THE PATHOGENESIS OF ALEUTIAN DISEASE OF MINK

I. IN VIVO VIRAL REPLICATION AND THE HOST ANTIBODY RESPONSE TO VIRAL ANTIGEN*‡

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Aleutian disease (AD) is a common chronic virus disease of mink, which may cause serious economic losses to commercial ranchers. The most consistent and striking feature of AD is that once a mink has been infected with Aleutian disease virus (ADV), infectious virus may be recovered from the serum, organs, and urine for the remainder of the animal's life (1-4). ADV in the serum of such mink has been shown to exist as an infectious virus–antibody complex (5). The primary lesion of AD is a systemic proliferation of plasma cells. A marked hypergammaglobulinemia, which may change into a monoclonal gammopathy, is secondary to the plasma cell proliferation (6-8). Glomerulonephritis, degenerative arterial lesions, and proliferation of intrahepatic bile ducts are frequently seen in AD (9-11). The markedly increased gamma globulin in AD is the result of overproduction, and has been found to have some degree of specificity (7, 12, 13). However, except for the infectious complexes, antibody to ADV antigens has not been previously demonstrated.

The present study was undertaken to define the early and late stages of ADV replication in mink in an attempt to elucidate the pathogenesis of ADV infection. Since a reproducible in vitro assay for ADV was initially not available, virus replication was determined by quantitative in vivo virus titrations in mink, patterned after Fenner's work on mouse pox (14). During the course of the present study a direct immunofluorescence test capable of detecting

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viral antigens in infected mink cells and an indirect immunofluorescence test for mink antibody to ADV were developed.

Materials and Methods

Mink.—Ranch-raised mink (*Mustela vison*) were obtained from the Fur Breeders Agricultural Cooperative, Midvale, Utah. A total of 1018 mink were used in the experiments exclusive of normal controls for the titration experiments. Since genetic factors are thought to be important in ADV infection (4), two genotypes of mink were used in the viral growth and immunofluorescence studies. Of the two types used, violet mink are homozygous for the recessive Aleutian gene (aa), while pastels may be heterozygous or not carry the gene (Aa or AA). The mink were 2.5-5 months of age when used, and were sacrificed at the end of the experiments. All mink were routinely immunized with distemper, viral enteritis, and type E *Clostridium botulinum* toxoid vaccines.

Electrophoresis.—Serum protein electrophoresis was done on cellulose acetate strips with a model R-100 system and R-110 densitometer (Beckman Instruments, Inc., Palo Alto, California). Two control sera with a mean gamma globulin of 9.0 and 15.2% of the total serum protein were applied to each strip. The electrophoresis was considered satisfactory if the amount of control serum gamma globulin varied no more than ±2% from the mean value. Mink which had a serum gamma globulin of 14.9% or less of the total serum protein were considered to be normal (10).

Aleutian Disease Virus.—The original virus stock was a 10% weight/volume (w/v) spleen homogenate prepared from 30 naturally infected mink in 1963. This stock had a titer of 10^8 ID₅₀/ml and was stored at −70°C. Passage 1: four pastel mink were infected with 1 ml of this stock and the spleen and serum harvested 4 months later. Infectious virus-antibody complexes were present in the serum (5), and the virus in the spleen homogenate, with a titer of 2 × 10⁵ ID₅₀/ml, was considered to be complexed since free antibody was demonstrable in the homogenate by the indirect immunofluorescence test described below. Passage 2: two violet mink were infected with 1 ml of passage 1 virus, and the mink were sacrificed 9 days later. A 10% w/v spleen homogenate was prepared, which had a titer of 1 × 10⁴ ID₅₀/ml. This virus was considered not to be complexed with antibody since neither animal had antibody in the serum nor in the spleen homogenate.

