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Passive Transfer of Antiviral Antibodies Restricts Replication of Aleutian Mink Disease Parvovirus In Vivo

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When mink kits were infected neonatally with a highly virulent strain of Aleutian disease virus (ADV), 100% of both Aleutian and non-Aleutian genotype mink died of interstitial pneumonia characterized by permissive ADV infection of alveolar type II cells. Treatment of infected kits with either mink anti-ADV gamma globulin or mouse monoclonal antibodies against ADV structural proteins reduced mortality by 50 to 75% and drastically reduced the severity of clinical signs. Interestingly, mink kits that survived the acute pulmonary disease all developed the chronic form of immune complex-mediated Aleutian disease. Thus, the antibodies directed against ADV structural proteins were capable of modulating the in vivo pathogenicity from an acute fulminant disease to a chronic immune complex-mediated disorder. The mechanism of this modulation was examined by strand-specific in situ hybridization. We found that the number of ADV-infected type II cells was the same in both untreated and antibody-treated kits. However, in the treated kits, viral replication and transcription were restricted at the cellular level. These data suggested that antibodies prevented acute viral pneumonia by restricting the intracellular level of viral replication and that the relevant antigenic determinants were contained within the viral structural proteins. The restricted levels of viral replication and transcription seen in antibody-treated mink kits resembled the levels observed in infected adult mink and suggested a role of antiviral antibodies in development of persistent infection and chronic immune complex disease.

Aleutian disease virus (ADV) is a nondefective parvovirus (11) which causes a persistent infection leading to a usually fatal, immune complex-mediated disease in adult mink. This disease, classic Aleutian disease (AD), is characterized by plasmacytosis, hypergammaglobulinemia, and immune complex-mediated glomerulonephritis and arteritis (29, 41, 43). Although the mink produce massive amounts of anti-ADV antibodies, the virus infection is persistent in vivo, and moreover, the neutralizing ability of these antibodies in vitro is not easily demonstrated (39, 43, 51; M. E. Bloom, unpublished results).

In 1982 an entirely new type of ADV-associated disease was found in mink kits from four Danish mink ranches that had previously been free of AD. The infection caused interstitial pneumonia in newborn kits (36), and we have previously shown that this pneumonia can be experimentally reproduced by inoculating ADV into neonatal mink from ADV antibody-negative females (3, 5, 7). Although ADV infection in mink has been known to occur for more than 30 years (31), the interstitial pneumonia was not observed until 1982. In most countries ADV is endemic and most mink are infected; however, in Denmark, as a result of an efficient eradication program, more than 50% of Danish mink farms are free of ADV infection as measured by lack of antibodies to the virus (2). Consequently, a major change in the immune status of mink dams occurred in Denmark, and the appearance of pneumonia correlated with this change in immune status. Although kits born to ADV-infected dams do develop classic immune complex-mediated disease (3; S. Alexandersen, unpublished results), the accumulated information suggested that perhaps maternally transferred antibodies masked the development of pneumonia.

By using immunofluorescence, Southern blot analysis, and strand-specific in situ hybridization, we previously showed that the acute pneumonia is caused by cytopathic replication of ADV in lung alveolar type II cells of newborn ADV antibody-negative mink kits. This infection is associated with high levels of viral antigen, viral DNA, the viral replicative forms (RFs) of DNA, and viral mRNA in each infected cell (7). In contrast, in mink infected as adults, viral replication and transcription are dramatically altered. The target cells lie within lymphoid organs (probably lymphocytes), and ADV expression is markedly restricted at the cellular level. That is to say, the level of viral RF DNA and mRNA is decreased by a factor of 10 to 100 and intranuclear viral protein, which is easily detected in infected mink kits, cannot be detected (6).

One difference between adult and newborn mink is the rapid development of anti-ADV antibodies in adults, and we previously speculated that the restricted pattern seen in adults might be a result of the anti-ADV antibody response (6). Similarly, we wondered whether the failure to observe pneumonia in ADV antibody-positive kits was due to some effect of maternally transferred antibodies. In the present study, we directly examined the effect of passive anti-ADV antibodies on the replication of ADV and development of disease in neonatally infected mink kits. Our results showed that antibodies restrict viral replication at the cellular level, preventing the acute but not the chronic disease caused by ADV.

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MATERIALS AND METHODS

Virus. In vivo-passaged ADV-Utah I virus (14), obtained from United Animal Division (Middleton, Wis.), was passaged once in newborn ADV antibody-negative mink kits. An inoculum was prepared from their lungs, spleens, and livers and adjusted to contain 10^7 adult mink 50% infective doses per ml as described before (7).

