Pathogenesis of Aleutian Disease of Mink: Identification of Nonpersistent Infections

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Aleutian disease virus usually produces a persistent infection and progressive immune complex disease in mink of the Aleutian genotype. Study of Aleutian disease virus infection in non-Aleutian mink showed that about one-quarter developed nonpersistent infections by the virus, and that the nonpersistence was not genetically determined by the host. The nonpersistently infected mink developed only a transient elevation of serum gamma globulin, and much lower specific Aleutian disease virus antibody titers than persistently infected mink. No lesions were found in the nonpersistently infected mink.

Aleutian disease virus (ADV) replicates rapidly in vivo and peak viral titers are found 10 days after experimental infection; most mink become persistently infected and have virus present in the viscera, serum, and urine for the remainder of their lives (2, 9). Antibody to ADV antigen appears 9 to 10 days after infection and reaches very high levels (1, 4, 9). ADV in the circulation of persistently infected mink is in the form of infectious virus-antibody complexes (8), and the arterial and glomerular lesions of Aleutian disease are caused by deposition of immune complexes with a subsequent inflammatory response (3, 6, 11).

During quantitative titrations of ADV in mink heterozygous for or lacking the Aleutian gene, we noted that some mink failed to develop tissue lesions despite serological evidence of ADV infection. The present study was undertaken to define ADV infection without lesions and to ascertain if nonpersistent infections were genetically determined.

Materials and Methods

Mink. Ranch-raised mink (Mustela vison) were obtained from the Fur Breeders' Agricultural Cooperative, Midvale, Utah. All mink used in the study of nonpersistent infection had a pastel coat color and were heterozygous for or did not carry the recessive Aleutian gene. Mink which received serum for assay of ADV infectivity had a violet coat color and were homozygous for the Aleutian gene; we have not noted nonpersistent ADV infection in this genotype. The mink were 5 months of age when they were infected with ADV, and were caged individually and fed a standard ranch diet. The mink were routinely immunized with distemper (live), viral enteritis (formalin killed), and Clostridium botulinum type C toxoid vaccines.

Laboratory techniques. Serum protein electrophoresis was performed and controlled as previously described (9) (Microzone system, Beckman Instruments, Inc.). ADV antibody was determined by indirect immunofluorescence using liver sections of acutely infected mink as a source of ADV antigen (9). The controls used in the ADV antibody tests were the same as previously described. Criteria for the gross and histological diagnosis of Aleutian disease were the same as previously used in our laboratory (7).

Aleutian disease virus. The passage history and the method of in vivo titration of the ADV stocks has been described (9, 10).

Experimental design. A group of 150 5-month-old pastel mink of both sexes with a serum gamma globulin of 14.9% or less as determined by electrophoresis was infected by intraperitoneal inoculation of 10^8 pastel mink mean infective doses of ADV. Two animals were later excluded from the experiment due to preexisting ADV antibody. Cardiac punctures for blood samples performed under pentobarbital anesthesia caused about a 2% mortality per bleeding. Serum protein electrophoresis was performed before and 30 and 66 days after infection, and ADV antibody determination was done before and 66 days after infection. Mink having a serum gamma globulin that was greater than 20% or that increased from day 30 to day 66 were tentatively assigned to the persistently infected group and the remaining mink were tentatively assigned to the nonpersistently infected group. At day 90, the mink assigned to the persistently infected group were sacrificed for pelts and examined for lesions of Aleutian disease; eight randomly chosen mink of the nonpersistently infected group were similarly examined. Thirty-two of the nonpersistently infected group were kept for breeding purposes, and 1 ml of serum from each of these mink obtained 120 days after infection was inoculated into two indicator violet mink, which were then followed for a 5-month period for development of the characteristic serum protein changes or morphological changes of Aleutian disease, and for development of ADV antibody.
The 32 remaining mink judged to be nonpersistently infected were mated with each other by standard mink ranching procedures. Each nonpersistently infected female was mated on two occasions within 1 week with different nonpersistently infected males; 92 offspring resulted from these matings. Sequential mating results in a higher pregnancy rate in mink than does a single mating; the offspring are usually the product of the second mating. At 3 months of age, none of the offspring had ADV antibody, indicating that no transplacental infection by ADV had occurred (5). When the offspring were 5 months of age, 56 were challenged with 10^4 mean infective doses of the same passage of ADV as used to infect their parents. The challenged offspring were followed by serum protein electrophoresis and for the development of ADV antibody, and were sacrificed for morphological examination 3 months after ADV challenge.

RESULTS

Of the 150 pastel mink initially selected, 140 were ADV antibody-negative before experimental infection and survived 90 days after infection with ADV. At that time, all 99 mink which had a serum gamma globulin of 20% of the total serum protein 66 days after infection or which had an increase in serum gamma globulin from 30 to 66 days after infection were sacrificed for pelts. Each of these 99 mink had typical gross or microscopic tissue lesions of Aleutian disease and were tentatively assigned to the persistently infected group. Eight of the 41 mink which had a serum gamma globulin less than 20% of the total serum protein 66 days after infection and which did not have an increase in serum gamma globulin from 30 to 66 days after infection were necropsied 90 days after ADV infection. None of these eight mink had gross or microscopic lesions of Aleutian disease; therefore, the remaining group of 33 mink with nonprogressive serum gamma globulin changes were tentatively assigned to a nonpersistently infected group.