Growth Curves.—Mink with a normal gamma globulin of 14.9% or less and no antibody to ADV as determined by the indirect immunofluorescence test described below were given 0.5 ml of undiluted passage 1 or 1 ml of a 1:10 dilution of passage 2 virus intraperitoneally. Each inoculum thus contained 1 × 10⁸ ID₅₀ of ADV. Pairs of mink of the same color given the same inoculum were sacrificed 1-18 days later. Serum and both frozen and formalin-fixed sections of liver, spleen, lymph node, kidney, lung, thymus, and salivary gland were collected. Equal weights of spleen of the two mink were homogenized at full speed in an Omnimixer (Ivan Sorvall, Inc., Norwalk, Conn.) for 1 min in Eagle’s medium to make a 10% w/v suspension. This suspension was further disrupted in a 10 kHz sonic oscillator (Model DF101, Raytheon Co., Waltham, Mass.) for 30 sec at 1.1 amp. The homogenate was clarified at 2000 g for 15 min and serial 10-fold dilutions of the supernatant were made in Eagle’s medium. Homogenates of liver were prepared in the same fashion as spleen homogenates. Serum for virus titration was serially diluted in Eagle’s medium. The dilutions were kept at −70°C and shipped to Midvale for infectivity titrations within 1 month of the time they were prepared.

Infectivity titrations were carried out in pastel mink. Three mink per dilution were inoculated intraperitoneally with 1.0 ml of test material using individual syringes. Each preparation was tested over at least the dilution range of 10⁻⁸–10⁻¹, regarding the 10% homogenate as a 10⁻¹ dilution. All inoculated mink initially had a serum gamma globulin of 14.9% or less. Mink were bled from the toe approximately 30, 60, and 90 days postinoculation, and serum gamma
globulin determined by electrophoresis. Mink with a serum gamma globulin of 18% or more at 30 or 60 days, which did not fall below 18% by 90 days, were considered to have been infected by ADV. All mink were caged individually. Uninoculated mink and mink given normal mink spleen homogenate were placed in the same areas to evaluate possible random transmission of the virus. (Such random transmission of ADV was not noted during these experiments.) Infectivity was calculated by the method of Reed and Muench (15) and expressed as mg per gram of spleen, of liver, or per milliliter of serum. When the mink which received test materials were sacrificed, they were examined for gross lesions of AD.

Virus Neutralization Test.—IgG from normal and antibody-containing mink sera was prepared by diethylaminoethyl cellulose chromatography, a procedure which separates infectivity from the IgG (16). Four virus preparations were used: (a) Passage 1 spleen homogenate. (b) Passage 1 spleen homogenate extracted twice with 1/3 volume Freon 113. (c) Passage 1 spleen homogenate was sedimented 1 hr at 100,000 g; the pellet suspended in 0.1 M glycine-HCl buffer, pH 3, incubated 15 min, and recentrifuged. The final pellet was suspended in Eagle’s medium. (d) Passage 2 spleen homogenate.

To serial 10-fold dilutions of the four virus preparations a final concentration of 2 mg/ml of mink anti-ADV IgG was added, and to (d) either 2 mg of normal or anti-ADV IgG was added. The mixtures were incubated 1 hr at 37°C, frozen at -70°C and shipped to Midvale. When thawed, the preparations were allowed to stand at room temperature for 1 hr. The virus preparations alone or with antibody added were assayed for infectivity by the procedure outlined above using three or five mink per dilution. The IgG preparations alone were also checked for possible infectivity.

Fluorescent Antibody Techniques.—Rabbit antisera to normal mink IgG and albumin were prepared and characterized as previously described (7). Rabbit antiserum to mink complement was prepared and characterized by the method of Mardiney and Müller-Eberhard (17), except that it was necessary to absorb this antiserum with normal mink IgG to remove antibody to this protein. Normal mink serum was a pool of sera unreactive for antibody to ADV using the indirect fluorescent antibody test. Mink serum with antibody to ADV was pooled from sera giving maximal inhibition of the direct fluorescent antibody test for ADV antigen at a dilution of 1:10. The pooled mink sera were centrifuged at 78,000 g for 2 hr to remove virus-antibody complexes in the antibody-containing serum; however, this step was found to be unnecessary.