Animals. Mink used in this study were either pastel mink (non-Aleutian, genotype *A/A*) or sapphire mink (Aleutian, genotype *a/a*) and were housed in commercial mink cages and fed a standard mink diet. ADV antibody-negative mink were obtained from a farm known to be free of ADV infection for at least the last 3 years. Sera from these mink were negative for circulating anti-ADV antibodies when tested by countercurrent immunoelectrophoresis (CCI) (15) and rocket line immunoelectrophoresis (8). Mink kits were kept as family groups with their dams until approximately 2 months old and then placed in pairs. Kits were inoculated intraperitoneally (i.p.) as newborns (within 16 h after birth) with 0.1 ml of virus inoculum containing 10^6 50% infective doses. Control kits were inoculated i.p. as newborns with 0.1 ml of phosphate-buffered saline (PBS). No distinction was made between female and male kits.

Gamma globulin preparation. To study the role of antibodies in the development of lung lesions, a virus-free anti-ADV gamma globulin pool was prepared as follows. Sera from 10 mink naturally infected with ADV-DK (1) and having an anti-ADV titer above 1:100 in CCI were obtained from mink at a Danish farm known to be heavily infected with ADV. These sera were pooled and sterile filtered, and an equal part of 50% (wt/vol) ammonium sulfate was added. The suspension was incubated for 1 h at 4°C and then centrifuged at 1,500 × *g* to pellet the precipitate. The pellet was washed twice with 1.75 M ammonium sulfate, suspended in the original serum volume of PBS, and ultracentrifuged at 150,000 × *g* for 2 h. The supernatant was reprecipitated with ammonium sulfate as described above, suspended in PBS to five times the original serum volume, and filtered through an Amicon XM 300 filter (300,000-dalton cutoff; Amicon Division, W. R. Grace & Co., Danvers, Mass.) at 10 lb/in^2. The filtrate was finally dialyzed against PBS with 20% polyethylene glycol (molecular weight 20,000) until a protein concentration of 2 mg/ml was obtained. The final product was tested in zone electrophoresis and thin-layer rocket electrophoresis against immunoglobulin class-specific antisera as described previously (4) and was found to be more than 95% immunoglobulin G (IgG). The preparation was also tested in CCI and had a titer of approximately 1:500 against ADV. To confirm that the IgG preparation was free of ADV, 1 ml of the preparation was inoculated i.p. into three adult sapphire mink. The mink were free of AD lesions and anti-ADV antibodies when killed 3 months after inoculation, indicating that the preparation did not contain infectious ADV.

Another gamma globulin preparation was made, as described above, from a pool of four anti-ADV monoclonal antibodies. These were ascites fluids produced in pristane-primed mice inoculated i.p. with 10^7 hybridoma cells. The four hybridomas (30, 68, B10, and 97) all had reactivity against ADV structural proteins and have been described before (46). This preparation was adjusted to contain 5 mg of total protein per ml and had an anti-ADV titer of approximately 1:500 in CCI.

Control gamma globulin preparations were made in the same way from the sera of normal adult ADV-negative mink or normal adult mice. These preparations were adjusted to contain 2 (mink) or 5 (mouse) mg of total protein per ml and were negative in CCI.

Antibodies were passively administrated to the kits by intramuscular injections of 0.1 ml of either of the gamma globulin preparations at birth or on the days indicated below.

Electrophoresis. CCI and rocket line immunoelectrophoresis were performed as described previously (8). Serum γ-globulin was quantitated by densitometric scanning of electrophoresed sera on agarose gels stained with amido black as described previously (4).

Dot-blot hybridization for the presence of ADV DNA. Serum samples to be analyzed by dot-blot hybridization were prepared by taking 20 μl of serum and adding 1 μg of salmon sperm DNA as carrier and 150 μl of 0.5 M NaOH–1.5 M NaCl to lyse the virus and denature the DNA. Denatured samples were then directly applied to a nylon filter (Hybond-N, Amersham) wet with 0.5 M NaOH–1.5 M NaCl with a vacuum manifold (Bethesda Research Laboratories) and washed with the same solution. The filters were subsequently washed in 2× SSPE (38), dried, and hybridized with an ADV plus-strand RNA probe as described previously (5, 12). Twofold dilutions of positive and negative standards were included in each blot, and calculations were done as described previously (10).

Light microscopy and in situ hybridization. At selected days, mink kits were exsanguinated under pentobarbital anesthesia. Dead kits were collected each day. Moribund kits were killed in extremis, and if severe lung lesions were observed by light microscopic examination, data for these kits were included in the calculations. Transversely cut segments of the middle part of the cranial and caudal lung lobes and pieces of liver, spleen, kidney, mesenteric lymph node, and gut (proximal jejunum) were fixed in periodate-lysine-paraformaldehyde-glutaraldehyde (PLPG) fixative and processed for examination by light microscopy and in situ hybridization as described previously (3, 6, 7). This sampling technique has given highly reproducible morphological and quantitative results in several previous studies (3, 5–7, 9). ADV-infected CRFK cells (7) and tissue sections from adult ADV-infected mink (6) were included in each hybridization and served as an internal standard by which the genome number could be calculated. This method of autoradiographic in situ hybridization with counting of developed silver grains and comparison with standards is highly accurate (6, 7, 26, 28).