Using the criteria outlined in Materials and Methods, the serum of 32 of the 33 mink assigned to the nonpersistently infected group failed to produce Aleutian disease or ADV antibody in indicator mink. The serum of one mink without progressive gamma globulin changes caused Aleutian disease in both indicator mink, and this mink was excluded from further use. Serum from 8 of the 99 mink assigned to the persistently infected group was tested for the presence of ADV, and all 16 recipients developed Aleutian disease.

Table 1 shows the serum gamma globulin response of mink to persistent and nonpersistent ADV infection. There was no significant difference of the preinfection gamma globulin levels between these two groups of mink, but both 30 and 66 days after infection there was a highly significant (P < 0.01) difference of the mean values. Although it could be argued that this finding was simply a result of the selection criteria applied to group the mink, Table 2 shows that the mean specific ADV antibody titer 66 days after experimental infection was 7.4-fold higher in the persistently infected mink as compared with the nonpersistently infected mink. The nonpersistently infected mink had a drop of the ADV antibody to very low levels by 500 days after infection. In contrast, the few persistently infected mink of this genotype that survive ADV infection for 500 days generally have antibody titers similar to or higher than those found 2 months after infection (unpublished data). When the nonpersistently infected mink were necropsied 500 days after infection, no lesions of Aleutian disease were found.

To test whether nonpersistent infection by ADV was genetically determined by the mink, 56 offspring of 32 nonpersistently infected mink were challenged with the same passage and dose

**Table 1. Serum gamma globulin response to Aleutian disease virus infection in pastel mink**

<table>
<thead>
<tr>
<th>Mink group</th>
<th>Preinfection</th>
<th>Day 30</th>
<th>Day 66</th>
<th>Day 500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent infection (N = 99)</td>
<td>12.69 ± 2.23</td>
<td>26.71 ± 4.33</td>
<td>29.95 ± 4.69</td>
<td>Not tested, sacrificed at day 90</td>
</tr>
</tbody>
</table>

**Table 2. Aleutian disease viral antibody response in pastel mink**

<table>
<thead>
<tr>
<th>Mink group</th>
<th>Reciprocal of geometric mean antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Nonpersistent infection (N = 41)</td>
<td>0</td>
</tr>
<tr>
<td>Persistent infection (N = 99)</td>
<td>0</td>
</tr>
</tbody>
</table>
of ADV given to their parents. Using the same criteria for persistence or nonpersistence of infection as applied to the parents, 12 developed nonpersistent infection and 44 developed a persistent ADV infection. Twenty-nine percent of the original unselected pastel mink developed a nonpersistent ADV infection, and 21% of mink whose parents both had a nonpersistent infection developed a nonpersistent infection upon ADV challenge. This result is not significantly different ($P > 0.80$) and suggests that in pastel mink the difference between animals which develop a persistent and nonpersistent ADV infection is not genotypic.

**DISCUSSION**

Aleutian disease of mink is generally considered to be an excellent example of a slow or persistent virus infection. This study demonstrates that approximately one-quarter of unselected pastel mink which are infected by ADV can clear the viremia and fail to develop lesions. This response to ADV infection appears not to be genetically determined by the mink, since offspring of the resistant mink had about the same number of nonpersistent infections.

It might be argued that the failure to demonstrate viremia in mink judged to have nonpersistent infections does not prove that ADV was not persisting in the tissues. The relatively low early ADV antibody response which fell to very low levels by 500 days after infection strongly suggests that there was either no continuing replication of viral antigens in these mink or that only small amounts of virus persisted in limited sites. Conversely, the data also suggest that the very high ADV antibody levels found in persistent ADV infections result from continuing antigenic stimulation by large amounts of replicating virus (1, 4, 9).

This study provides at least a partial explanation for irregular production by disease and death in non-Aleutian mink of ADV infections (2). Mink of the Aleutian genotype appear to be uniformly susceptible to progressive ADV infections (2, 9); however, reported experiments of the type performed here have not been performed with mink of the Aleutian genotype. Since the use of non-Aleutian mink in quantitative virus titrations may result in an underestimate of the ADV infectivity when the endpoint is increased gamma globulin, disease, or death (2, 9), it would appear preferable to use production of ADV antibody as the criterion for infection in future experiments.

The ability of ADV to cause self-limited as well as persistent infections in mink is of importance in understanding the natural history of Aleutian disease. Whether other viruses which typically produce slow or persistent infections in animal hosts also produce a significant incidence of self-limited infections is not known. Such self-limited infections might be identified by an irregular susceptibility to disease production as noted in the non-Aleutian mink.

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**LITERATURE CITED**