Labeling of antisera with fluorescein isothiocyanate, the staining procedures, and the controls used were all identical with those previously described for studies with lactic dehydrogenase virus (18). Cryostat sections of mink tissue were air-dried and fixed in acetone for 15 min. The immunofluorescence tests were performed as follows:

(a) Direct test for ADV antigen: ADV antigen was localized in mink tissue by application of labeled anti-ADV mink serum at a protein concentration of 5 mg/ml. Labeled normal mink serum served to control the test. The test was considered positive if more than 30 cells per tissue section of 0.3 cm² were stained with anti-ADV mink serum and if no reaction was observed with the normal mink serum.

(b) Indirect test for ADV antibody: ADV antibody in mink serum was determined by the indirect method using labeled rabbit anti-mink IgG at a protein concentration of 0.5 mg/ml. Screening tests for ADV antibody were done using 1:10 serum dilutions. Titters of ADV antibody were determined by reacting serial 2-fold (up to 18 days postinfection) or 10-fold (30 and 60 days postinfection) dilutions of serum made in 10% antibody-free mink serum with antigen-containing tissue. Liver sections of mink infected 9 days, having 1000 or more antigen-containing cells per section, served as the antigen-containing tissue. The antibody titer of a serum was taken as the reciprocal of the greatest dilution at which definite staining of infected cells occurred.

(c) Test for immune complexes in kidneys: Examination of kidneys for the deposition of im-
mune complexes was done by the direct method using labeled anti-IgG, anticomplement, anti-albumin and normal and anti-ADV mink sera at a protein concentration of 5 mg/ml.

**Elution of Kidney-Fixed IgG.**—Pools of normal and 90-day ADV-infected pastel mink kidneys were eluted by the method of Lambert and Dixon (19). The IgG concentration was estimated by the method of Fahey and McKelvey (20). The eluates were analyzed by indirect immunofluorescence for antibody to ADV antigen, to glomerular basement membranes, and to nuclei at a concentration of 37 μg IgG per ml for the normal eluate and 45 μg IgG per ml for the ADV kidney eluate.

![Graph showing growth curve of passage 1 Aleutian disease virus in violet and pastel mink spleen.](image)

**RESULTS**

**Time Course of Viral Replication.**—Fig. 1 shows the growth curves of passage 1 ADV in the spleen of violet and pastel mink. Virus was first found in the violet mink spleen 6 days after experimental infection at a titer of $2 \times 10^6$ ID$_{50}$ per g. During the interval of 9–18 days after infection, levels around $10^7$ ID$_{50}$ per g were found in the violet mink spleens. In the pastel mink, virus was first found in the spleen 9 days after infection when $10^6$ and $3 \times 10^6$ ID$_{50}$ per
g were recovered. At 12 and 15 days, the pastel mink had about $10^9$ ID$_{50}$ in the spleen, approximately the same as the violet mink inoculated with the same virus. Sera from these mink were assayed for the presence of ADV at 1, 3, 6, and 9 days after infection. No virus was found in the serum of pastel mink, nor in the serum of violet mink infected 1 or 3 days previously. The violet mink had $1 \times 10^4$ ID$_{50}$ per ml 6 days after infection and $2 \times 10^8$ at 9 days after infection. These levels in the serum represent only 5 and 2% of the amount of infectivity found in the spleen of the same violet mink at these times.

The growth curve of passage 2 ADV in violet mink spleen is shown in Fig. 2. Relatively small amounts of virus were recovered 4 and 6 days after inoculation. An amount of virus greatly in excess of the infectious inoculum was recovered at 8 days; at 10 days a peak titer of $5 \times 10^8$ ID$_{50}$ per g was observed. The amount of virus found in the spleens slowly fell at times later than 10 days. The individual livers of two violet mink given the same inoculum of

![Growth curve of passage 2 Aleutian disease virus in violet mink spleen. Each point represents the amount of infectivity recovered from a pool of the spleens of two violet mink at the indicated time after inoculation of the virus.](image)
passage 2 virus were titered, and $2 \times 10^8$ ID$_{50}$ and $1 \times 10^9$ ID$_{50}$ per g were found at 9 and 10 days, respectively.