Statistics. Data were analyzed by Student's *t* test and chi-square tests for statistical significance.

RESULTS

Clinical and histological characteristics of ADV-induced interstitial pneumonia. We first studied the clinical manifestations, mortality, and pathology of ADV-induced interstitial pneumonia and the effect of antibodies on these parameters. The clinical and pathological findings will only be described briefly, since they have been described in detail previously (3, 5).

When ADV-Utah I was given to either sapphire (*a/a*) or pastel (*A/A*) newborn mink kits, 100% of the kits died (10 of 10 in each group). All of these kits showed severe clinical signs of respiratory distress and were either killed in extremis or died within 24 h after the first clinical signs were observed. Infected kits had severe pulmonary lesions consisting of diffuse hypertrophy and hyperplasia of alveolar type II cells, interstitial edema, hyaline membrane formation, and intranuclear inclusion bodies in alveolar type II

TABLE 1. Summary of experiments with mink kits infected neonatally with ADV-Utah I

Mink	Infection	Gammaglobulin source	% of animals showing clinical signs	No. dead/no. tested	Response to infection[a]				AD status[b] (no. positive/ no. surviving)
					PID 8		PID 12		
					Antibody	Viremia	Antibody	Viremia	
Sapphire	Mock	None	0	3/22	− (2)	− (2)	− (2)	− (2/2)	0/19
	ADV	None	100	10/10	− (4)	5×10^8	− (2)	4×10^8	
	ADV	Normal mink	100	15/15	− (2)	2×10^8	− (2)	2×10^8	
	ADV	Normal mouse	100	13/13	− (2)	2×10^8	− (1)	2×10^8	
	ADV	ADV-infected mink	43	6/14	+ (2)	2×10^7	− (2)	6×10^7	NA[c]
	ADV	MAb[d]	25	6/24	+ (2)	3×10^7	− (2)	5×10^7	8/8
Pastel	Mock	None	0	2/20	− (2)	− (2)	− (2)	− (2/2)	0/18
	ADV	None	100	10/10	− (2)	3×10^8	− (2)	8×10^7	
	ADV	ADV-infected mink	33	3/12	+ (2)	4×10^6	− (2)	2×10^7	4/4

[a] The presence (+) or absence (−) of anti-ADV antibodies in serum is indicated. The number of animals tested is shown in parentheses; the same result was obtained for both (all) animals tested in all cases. For viremic animals, viremia is expressed as number of viral genomes per milliliter of serum. Viremia levels within each group were within one twofold dilution in all cases.

[b] Kits that survived to 10 months of age were bled, and their AD status was determined. Kits with a positive anti-ADV antibody titer and mor than 20% gamma globulin in serum (19) were considered positive for classic AD.

[c] NA, Survived neonatal period but died.

[d] MAb, Mouse anti-ADV monoclonal antibody.

cells. Randomly picked infected untreated kits killed at postinfection days (PIDs) 8 and 12 all had severe diffuse lesions, and no kits survived longer than PID 17.

On the other hand, when mink anti-ADV gamma globulin was administered to similarly infected kits on PID 0 (simultaneously with virus inoculation) and on PIDs 5 and 8, mortality was reduced to 43% (6 of 14) for sapphire kits and 25% (3 of 12) for pastel kits. The effect of mouse anti-ADV monoclonal antibodies was also tested by giving three injections (PIDs 0, 5, and 8) of a mixture of four monclonal antibodies directed against ADV structural proteins to infected sapphire kits. Treatment with monoclonal antibodies reduced mortality to 25% (6 of 24). Treated kits that eventually died in spite of the treatment showed severe clinical signs of lung disease and severe histological lesions as described above. Surviving mink showed no or very mild clinical symptoms, and histological examination of mink kits killed at PIDs 8 and 12 showed no detectable lung lesions. Treatment of infected kits with either normal mink immunoglobulin or normal mouse immunoglobulin did not decrease mortality (15 of 15 and 13 of 13, respectively), and all kits showed severe clinical symptoms and lung lesions. These experiments showed that pneumonia could be prevented in both Aleutian and non-Aleutian genotype mink and that both mink anti-ADV gamma globulin and mouse monoclonal antibodies directed against ADV structural proteins were effective in preventing fatal pneumonia.

