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Temporal Replication of the Pullman Strain of Aleutian Disease Virus in Royal Pastel Mink

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Information was sought on the temporal replication of Aleutian disease virus in 27 royal pastel mink. Groups of three were examined 8 to 126 days after they were inoculated subcutaneously with 10^3 50% lethal doses of the Pullman strain. Much individual variation was noted in the onset of infection, occurrence of viremia, and extent of virus replication in the tissues. Thus, virus was detected in lymph nodes regional to the site of inoculation in only some mink during the first 14 days after inoculation. During this period, virus was often present as well in the mesenteric lymph node and spleen. First detected on day 10, viremia was present in all mink examined on day 28 but occurred irregularly thereafter, even when virus was widespread in the tissues. Except in five mink succumbing to the disease, the tissue distribution of virus after day 28 tended to be more limited, and the titers were generally lower than they had been earlier. Even though present in the lymph nodes and spleen, virus was often absent from the kidney, liver, and intestine after day 28. Specific antibody was detected on day 28 and was present in all mink thereafter, ostensibly without any adverse effect on virus replication. In most mink, the infection was considered subclinical, for it was usually not accompanied by a rise in serum gamma globulin or by morphologic evidence of the disease. The virologic findings in this study have a bearing on the relationship of subclinical infections to both horizontal and vertical transmission of the virus.

The Pullman strain of Aleutian disease (AD) virus seldom causes disease in royal pastel mink (a non-Aleutian genotype), but it readily infects them, as indicated by the appearance of specific antibody (4, 7, 8). In some subclinically infected mink, a small amount of virus circulates in the blood for brief periods during the first 12 or so weeks after inoculation (8). Occasionally, viremia is accompanied by a slight and usually transient rise in the level of serum gamma globulin. Infectious virus only exceptionally persists in the spleen or mesenteric lymph node long after the period of active infection (6, 8). So far, however, little is known about the temporal replication and distribution of the virus in tissues of pastel mink infected with the Pullman strain. Such virologic information is crucial to a clearer understanding of subclinical infections with viral strains of low virulence, especially as this relates to both horizontal and vertical transmission of the virus—a matter of great practical concern in efforts to eradicate AD from ranch mink. Reported here are the results of a study that sought such information.

Experimental design. Twenty-seven 15-month-old female royal pastel mink, obtained for a local herd free of AD, were inoculated subcutaneously (behind the left elbow) with 10th-passage Pullman strain of AD virus (7). Each mink received 10^3 50% lethal doses of virus, as determined by intraperitoneal inoculation of sapphire mink. Three mink chosen at random were killed (exsanguinated by cardiac puncture while under ether anesthesia) each day on days 8, 10, 12, 14, 28, 43, 70, 99, and 126 after inoculation. In addition to blood, the following specimens were collected for the virus assay: peripheral lymph nodes (submandibular, medial retropharyngeal, prescapular, axillary, prefemoral, superficial inguinal, and popliteal), mesenteric lymph node, spleen, kidney, liver, and small intestine (three levels pooled). Through day 14, lymph nodes regional to the site of inoculation (left prescapular and left axillaries) were kept separate from the other peripheral lymph nodes. Thereafter, all peripheral lymph nodes were pooled. Specimens from each mink were stored separately at −65°C until the suspensions were prepared.

Serum gamma globulin, blood urea nitrogen, and specific antibody were determined each time three mink were killed. Serum gamma globulin was determined by electrophoresis on cellulose acetate, and total serum proteins were determined with a clinical refractometer. Amounts of gamma globulin greater than 1.3 g/dl of serum were considered elevated. Blood urea nitrogen was determined with diacetyl monoxime in an autoanalyzer (Technicon Instruments Corp.). Specific antibody was measured by counterimmunoelectrophoresis in serial fourfold dilutions of serum (4). Paraffin sections stained with hematoxylin and eosin were prepared from tissues fixed at necropsy in neutral buffered 10% Formalin.