No obvious illness was seen in the mink infected with ADV 1–18 days previously, despite the very high virus titers observed. Many of the pastel mink in which the virus was titered showed progressive lethargy and anorexia starting about 40 days after inoculation of virus-containing test material. Nearly all the mink used for the virus titrations which developed elevated gamma globulin levels showed gross lesions of AD, while those with normal gamma globulin levels had no lesions.

**Demonstration of ADV Antigen in Mink Tissue.**—Fluorescein-labeled normal and ADV-infected mink sera were used as a direct stain on tissue from 8 normal mink, the 44 used in the growth curve experiments, and 24 additional mink inoculated with passage 2 virus. The tissues were examined 1–18 and 60 days after infection of the mink. Viral antigen(s) was found in cells of the liver, spleen, and lymph nodes in 34 of 39 mink infected 8–18 days earlier, and in 8 of 14 mink infected 60 days previously, but not in any of the 17 mink infected 1–6 days earlier. In addition, none of the uninfected mink tissues reacted with the sera. In any given animal, if viral antigen was observed in the liver, spleen, or lymph node, it was present in all three organs. Typical Kupffer cells in the liver had a powdery cytoplasmic fluorescence when stained with AD mink serum, and no reaction was seen when normal mink serum was used as a stain. The antigen-containing Kupffer cells occurred in no particular relationship to the lobular architecture; occasionally clusters of 2–4 antigen-containing cells were seen, but more often only individual Kupffer cells contained ADV antigen. The number of ADV antigen–containing Kupffer cells per section of liver approximately 0.3 cm$^2$ in size is shown in Table I, and the appearance of such cells is shown in Fig. 4. The number of antigen-containing cells observed in the liver roughly paralleled the viral infectivity recovered from the spleens. No differences were noted in the pattern of fluorescence staining between violet and pastel mink, or between the two virus passages used.

The intensity of fluorescent staining in ADV antigen-containing cells was very strong 8–12 days after infection and considerably weaker at 15 and 18 days, even though relatively large numbers of infected cells were still present. In the 8 of 14 mink with ADV antigen found at 60 days, the staining was extremely faint.

In the spleen and lymph node, clusters of 10–25 ADV antigen-containing cells were found in the red pulp of the spleen and medullary areas of the lymph nodes. Such cells had the appearance of macrophages, and the ADV antigen was limited to the cytoplasm. A cluster of antigen–containing cells in a lymph node is shown in Fig. 5 $a$ and the lack of a reaction when the labeled normal mink serum was applied to the same tissue is shown in Fig. 5 $b$. In contrast to the liver, where the distribution of infected cells was relatively uniform, the
clusters of infected cells in the spleen and lymph node were frequently separated by several mm or more.

Spleen and lymph node of mink sacrificed 9, 10, and 12 days after inoculation of ADV showed staining of viral antigen in a crescentic cap or ring around some of the lymphoid follicles. It was impossible to discern whether such antigen was on the surface of the cells or within the cytoplasm. This type of staining is shown in Fig. 6.

No cells containing ADV antigen were noted in sections of heart, thymus, and salivary gland of mink infected 1–18 days previously. Rare antigen-containing mononuclear cells were seen in the lumen of pulmonary vessels, but

<table>
<thead>
<tr>
<th>Days post inoculation with $1 \times 10^4$ dose</th>
<th>No. of mink having indicated</th>
<th>No. of infected cells per 0.3 cm$^2$ section of liver</th>
<th>No. of mink with ADV antigen/No. examined</th>
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<tr>
<td>0-30</td>
<td>0/4</td>
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<td>4</td>
<td>4</td>
<td>0/4</td>
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<tr>
<td>60</td>
<td>6</td>
<td>1</td>
<td>2/3</td>
</tr>
<tr>
<td>Uninoculated control</td>
<td>8</td>
<td>0/8</td>
<td></td>
</tr>
</tbody>
</table>

TABLE I

Time Course of the Appearance of Aleutian Disease Virus Infected Kupffer Cells in the Liver of Mink

none in the pulmonary parenchyma. Traces of ADV antigen were seen in the glomeruli of three mink (see below), but no antigen was present in other portions of the kidney.