Detection of viremia and anti-ADV antibodies. ADV pneumonia is characterized by high levels of viremia and low or undetectable levels of anti-ADV antibodies, and consequently we wanted to study the effects of passively transferred antibodies on these parameters. Therefore, one to four randomly selected kits were killed on PIDs 8 and 12. Sera from these kits were tested by CCI for the presence of antibodies against ADV and by dot-blot hybridization analysis for the presence of ADV viremia. Mink kits that received antibodies, either as mink gamma globulin or mouse monoclonal antibodies, had detectable anti-ADV antibodies at PID 8 but not at PID 12. Kits not treated with antibodies or treated with normal mink or mouse gamma globulin had no detectable circulating anti-ADV antibodies by CCI at either day, indicating that the antibodies detected in treated kits represented passively transferred antibodies.

Dot-blot hybridization analysis of sera showed that the level of viremia in infected, antibody-treated kits, especially on PID 8, was decreased by a factor of 10 to 100 compared with that in infected, untreated kits. This decrease in viremia in kits treated with antibodies compared with untreated or normal gamma globulin-treated kits was statistically significant by Student's t test ($P < 0.05$). The viremia was decreased most in pastel kits, consistent with the low mortality in this group. The difference between pastel and sapphire kits treated with gamma globulin was statistically significant ($P < 0.05$). At PID 12, the differences between groups became less pronounced and were not significantly different. All of these results are summarized in Table 1.

In situ hybridization analysis of viral replication. Tissue samples from the mink kits were also examined by strand-specific in situ hybridization. We previously showed that approximately 15 to 20% of the alveolar type II cells (ca. 2% of total lung cells) replicated ADV to high titers in infected neonatal mink (5, 7). Surprisingly, the number of lung alveolar type II cells positive for viral replication was the same regardless of whether the kits had received antibodies. However, quantitative analysis of replication within single cells showed that the replication of ADV on PID 8 and to a lesser extent on PID 12 was decreased by a factor of 10 or more in kits treated with mink anti-ADV gamma globulin or with mouse monoclonal antibodies (Table 2). This decrease in replication, as measured by a depression in the content of viral RFs of DNA and mRNA per positive cell, was most easily detected in alveolar type II cells of the lung (Fig. 1), although replication in mesenteric lymph node (Table 2) and other organs (data not shown) was also decreased. Because the absolute number of cells replicating virus was not decreased, these observations indicated that the antibodies were operating at the level of individual infected cells, directly decreasing the replication of ADV.

In situ hybridization with a plus-strand ADV probe, which preferentially detects viral sequestration (6, 7), showed moderate (two- to fivefold) reduction in viral single-stranded DNA production or sequestration in the lung, mesenteric lymph node, and liver (Table 2 and data not shown). In contrast, the level of viral sequestration in kidney glomeruli was decreased more than 50-fold in antibody-treated kits compared with untreated kits (Fig. 2). This suggested that antibodies bound to the surface of the virus may actually have improved virus clearance by cells of the reticuloendo-

TABLE 2. In situ hybridization of tissue from mink kits infected neonatally with ADV[a]

Cell type	Gamma globulin treatment	% Positive cells[b]		No. of viral genomes/positive cell[c]	
		RF + RNA	Virion	RF + RNA	Virion
Alveolar type II	−	2	2	400,000	100,000
	+	2	2	20,000	30,000
Mesenteric lymph node cells[d]					
Kits	−	0.05	1	300,000	60,000
	+	0.05	1	20,000	30,000
Adults	−	2	5	10,000	12,500

[a] Four newborn sapphire mink kits were inoculated with ADV-Utah I, and two of these were treated with gamma globulin. At PID 8, the kits were killed and tissues were processed for in situ hybridization analysis as described in Materials and Methods.

[b] The number of positive cells and number of grains over each positive cell were recorded for an area containing at least 20 positive cells. Cells were scored with either the minus-strand probe (detects ADV RFs of DNA and mRNA) or the plus-strand probe (detects virion DNA).

[c] The number of grains per positive cell was correlated to the number of genomes by comparison with a standard (infected CRFK cells) as previously described (6, 7).

[d] Mesenteric lymph node cells from neonatally infected kits and infected adult mink. The figures for adult mink are estimated from calibrated adult mink sections included in each hybridization experiment and are included for comparison. For experimental details on adult mink ADV infection, see Alexandersen et al. (6).

thelial system in organs other than kidney or may have interfered with trapping of virions in the glomeruli.

No significant differences were detected between kits treated with normal gamma globulin and untreated kits or between kits of the pastel and sapphire genotypes.