Assay of virus. By using a mortar and pestle and a refrigerated centrifuge, we prepared 10% suspensions of tissues collected at necropsy in an 0.85% NaCl solution containing 10% heat-inactivated fetal bovine serum and antibiotics (100 U of penicillin, 100 μg of streptomycin, and 0.25 μg of amphotericin B per 1 ml of diluent). All assays were done in male or female sapphire mink inoculated intraperitoneally with 0.5 ml of serial 10-fold dilutions of tissue suspensions or serum, one mink per dilution. Undiluted serum was used in the initial titration. When a titration was extended to reach the endpoint, the highest positive dilution in the starting sequence was repeated in the succeeding sequence of 10-fold dilutions. Criteria of infectivity

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TABLE 1. Temporal replication of Pullman strain AD virus in pastel mink inoculated subcutaneously

Day after inoculation	Mink no.	Laboratory and necropsy findings					Virus detected in[a]:							
		Total serum proteins (g/dl)	Serum gamma globulin (g/dl)	Antibody[b]	Blood urea nitrogen (mg/dl)	Presence of disease[c]	Serum	Regional lymph nodes	Peripheral lymph nodes	Mesenteric lymph node	Spleen	Kidney	Liver	Intestine
8	65	7.4	0.9	—	15	−	ND	3	—	1	3	—	1	—
	71	7.0	0.8	—	28	−	ND	—	—	—	—	ND	ND	ND
	81	7.0	0.6	—	14	−	ND	2	—	—	—	ND	ND	ND
10	66	7.2	0.9	—	17	−	ND	—	—	1	—	ND	ND	ND
	83	6.8	0.8	—	31	−	1	5	1	2	1	—	2	—
	86	6.6	0.8	—	14	−	ND	—	—	—	—	ND	ND	ND
12	73	7.6	0.8	—	20	−	UN	1	1	1	—	—	2	—
	75	7.7	0.9	—	23	−	UN	1	1	2	2	—	2	1
	78	7.0	0.9	—	21	−	ND	—	—	—	—	ND	ND	ND
14	70	7.6	1.2	—	18	−	2	1	1	2	1	1	3	2
	76	7.9	0.6	—	37	−	—	1	—	—	—	ND	ND	ND
	89	7.0	0.9	—	27	−	2	—	2	2	2	—	2	—
28	79	7.8	1.3	64	23	−	3		6	5	6	3	6	4
	84	7.7	1.1	16	34	−	2		4	5	5	3	5	3
	91	8.2	1.3	64	22	−	2		6	6	5	5	6	5
43	68	7.9	1.4	256	30	−	—		5	6	4	2	1	4
	85	8.5	2.0	256	48	+	3		5	8	6	5	5	6
	90	6.6	0.4	16	18	−	—		2	3	1	—	—	—
70	72	7.3	0.7	256	12	−	UN		5	4	3	—	2	3
	74	7.5	1.0	256	23	+	1		4	6	6	3	4	5
	80	6.6	0.6	256	15	−	—		3	4	4	3	—	2
99	77	7.7	0.8	16	18	−	—		1	1	3	—	—	—
	82	7.2	0.9	256	12	−	—		3	3	2	—	—	—
	88	9.2	2.2	1,024	54	+	2		<3	6	5	2	<2	3
126	67	7.5	1.3	256	30	−	—		2	4	1	—	—	3
	69	8.9	2.5	1,024	30	+	UN		5	6	6	4	5	7
	87	11.2	4.0	1,024	144	+	3		5	6	5	5	5	5

[a] Numbers represent the $\log_{10}$ reciprocal of the highest dilution containing virus. ND, Not done. UN, Undiluted serum. —, Not detected. Regional lymph nodes were not kept separate from other peripheral lymph nodes after day 14.
[b] Numbers represent the reciprocal of the dilution. —, Not detected.
[c] −, Disease absent; +, disease present.

included typical clinical and necropsy findings of AD, as previously described (6). As found in another study (unpublished data), traces of blood in tissues of mink exsanguinated by cardiac punture had no measurable effect on the concentrations of virus detected in the tissues, at least when the blood contained a moderate amount of virus.