The only histologic lesion noted during the first 18 days of ADV infection was necrosis of small numbers of Kupffer cells 9 days after infection or later. Such necrotic cells were surrounded by mononuclear cells 12–18 days after infection.

Development of Antibody to ADV in Infected Mink.—Liver sections of mink infected 9 days earlier were chosen as the source of antigen for indirect immunofluorescence tests for ADV antibody. This tissue was suitable since the distribution of infected cells was uniform, and since few or no immunoglobulin-containing cells were present. It is not known whether the tests measure antibody directed against virion or other viral-specific antigens, or both.

To establish the validity of the assay, tests for ADV antibody were initially
done using mink sera from three ranches with a high incidence of ADV infection. The correlation between the antibody tests and the presence or absence of histologic lesions of AD and hypergammaglobulinemia is given in Table II. Only 7 of 100 sera from mink with a gamma globulin of 14.9% or less and no lesions had ADV antibody. In contrast, 9 of the 10 mink with elevated gamma globulin and no lesions had antibody, as did all of the 34 mink with both lesions and hypergammaglobulinemia. Also, all of 24 sera from mink with the monoclonal gammopathy of AD and 11 of 14 sera of ferrets housed with infected mink had such antibody.

None of the mink used in the viral growth experiments initially had ADV antibody, and none of 19 mink sacrificed within 8 days after infection developed such antibody. All mink sacrificed 10 days or later after infection and 4 of 13 mink sacrificed on day 9 had ADV antibody. The results of antibody titrations in this group of mink are given in Table III. Significant differences

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**TABLE II**

*Correlation of ADV Antibody Test with Hypergammaglobulinemia and Histologic Lesions in Mink and Ferrets Naturally Infected with Aleutian Disease Virus*

<table>
<thead>
<tr>
<th>Group</th>
<th>γ globulin</th>
<th>Histologic lesions of AD</th>
<th>Antibody positive/No. tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mink</td>
<td>14.9 or less</td>
<td>0/100</td>
<td>7/100</td>
</tr>
<tr>
<td>Questionable early AD</td>
<td>15.0 or more; mean = 21.8</td>
<td>0/10</td>
<td>9/10</td>
</tr>
<tr>
<td>Typical AD</td>
<td>15.0 or more; mean = 40.6</td>
<td>34/34</td>
<td>34/34</td>
</tr>
<tr>
<td>Monoclonal gammopathy of AD</td>
<td>15.0 or more; mean = 58.8</td>
<td>24/24</td>
<td>24/24</td>
</tr>
<tr>
<td>Ferrets housed with AD-infected mink</td>
<td>Variable 11/14 below 20</td>
<td>2/14</td>
<td>11/14</td>
</tr>
</tbody>
</table>

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**TABLE III**

*The Development of Antibody to Aleutian Disease Virus Antigen in Mink*

<table>
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<tr>
<th>Days postinoculation with 10⁴ dose</th>
<th>No. tested/No. with 10⁻⁸ positive</th>
<th>Geometric mean titer</th>
<th>Gamma globulin mean %</th>
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<td>1-8</td>
<td>0/19</td>
<td>0</td>
<td>Not tested</td>
</tr>
<tr>
<td>9</td>
<td>4/13</td>
<td>3</td>
<td>7.2</td>
</tr>
<tr>
<td>10</td>
<td>7/7</td>
<td>24</td>
<td>6.7</td>
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<tr>
<td>12</td>
<td>7/7</td>
<td>40</td>
<td>9.4</td>
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<td>15</td>
<td>7/7</td>
<td>145</td>
<td>10.6</td>
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<td>18</td>
<td>3/3</td>
<td>509</td>
<td>8.7</td>
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<td>30</td>
<td>14/14</td>
<td>4,400</td>
<td>25.8</td>
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<td>60</td>
<td>14/14</td>
<td>100,000</td>
<td>31.6</td>
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between mink given passage 1 and 2 virus, or between violet and pastel mink were not found, so the data were combined. The geometric mean ADV antibody titer rose from 3 on day 9 to 40 on day 12 and 145 on day 15. By day 30, when hypergammaglobulinemia was evident, the titer was 4400. On day 60, the extraordinarily high titer of 100,000 was found. A general correlation between antibody titers and gamma globulin levels was noted.