Development of classic AD in surviving mink kits. Eight infected sapphire kits treated with monoclonal antibodies and four pastel kits treated with mink anti-ADV gamma globulin survived longer than 10 months after virus inoculation. These mink were bled and their sera were tested by CCI for anti-ADV antibodies and in agarose electrophoresis for γ-globulin quantitation at 10 months postinfection. All 12 mink had high titers (>1:100) against ADV and all had elevated γ-globulin values (more than 20% of total serum protein [Table 1]). Two sapphire and two pastel mink were randomly picked from this group and killed for histopathological and in situ hybridization examination. All four had immune complex lesions typical of AD (43). No evidence of residual lesions from previous acute pneumonia were found. This indicated that antibody-treated kits surviving neonatal ADV infection had only had mild or no lung lesions as kits, since severe lung lesions usually result in chronic residual lesions and fibrosis (17, 18, 33). In situ hybridization analysis showed significant amounts of virion DNA in the liver, spleen, and mesenteric lymph nodes (50 to 125 viral genomes per total cell) of all four mink. A few (0.01%) of the cells in the mesenteric lymph nodes were also positive for ADV RFs of DNA and mRNA (about 3,000 copies per positive cell). The lungs of these mink were negative with either probe.

Because ADV-Utah I is generally considered highly virulent and because survival for more than 5 months is very unusual, we tested samples from survivors from the present study. When 0.1 ml of a 10% homogenate of spleen and mesenteric lymph node tissues from these mink was inoculated i.p. into adult ADV-negative sapphire mink, all developed high anti-ADV antibody titers and severe AD lesions within 2 months after inoculation. Thus, the virus retained its virulence even after persisting for a long time in these mink and had not become attenuated during in vivo persistence. These results indicated that mink kits can be protected from neonatal ADV-induced pneumonia by antibody treatment but that they nevertheless develop persistent infection, hypergammaglobulinemia, and immune complex lesions. Moreover, the results also indicated that some element of the host immune response was important for the pace of immune complex disease development.

DISCUSSION

Infection of seronegative newborn mink kits with ADV results in an acute, fatal interstitial pneumonia. Treatment of such infected newborn mink with anti-ADV gamma globulin reduces the acute mortality in both Aleutian and non-Aleutian genotype mink. However, the mink that survive are not "cured" but go on to develop persistent infection and the classic form of AD, which is a chronic disorder of the immune system characterized by plasmacytosis and immune complex disease. The present study attempted to illuminate the mechanism by which passive anti-ADV antibody treatment modulates the acute disease into a protracted disorder of the immune system.

The prevention of fatal pneumonia was clearly due to antiviral antibodies, because control preparations were totally ineffective. In the absence of anti-ADV antibodies, alveolar type II cells undergo a cytopathic permissive infection by ADV that results in the development of hyaline membranes and respiratory distress. Although the exact genesis of the respiratory distress is not clear, we have speculated that infection of the type II cells leads to decreased synthesis of pulmonary surfactant by these cells (7). The observation that infected type II cells are hypertrophic may implicate disordered surfactant metabolism. On the other hand, the extensive interstitial edema may also play a role in compromising pulmonary function. Nevertheless, the situation is drastically different if antibody treatment is employed. In antibody-treated kits, the lungs are histologically normal, but interestingly, the overall number of infected type II cells in the lung is identical in both treated and untreated kits. However, in antibody-treated kits, the level of intermediates of ADV replication (RFs of DNA and mRNA) in infected cells is depressed by at least a factor of 10, and the infection appears to be converted from a highly permissive one to one in which viral replication is severely restricted. Whatever the exact nature of the functional impairment, antibody treatment is able to alleviate it without totally suppressing ADV infection of type II cells. Perhaps permissive ADV infection of type II cells interferes with normal surfactant metabolism, but the restricted infection induced by antibody treatment does not. Now that the genes for various components of surfactant have been identified (32, 52, 54), it may be worthwhile to observe levels of these components in the ADV pneumonia system.