Responses of mink. Considerable individual variation occurred in the virologic findings, especially during the first 14 days after inoculation (Table 1). Thus, virus was detected in the regional lymph nodes in two mink on day 8, in one on day 10, in two on day 12, and in two on day 14. It was present as well in the mesenteric lymph node, spleen, and liver in one of the two mink positive on day 8. Virus was also distributed in much the same way in those mink whose regional lymph nodes contained virus on days 10, 12, and 14. With one exception, titers in all these mink were low (10^{-1} to 10^{-3}). In contrast, virus was not detected anywhere in one mink from each group examined at the first three intervals and was present only in the peripheral lymph nodes (other than the regional ones) in one mink on day 14. Virus was found in the serum in one mink on day 10, in two on day 12, and in two on day 14, always in low titers. On day 28, all three mink had virus in the serum, as they did in every tissue assayed. Thereafter, viremia was irregularly present, usually occurring when virus was widespread in the tissues in moderate to high titers (10^{-4} to 10^{-6}). Except in mink succumbing to AD, the distribution of virus after day 28 tended to be more limited, and titers were generally lower. At each later interval, virus was often absent from the kidney, liver, and intestine.

Morphologic evidence of AD was present in one mink on day 43, in one on day 70, in one on day 99, and in two on day 126. All were viremic and had virus in every tissue assayed, usually in moderate to high titers. In all five, the liver, spleen, and abdominal lymph nodes contained infiltrates of plasma cells characteristic of the disease. Except for the mink on day 70, in which the microscopic changes were mild, all had typical renal lesions of AD. The most severe were in one mink on day 126. Like all other mink in the experiment, these AD-affected mink had remained clinically normal and in a good to excellent state of nourishment.

Antibody was first detected on day 28, somewhat later

than anticipated, and was present in all mink examined thereafter. The highest titers (1:1,024) were in three mink succumbing to the disease.

Serum gamma globulin levels remained within normal limits through day 28. Thereafter, except for a slight rise (1.4 g/dl) in one mink on day 43, the levels were elevated only in four of the five mink in which AD had supervened. The greatest increase (4.0 g/dl) was in one mink on day 126, by which time the disease was well developed, as indicated by the presence of pronounced renal lesions and a greatly elevated level of blood urea nitrogen (144 mg/dl). In four AD-affected mink, the elevated levels of serum gamma globulin were reflected in the increased amounts of total serum proteins (8.5 to 11.2 g/dl).

Comments. The detection of virus in regional lymph nodes and sometimes in other organs as well on days 8, 10, and 12 was consistent with the conclusion that AD virus replicates fairly soon after a mink is exposed to it, even though the disease that may supervene characteristically evolves slowly (6, 9, 14, 16). Such early replication, however, occurred in only some mink. But, in view of the virologic and serologic findings after day 14, this discrepancy no doubt is indicative only of the wide individual variation in the onset of the infection and not of its failure to occur at all in some mink. Presumably, infection would have occurred eventually in the mink without virus on days 8, 10, and 12, for the dose of virus used was large enough to ensure this happening.

The early appearance of virus in the mesenteric lymph node and spleen attested to their being favored sites of replication, probably after any route of exposure (6, 14). Virus replicating to moderate or high titers became widespread in the body while the mink remained clinically normal and free of lesions indicative of AD. From our earlier findings (7, 8), we assumed that the infection was subclinical in most of the 27 mink inoculated. Whether the infection was subclinical or was giving rise to disease, the appearance of specific antibody, beginning on day 28, did not have any obvious adverse effect on virus replication, for titers remained moderate to high in many sites. For reasons not understood, such widespread replication of virus was not always accompanied by viremia, which typically occurs fairly early in the course of the infection (6, 8, 14).