Of the large number of mink sera studied by indirect immunofluorescence tests, only two gave weak antinuclear reactions and one reacted with cell membranes. No prozone phenomena were encountered in sera with a high ADV antibody titer.

**Attempted Neutralization of ADV.**—Attempts to neutralize four preparations of ADV with antibody-containing mink IgG which lacked infectivity are summarized in Table IV. A constant amount of IgG and varying amounts of ADV were used. Infectivity is expressed as the amount per milliliter of virus stock rather than per gram of tissue as previously. No neutralization of ADV was found when the virus-antibody mixtures were inoculated intraperitoneally.

**Examination of Kidneys for the Presence of Immune Complexes.**—Sections of the kidneys of eight control mink, 24 used in the growth curve experiments, and 14 taken 60 days after infection, were examined for the presence of host IgG, complement and albumin and for ADV antigen in the glomeruli. Neither the control mink or those infected 1–8 days had localization of these proteins in the glomerular capillaries. All mink examined which were infected 9–18 days showed a slight to moderate amount of IgG and complement deposited in a granular distribution along the glomerular capillary walls. An extremely faint stain for ADV antigen in the same distribution was present in the glomeruli of three mink infected 9 days; no ADV antibody was detectable in the serum of these three mink. Labeled anti-albumin and normal rabbit and mink sera did not stain glomeruli in this pattern. Staining of the glomerular deposits was abolished by addition of specific antigen to the labeled antibody preparation.

Of the 14 mink kidneys examined 60 days after infection, all of 7 violet and
5 of 7 pastel mink kidneys had histologic glomerulonephritis. Deposition of mink IgG in a lumpy pattern along the glomerular capillary walls was seen in 5 of 7 violet and in 2 of 7 pastel mink kidneys; the remaining 7 showed only mesangial localization of IgG. Deposits of mink complement in a granular to lumpy pattern along the glomerular capillaries were present in all of 7 violet and 6 of 7 pastel mink kidneys. The amount of complement in the violet mink kidneys was far greater than that in the pastel mink kidneys, and the amount present closely approximated the degree of histologic glomerulonephritis observed. Fig. 7 a and 7 b show the typical appearance of a violet mink kidney stained for mink IgG and complement 60 days after ADV infection. It should be noted that the anticomplement staining had a very low background on mink tissue, while the anti-IgG stain had a high background in hyperglobulinemic but not in normal mink tissues.

12 and 14 μg of IgG per g of tissue were eluted from a pool of normal and a pool of 90-day infected pastel mink kidneys, respectively. Neither eluate stained normal mink liver or gave an antinuclear staining reaction. The eluate from the infected mink kidneys gave a strong stain on infected mink Kupffer cells, and the normal kidney eluate gave no reaction. The pattern of staining of the ADV kidney eluate was the same as that given by serum from infected mink. Both eluates gave a weak linear stain on normal mink glomerular capillary walls.

DISCUSSION

Aleutian disease of mink is generally regarded as a “slow-virus” disease (4), using the criteria outlined by Sigurdsson (21). The results of the viral growth experiments obtained in the present study indicate that amounts of Aleutian disease virus greatly in excess of the infecting inoculum can be recovered from mink 8–18 days later. It is evident that this “slow-virus” replicates as rapidly in vivo as do many viruses responsible for acute infections. The passage 2 virus consistently produced higher infectivity titers than did passage 1 virus in violet mink. This may be due to complexing of passage 1 virus with antibody, to an increase of virulence of passage 2 virus with rapid in vivo passage, or to both factors.