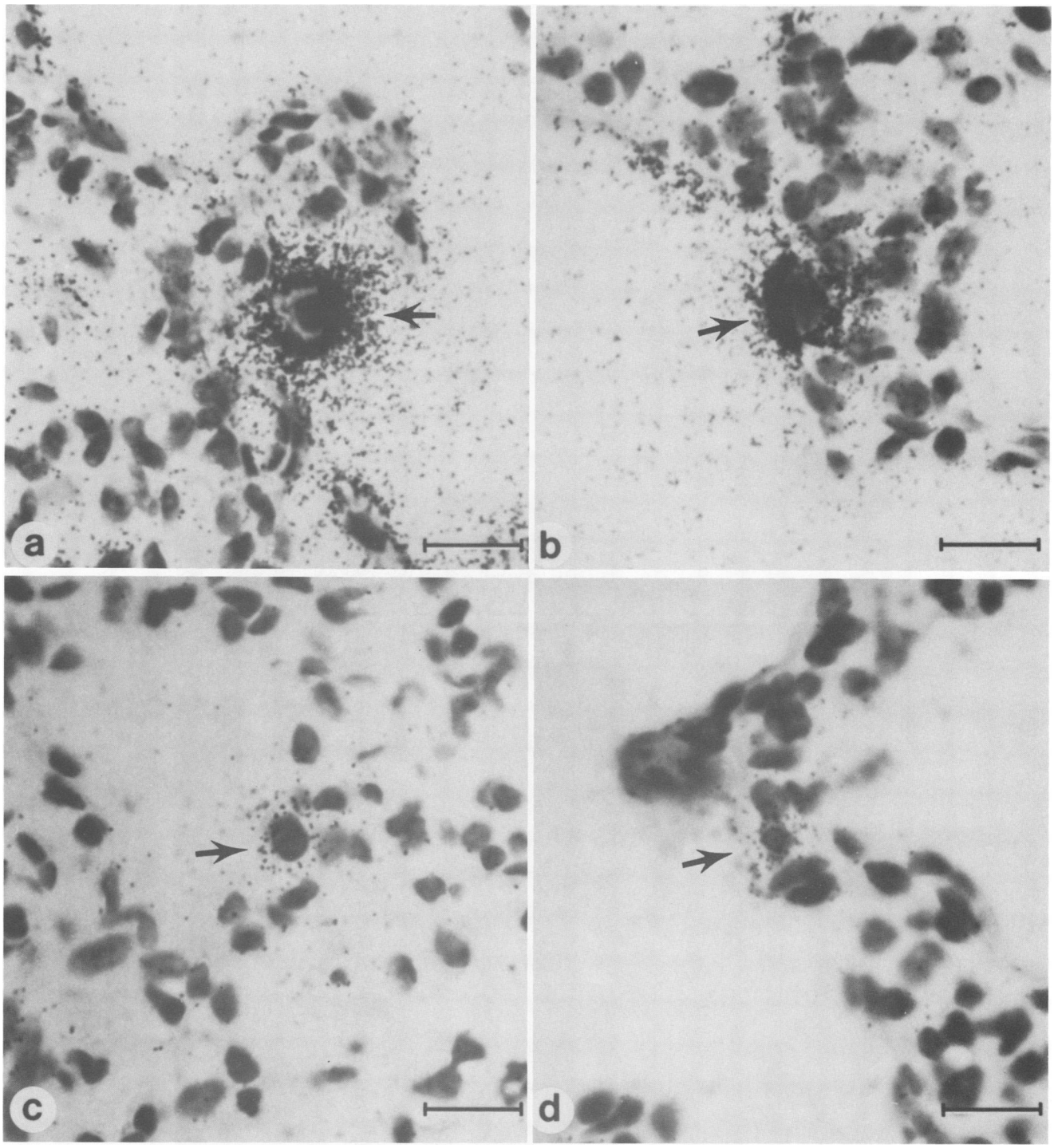

FIG. 1. In situ hybridization analysis for mRNA and RFs of ADV DNA on PLPG-fixed, paraffin-embedded lung sections from infected mink kits with the minus-strand probe. (a and b) In situ hybridization on lung section from a sapphire kit infected as a newborn with ADV-Utah I and killed at PID 8. The section was not treated with RNase. Heavy grain production is seen primarily over the cytoplasm of alveolar type II cells (arrows) and represents hybridization to ADV RFs of DNA and mRNA. (c and d) In situ hybridization on lung section from a sapphire kit infected as a newborn with ADV-Utah I. The kit was treated with anti-ADV monoclonal antibodies at PIDs 0 and 5 and killed at PID 8. The section was not treated with RNase. Light grain production is seen over the cytoplasm of alveolar type II cells (arrows). Bars, 17 μm.

How the antibodies are able to modify ADV replication in the type II cells is not clear. The restriction of ADV replication could be observed after treatment with both mink antibodies and mouse anti-ADV monoclonal antibodies reactive against ADV structural proteins. The fact that these monoclonal antibodies are able to modulate the infection strongly suggests that at least some of the relevant antigenic determinants must be present as components of the virus