The frequent failure to find virus in the serum after day 43 may mean that viremia never occurred or that if it had occurred earlier it persisted only briefly. In view of previous findings (8), both possibilities no doubt accounted for the absence of viremia in some mink examined at later intervals when moderate to high concentrations of virus indicative of active infection were still present in many sites.

Despite its epidemiologic importance, the duration of active subclinical infections is poorly understood. In one study (2), four subclinically infected pastel mink from a commercial herd still had small amounts of virus in the mesenteric lymph node, spleen, kidney, and liver 10 months after antibody was first detected. In another study (8), moderate amounts of virus were found in the mesenteric lymph node and spleen of a subclinically infected pastel mink 8 months after it had been inoculated with the Pullman strain. However, as found in other pastel mink subclinically infected with this strain (6, 8, 9), virus replication eventually subsides, and in all but an occasional mink virus is cleared from the body in a way not understood. Thus, apart from these few exceptions when trace amounts of infectious virus remain hidden for many months or years in the mesenteric lymph node or spleen, AD virus infection is self-limiting in some pastel mink; it persists only in those in which disease supervenes. General statements about the persistence of AD virus infection seldom make that clear (1, 18). Whether the lower titers in the presumed subclinically infected mink examined at later intervals indicated waning replication is uncertain but is likely.

Subclinically infected mink identified in the field only by the persistent telltale antibody are the ones whose epidemiologic importance in an infected herd continues to bewilder commercial breeders trying to eradicate the disease (5). Such mink may be a source of virus transmitted horizontally (directly by contagion or indirectly by fomites) or vertically (3). Yet, the extent of this threat in a commercial herd remains poorly understood. Our findings offer some insight into the problem.

Thus, the absence of virus from kidney, liver, and intestine in several mink after day 28 suggests that some subclinically infected mink do not shed virus in urine or feces and therefore would not be likely sources of virus for horizontal transmission. Moreover, the absence of viremia in subclinically infected mink, as in some after day 43, would reduce the chances of contaminating catching gloves with virus-laden blood from those mink that ordinarily bleed readily from the mouth when handled. The absence of virus from the blood also might have a bearing on the possible transmission of virus by biting during breeding time—the only occasion when two adult mink are housed together (10).

Viremia also has another, more important, epidemiologic implication. For example, transplacental transmission of the virus is thought to account for the common familial occurrence of AD (12). The precise conditions necessary for such fetal infection to take place in mink, however, are unknown. Experimental studies on the infection of mink fetuses with AD virus offer no good clues (15). As a general rule, though, a large amount of virus circulating in the blood of the mother for a sustained period is thought necessary for most viral infections to occur in the fetus (11). Although that condition exists in mink suffering from AD, it does not in subclinically infected mink in which viremia is either absent or present in low titers and is transient. Perhaps even when moderate amounts of virus are present in lymphoid tissues during active infection, mink fetuses do not become infected unless viremia of a moderate titer supervenes and persists for more than a brief period.

The occurrence of AD in only 5 of the 27 mink is consistent with findings from previous studies in which pastel mink were inoculated with the Pullman strain of AD virus (4, 6–9). Yet, manifestations of AD might have been detected in some of the 15 mink killed before day 43 if they had been examined later. Evidence of AD in one mink on day 43 seemed unusually early for pastel mink inoculated with the Pullman strain of AD virus. Nevertheless, as found in other studies (6, 9), the disease still usually evolves slowly in pastel mink, especially when they are infected with virus of low virulence for them. As expected, virus was widespread and titers were generally high in mink succumbing to the disease.

Our limited findings provide at least some insight into the virologic events occurring in pastel mink infected with AD virus of low virulence. A more complete understanding of these events awaits the advent of a less cumbersome, less time-consuming, and less costly way to assay the virus than by inoculating mink of the Aleutian genotype (e.g., sapphire color phase), however sensitive that method is in detecting it. So far, cell culture (13, 17) has not fulfilled this pressing need in efforts to learn more about AD as an infectious disease.

