would not be detected by this test. Even though specific antibody is present in such mink, levels of serum γ-globulin might remain within normal limits. Moreover, antibody can be demonstrated by CIEP 4 to 6 weeks before a rise in serum γ-globulin indicative of AD appears. Thus, some infected mink could be identified and culled much sooner by use of CIEP. Mink that have specific antibody but do not become hypergammaglobulinemic and affected with AD may harbor virus in the mesenteric node or spleen for at least 24 months (2). Whether such mink are a source of virus for other mink is not certain, but their continued presence in a herd would seem to be a considerable threat and would impede efforts to eliminate the disease.

In summary, our study showed that sapphire and pastel mink do differ in kinetics of the specific antibody response to Pullman strain of ADV; however, this difference could be secondary to differences in viral persistence rather than a primary cause of the resulting clinical disease. The mechanisms of the host control of viral infection are unknown and remain a topic of current investigation in this laboratory.

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