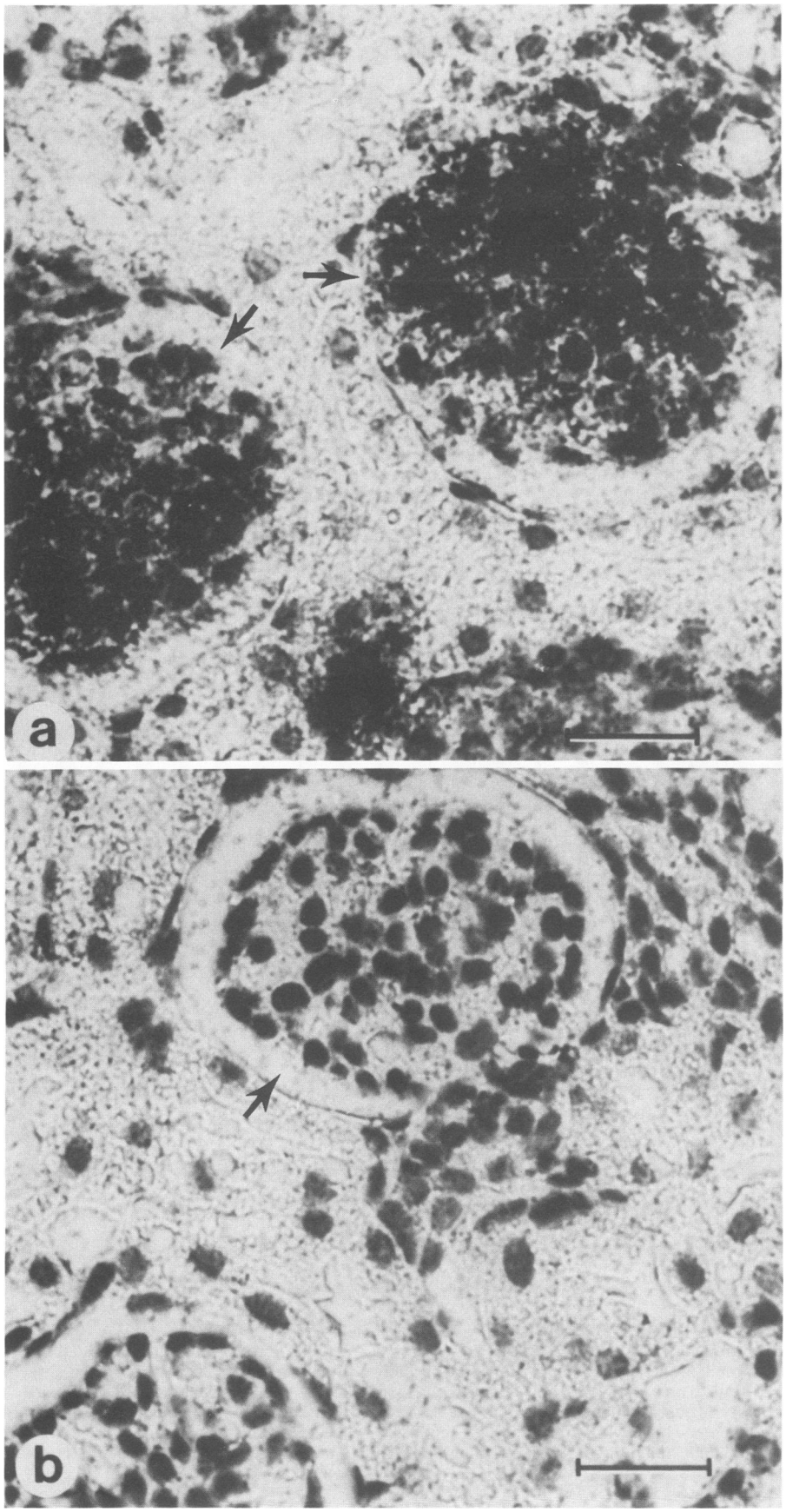

FIG. 2. In situ hybridization analysis for virion DNA on PLPG-fixed, paraffin-embedded kidney sections from infected mink kits with the plus-strand probe. (a) In situ hybridization on kidney section from a sapphire kit infected as a newborn with ADV-Utah I and killed at PID 8. The section was not treated with RNase. Heavy grain production is seen over the glomeruli (arrows) and represents hybridization to sequestered virion DNA. (b) In situ hybridization on kidney section from a sapphire kit infected as a newborn with ADV-Utah I. The kit was treated with anti-ADV monoclonal antibodies at PIDs 0 and 5 and killed at PID 8. Light grain production is seen over the glomeruli (arrow). Bars, 27 μm.

capsid. It has been shown that efficient replication and transcription of the related parvovirus minute virus of mice require a certain amino acid motif on the incoming viral capsid (22). If such a motif is important for permissive replication of ADV in type II cells, maybe the antibodies change or mask it. We are currently examining the modulating effect of other anti-ADV monoclonal antibodies to see whether certain epitopes govern permissibility in this virus-cell system. Experiments with Fab fragments will further show whether the antibody-binding site on the immunoglobulin molecule is the important moiety.

Another mechanism for antibody action could be, as shown for other viruses, that antibodies binding to the surface of the virions result in conformational changes in the virion structure and that such changes, although not affecting viral attachment and penetration, may restrict viral replication and transcription (16, 21, 23, 24, 37). Finally, it is possible that antibody-coated virions are more efficiently and rapidly cleared from the circulation by the reticuloendithelial system or that antibodies block specific receptors necessary for binding of the virus to target cells. However, either of the two latter mechanisms would probably reduce the number of cells replicating the virus instead of restricting replication on a per-cell basis. Regardless of the exact mechanism, clearly anti-ADV antibodies can drastically change the pattern of ADV replication and the nature of the disease induced.