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LITERATURE CITED

1. **An, S. H., F. J. DePauli, P. Wright, and D. G. Ingram.** 1978. Characteristics of inapparent Aleutian disease virus infection in mink. Res. Vet. Sci. **24**:200–204.
2. **An, S. H., and D. G. Ingram.** 1977. Detection of inapparent Aleutian disease virus infection in mink. Am. J. Vet. Res. **38**:1619–1624.
3. **An, S. H., and D. G. Ingram.** 1978. Transmission of Aleutian disease from mink with inapparent infections. Am. J. Vet. Res. **39**:309–313.
4. **Bloom, M. E., R. E. Race, W. J. Hadlow, and B. Chesebro.** 1975. Aleutian disease of mink: the antibody response of sapphire and pastel mink to Aleutian disease virus. J. Immunol. **115**: 1034–1037.
5. **Cho, H. J., and J. Greenfield.** 1978. Eradication of Aleutian disease of mink by eliminating positive counterimmunoelectrophoresis test reactors. J. Clin. Microbiol. **7**:18–22.
6. **Eklund, C. M., W. J. Hadlow, R. C. Kennedy, C. C. Boyle, and J. A. Jackson.** 1968. Aleutian disease of mink: properties of the etiologic agent and the host responses. J. Infect. Dis. **118**:510–526.
7. **Hadlow, W. J., R. E. Race, and R. C. Kennedy.** 1983. Comparative pathogenicity of four strains of Aleutian disease virus for pastel and sapphire mink. Infect. Immun. **41**:1016–1023.
8. **Hadlow, W. J., R. E. Race, and R. C. Kennedy.** 1984. Royal pastel mink respond variously to inoculation with Aleutian disease virus of low virulence. J. Virol. **50**:38–41.
9. **Henson, J. B., J. R. Gorham, J. C. McGuire, and T. B. Crawford.** 1976. Pathology and pathogenesis of Aleutian disease, p. 175–205. *In* R. H. Kimberlin (ed.), Slow virus diseases of animals and man. Elsevier/North-Holland Publishing Co., Amsterdam.
10. **Larsen, A. E.** 1966. AD research progress report of Utah Fur Breeders Ag. Co-op. Am. Fur Breeder **39**:12–15, 44.
11. **Mims, C. A.** 1968. Pathogenesis of viral infections of the fetus. Prog. Med. Virol. **10**:194–237.
12. **Padgett, G. A., J. R. Gorham, and J. B. Henson.** 1967. Epizootiologic studies of Aleutian disease. I. Transplacental transmission of the virus. J. Infect. Dis. **117**:35–38.
13. **Porter, D. D., A. E. Larsen, N. A. Cox, H. G. Porter, and S. C. Suffin.** 1977. Isolation of Aleutian disease virus of mink in cell culture. Intervirology **8**:129–144.
14. **Porter, D. D., A. E. Larsen, and H. G. Porter.** 1969. The pathogenesis of Aleutian disease of mink. I. *In vitro* viral replication and the host antibody response to viral antigen. J. Exp. Med. **130**:575–593.
15. **Porter, D. D., A. E. Larson, and H. G. Porter.** 1977. Reduced severity of lesions in mink infected transplacentally with Aleutian disease virus. J. Immunol. **119**:872–876.
16. **Shahrabadi, M. S., and H. J. Cho.** 1977. Detection and localization of Aleutian disease virus and its antigens *in vivo* by immunoferritin technique. Can. J. Comp. Med. **41**:435–445.
17. **van Dawen, S., O.-R. Kaaden, and S. Roth.** 1983. Propagation of Aleutian disease parvovirus in cell line CCC clone 81. Arch. Virol. **77**:39–50.
18. **Wright, P. F., and B. N. Wilkie.** 1982. Detection of antibody in Aleutian disease of mink: comparison of enzyme-linked immunosorbent assay and counterimmunoelectrophoresis. Am. J. Vet. Res. **43**:865–868.