Eklund and coworkers (4) have also noted viremia in mink as early as 1 wk after infection. These authors found that aa mink were more susceptible to ADV infection than other genotypes when death from infection was used as the endpoint. In the present study, similar ADV titers were found both in violet (aa) and in pastel (Aa or AA) mink. It appears that genetic factors influence the severity of the renal lesions, and hence death, to a greater extent than they influence virus titers early in infection.

Earlier studies have indicated that the hypergammaglobulinemia of AD has a considerable degree of specificity, and is not due to indiscriminate proliferation...
of all clones of plasma cells (7, 12, 13). The increased 6.4 S IgG was shown to be
the result of overproduction rather than a defect in catabolism (7). The fairly
frequent conversion to a monoclonal gammopathy in AD also indicates the
participation of relatively few clones of plasma cells in the hypergammaglobul-
linemia (8). In the present study we have found that all mink made antibody to
ADV antigen by 10 days after infection, and by 60 days, the geometric mean
antibody titer determined by indirect immunofluorescence was 100,000. Such a
titer is far greater than that found in other viral infections with immunofluores-
cence techniques (18, 22). While there is likely to be more than one viral antigen
associated with ADV, the available evidence suggests that the hyperglobuline-
mia probably represents an extremely large antibody response to ADV
antigen(s).

Relatively little morphologic damage to the mink takes place during the first
18 days of ADV infection, although the virus titers are quite high. The host
response to viral antigen, resulting in immune complexes and glomerulonephri-
tis, ultimately leads to the death of the mink. A similar sequence of events has
been noted in neonatal lymphocytic choriomeningitis virus infection by
Oldstone and Dixon (23). These authors have referred to disease of this type as
chronic, a word probably more descriptive of the actual situation than Sigurds-
son's (21) designation of slow.

Light and electron microscopic observation of naturally infected mink has
shown severe glomerulonephritis, particularly in the aa genotype (9, 11, 24,
25). Electron-dense subendothelial deposits were present in the glomerular
capillaries. Henson and coworkers (26, 27), in a sequential study of experimen-
tally infected aa mink, have shown the deposition of host IgG and complement
in granular pattern consistent with the localization of the electron-dense de-
posits. Previously, we concluded that only mesangial deposition of IgG could be
found in the glomeruli of AD-infected pastel (Aa or AA) mink (7), but later
found that if kidney sections were washed in saline prior to fixation, small
deposits of IgG could be seen in the glomerular capillary walls (5). In the present
study, 5 of 7 violet and only 2 of 7 pastel mink were found to have an immune
complex type of IgG deposition in the glomeruli 60 days after ADV infection. In
contrast, 13 of these same 14 mink were found to have moderate to marked
deposits of complement in the same location, although the violet mink had much
greater deposits of complement than did the pastel mink. Our earlier failure to
demonstrate immune complexes in the glomeruli of ADV-infected mink ap-
parently was due to using the less affected genotype of mink. Elevated IgG
levels and proteinuria evidently resulted in sufficient IgG in the mesangial areas
of glomeruli to obscure the presence of small to moderate amounts of complexes.
The anticomplement staining reaction, which has an extremely low background,
appears to be much more sensitive for detecting moderate amounts of immune
complexes in hypergammaglobulinemic mink than is the anti-IgG stain.
tendency of aa mink to die of AD much earlier than other genotypes (4) probably is the result of early renal failure due to a considerably greater deposition of immune complexes. However, we have found that antibody to ADV can be eluted from the pastel mink kidneys.

It appears clear that the glomerulonephritis of Aleutian disease is largely due to deposition of immune complexes in the glomeruli. The studies of Dixon and coworkers (28) and Cochrane and Hawkins (29) have indicated that vascular damage is initiated by immune complexes formed in antigen excess. The active complexes have ranged in size from 19S to those almost large enough to spontaneously precipitate. The ADV-infected mink are in far antibody excess for the major viral antigen, but may be in antigen excess for one or more other viral antigens. Complexes of the appropriate size to cause vascular damage, i.e. 22-25S, have been demonstrated in the serum of mink with ADV infection by analytical ultracentrifugation (7). It also seems possible that sufficiently large quantities of complexes formed in antibody excess might be responsible for the glomerular damage.