The decreased levels of viremia and viral replication in antibody-treated kits resembled the levels observed in adult mink infected with ADV (Table 2). Adult mink, in contrast to neonatal mink, produce a fast and vigorous antibody response, and the findings presented in the present report further support the idea that antibodies in adult mink may restrict virus replication and transcription to a certain extent (6). This modulation might operate by restricting replication and transcription at the cellular level, as it does in mink kits, and thereby prevent cell death and overt acute disease. However, the antibodies are only capable of preventing acute disease; mink kits surviving neonatal infection as well as mink infected as adults do develop chronic immune complex disease caused by persistent infection with the virus (3). It would be interesting to see whether neutralizing antibodies in other viral diseases work by a similar mechanism to restrict virus replication. Perhaps such antibodies, often capable of preventing both acute disease and development of persistent infection, are able to abolish virus replication completely. Another possible reason for the greater efficiency of some neutralizing antibodies in preventing persistent infection may relate to cellular defense mechanisms supporting virus clearance (13, 20, 35, 50). This would suggest that missing cellular defense mechanisms, perhaps lost due to direct viral infection of effector cell populations (6), have a role in the development of persistent ADV infection.

The development of chronic disease in antibody-treated infected neonatal mink was a consistent finding. However, there were some differences between the survivors and mink infected as adults. Sapphire mink infected as adults with ADV-Utah I have peak viral replication at PID 10 and undetectable levels at PID 60 and usually die within 5 months after inoculation (6, 29, 40). In the present study, kits were inoculated as newborns and treated with gamma globulin to prevent pneumonia. These mink lived for more than 10 months and still had detectable levels of viral replication at that time. This correlates well with the findings of Porter et al. (42), which showed prolonged development of lesions in mink infected in utero. In the same study it was shown that such transplacentally infected mink had lower ADV antibody titers at 83 days after infection and higher anti-ADV titers than mink infected as adults. These data further substantiate the idea that antibodies in adult mink are also capable of decreasing viral replication. Furthermore, the results of our present study might provide an explanation for the discrepancy found in virus distribution and titers between adult mink experimentally infected as adults (6, 10, 12) and naturally, presumably transplacentally, infected mink (25, 48). That is, naturally infected mink may have higher titers and more widespread replication of the virus than mink experimentally infected as adults.

Another interesting observation that emerged from these studies relates to the pattern of viral sequestration. In untreated mink kits, a high proportion of virus is sequestered in the kidney glomeruli, while the sequestration at this site is reduced dramatically in antibody-treated kits, suggesting that antibody actually prevents sequestration in glomeruli under certain circumstances. Together with the findings for mink infected as adults (6), these findings suggest that the physicochemical properties of the virus, the antibodies, and the virus-antibody complexes, especially the ratio of antibody to virus, are important in the pathogenesis of immune complex-mediated disease during ADV infection in mink.

The mortality in mink kits treated with mink anti-ADV gamma globulin was higher in kits of Aleutian genotype (sapphire) than of non-Aleutian genotype (pastel), and this difference between the two genotypes was significant. In addition, the level of viremia in antibody-treated kits was significantly lower in pastel than in sapphire kits. Many factors may account for the poorer survival of sapphire mink than of pastel mink. For example, sapphire mink are affected by the Chediak-Higashi syndrome (44), which may make them more susceptible to ADV infection due to decreased phagocytic potential of the reticuloendothelial system (45). However, mortality could be decreased in sapphire kits to a level resembling pastel kits by treatment with mouse anti-ADV monoclonal antibodies, indicating that the differences between genotypes are of a quantitative nature.

Antibodies clearly play a role in disease manifestations caused by ADV infection. Our accumulated data suggest that development of severe acute ADV-induced disease is linked to low or absent antibody titers paired with high levels of permissive viral replication. In contrast, chronic disease is correlated with high antibody titers and low levels of restricted viral replication. This speculation may imply that ADV belongs in the group with prominent human diseases such as herpesvirus infection and subacute sclerosing panencephalitis, diseases in which the host immune response may convert lytic virus infections into latent or chronic disease (24, 28, 49). Moreover, the restricted levels of ADV replication and transcription resemble features of other slow viral diseases, such as visna of sheep and acquired immune deficiency syndrome of humans, both infections in which host immune responses have been implicated in the slow development of clinical disease (27, 28, 30, 34). In addition, epidemiological evidence may link the human B19 parvovirus to chronic arthropathies (47, 53). Perhaps such chronic manifestations of the normally lytic B19 parvovirus may also be modulated by the host response, as implied here for ADV infection. Further experiments are in progress to study ADV infection, an intriguing animal model of acute versus chronic disease, and to study the interrelationships between acute cellular injury, antibody-mediated restriction of viral repli-

cation, and development of chronic immune-mediated disease due to persistent viral infection.

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