The in vivo persistence of ADV in the form of infectious virus–antibody complexes (5), and the growth of the virus in cells with a phagocytic function, suggest the hypothesis outlined in Fig. 3. It would appear from the data obtained on the distribution of ADV antigen(s) that ADV replicates in the macrophage, which may also serve an antigen-processing function (30, 31). A humoral antibody response to at least one ADV antigen ensues which in turn results in antigen-antibody complexes, some of which cause tissue damage. Macrophages have
been shown to be attracted to and ingest antigen-antibody complexes in the studies of Ward (32) and Benacerraf and coworkers (33). If virion-antibody complexes can be split in the macrophage, the resulting reactivated virus could undergo a cycle of replication in the new cell. The lack of neutralizing capacity of the antibody in ADV infection would be in favor of this hypothesis. If such a mechanism exists, the host antibody response might actually help perpetuate the infection by aiding virus reactivation from phagocytized complexes rather than aid in recovery from disease. This proposal may be equally applicable to lactic dehydrogenase virus infection of mice (18, 34–36).

SUMMARY

Mink inoculated with $1 \times 10^5$ ID$_{50}$ of Aleutian disease virus revealed very high virus titers in the tissues 8–18 days later. The highest virus titers observed were $5 \times 10^6$ ID$_{50}$ per g of spleen and $1 \times 10^8$ ID$_{50}$ per g of liver 10 days after inoculation. Concomitant with the increase in infectious virus titers, viral antigen(s) was found in the cytoplasm of macrophages in the spleen and lymph nodes and in Kupffer cells in the liver. Antiviral antibody was assayed by indirect immunofluorescence, using sections of infected liver as the source of antigen. A few mink infected for 9 days and all those infected 10 days or more developed antibody to Aleutian disease virus antigen(s). By 60 days after infection, when hypergammaglobulinemia was marked, the mink had an exceptionally high mean antibody titer of 100,000.

The pathogenesis of the glomerulonephritis of Aleutian disease is apparently related to formation of viral antigen-antibody-complement complexes which lodge in glomerular capillaries. No evidence was found that viral infection of the kidney took place, and no autoimmune responses were found.

In this “slow-virus” disease the virus replicates rapidly and the morphologic and biochemical manifestations of disease are apparently due to the continuing interplay between a replicating antigen and the host immune response.

The expert technical assistance of Mrs. Joan Brown and Mrs. Alice Karpowitz and the animal management by Mr. Robert Evans was essential to this work.

BIBLIOGRAPHY


Fig. 4. Liver from violet mink inoculated with Aleutian disease virus 10 days previously, stained with fluorescein-labeled mink antibody to ADV. The granular to powdery fluorescence is limited to the cytoplasm of Kupffer cells. Labeled normal mink serum did not stain this tissue. Fluorescence micrograph. × 320.
Fig. 5. Medullary area of a lymph node from a violet mink inoculated with ADV 9 days previously, and stained with (a) fluorescein-labeled mink antibody to ADV, and (b) labeled normal mink serum. A cluster of macrophages show powdery cytoplasmic staining with ADV antibody, but no reaction is seen with the normal mink serum. Fluorescence micrographs. × 320.
Fig. 6. Spleen from violet mink inoculated with ADV 10 days previously, and stained with labeled mink antibody to ADV. A ring of ADV antigen is seen around a lymphoid follicle. Fluorescence micrograph. × 200.
Fig. 7. Renal glomeruli from a violet mink inoculated with ADV 60 days previously and stained with rabbit antibody to (a) mink IgG and (b) mink complement (β1c globulin). Immunoglobulin and complement are deposited along the glomerular capillary walls in a granular to lumpy pattern. This mink had an ADV antibody titer of $10^5$ at the time of sacrifice. Fluorescence micrographs. × 200.