JOURNAL OF VIROLOGY, Sept. 1992, p. 5305–5312
0022-538X/92/095305-08$02.00/0

Vol. 66, No. 9

Identification of Aleutian Mink Disease Parvovirus Transcripts in Macrophages of Infected Adult Mink

HIROYUKI KANNO, JAMES B. WOLFINBARGER, AND MARSHALL E. BLOOM*

Laboratory of Persistent Viral Diseases, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratories, Hamilton, Montana 59840

Received 26 March 1992/Accepted 12 June 1992

We examined Aleutian mink disease parvovirus (ADV) mRNA expression in lymph nodes of adult mink infected with ADV by Northern (RNA) blot and in situ hybridization. In Northern blot analysis, ADV transcripts were detected in the poly(A) RNA fraction extracted from mesenteric lymph nodes of two of five mink 10 days after intraperitoneal inoculation with the virulent Utah I strain of ADV. In strand-specific in situ hybridization, ADV DNA and mRNA were detected in some macrophagelike cells located in the medullary sinus in mesenteric lymph node sections from two of six infected mink by using biotinylated probes. In suspensions of lymph node cells, about 30% of the cells phagocytic for latex particles contained ADV DNA and about 1% of these cells contained ADV mRNA. In peritoneal exudate cells, about 20% of the macrophagelike cells contained ADV DNA and about 2% of these cells contained ADV mRNA. These results indicated that some macrophages in ADV-infected mink contained ADV mRNA and were target cells in ADV infection.

Aleutian mink disease parvovirus (ADV) causes a chronic infection associated with a severe disorder of the immune system. The disease in adult mink, known as classical Aleutian disease (AD), is characterized by marked hypergammaglobulinemia, plasmacytosis, arteritis, and fatal immune complex glomerulonephritis (1, 22, 26, 29–31). Although AD has been studied for more than 30 years, the mechanism by which ADV induces this severe disorder of immune function and establishes a persistent infection remains unclear.

To clarify the pathogenesis of AD, identification of the target cells for ADV replication is a logical first goal. ADV antigens have been detected by immunofluorescence in macrophages in lymph nodes and spleen tissue and in Kupffer cells in liver tissue (29, 34). However, because these cells belong to the mononuclear phagocytic system and ADV is exceedingly stable (16, 28), it is necessary to distinguish precisely actual viral replication from sequestration of virions.

Because of the nature of parvovirus replication, strand-specific hybridization probes can discriminate intermediates of viral replication (6, 10). Plus-sense probes hybridize with virion DNA (minus in sense) and replicative-form DNA (plus and minus in sense), whereas minus-sense probes hybridize with replicative-form DNA (plus and minus in sense) and viral mRNA (plus in sense). However, minus-sense probes may also hybridize with the small portion of single-stranded virion DNA that is plus in sense (10). Thus, the minus-sense probe signals in in situ hybridization (ISH) specimens may not unequivocally indicate the replication site of ADV. Fortunately, sensitivity to RNase treatment can be used to determine the site of viral mRNA production and consequently we can define viral mRNA as RNase sensitive, minus-sense probe signals. The presence of viral mRNA in a tissue or cell type indicates that ADV gene expression occurs there.

We have previously applied these probes by ISH to define the replication sites in vivo (4, 6, 24). The results indicate that B lymphocytes, macrophages, and follicular dendritic cells may be potential target cells in lymph nodes of infected mink. However, the low resolution of autoradiographs of ISH prevents accurate identification of the replication site in tissue sections.

In this study, we focused on the detection of viral mRNA as a firm indicator of ADV replication and gene expression. In Northern (RNA) blot analysis, ADV transcripts were detected in the poly(A) RNA fraction extracted from mesenteric lymph nodes of some mink infected with the virulent Utah I strain of ADV. Viral mRNA was detected in cells which had macrophagelike morphology and were located in medullary sinuses in lymph nodes of infected adult mink by ISH with a biotinylated probe. In suspensions of lymph node cells, some cells containing ADV mRNA were phagocytic for latex particles. Moreover, macrophagelike cells in peritoneal exudate cells also contained ADV mRNA. These results indicate that macrophages are the target cells in ADV infection.

MATERIALS AND METHODS

Cells and viruses. Cell culture-adapted ADV-G (9) was grown at 32°C in Crandell feline kidney (CRFK) cells (15) in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum (Hyclone Laboratories, Logan, Utah) and 0.5% lactalbumin hydrolysate (1×; GIBCO Laboratories, Grand Island, N.Y.). CRFK cells were infected at a multiplicity of infection of 1.0 fluorescence-forming unit per cell (9, 28) and harvested at 48 h postinoculation (p.i.). At the time of harvest, monolayers were scraped with a syringe plunger for mRNA extraction or detached with 0.05% trypsin and 0.02% EDTA for cytocentrifuge slide preparation.

A 10% suspension of ADV Utah I (13, 28) was prepared from the spleens of adult sapphire mink and adjusted to contain 10^7 adult mink 50% infective doses per ml.

Animals. All of the mink used in this study were maintained as described previously (16). Mink were inoculated intraperitoneally with 0.5 ml of a standard ADV Utah I inoculum. They were killed on day 10 p.i. by exsanguination

* Corresponding author.

under ketamine anesthesia. Peritoneal exudate cells, collected by peritoneal lavage with 250 to 350 ml of Hanks balanced salt solution containing 1 U of heparin (United States Biochemical, Cleveland, Ohio) per ml, were fixed in a suspension with 0.01% glutaraldehyde (Sigma, St. Louis, Mo.) in PBS at room temperature for 10 min and then cytocentrifuged onto slides.

Mesenteric lymph nodes were embedded in OCT compound (Miles, Elkhart, Ind.), frozen in a mixture of acetone and dry ice without fixation, and stored at −70°C until use. In some mink, mesenteric lymph nodes were quickly frozen in liquid nitrogen and used for mRNA extraction. For the negative control of ADV infection, some mink were injected with a normal mink spleen homogenate prepared in the same manner used for the ADV Utah I preparation and the samples were collected from mink in the same manner.

Serum was collected and frozen at −30°C. The presence or absence of AD was confirmed by development of characteristic anti-ADV antibodies in countercurrent immunoelectrophoresis (8).

Latex particle phagocytosis. Lymph node cell suspensions were prepared by using a stainless steel mesh. Lymph node cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal calf serum at 5 × 10^5 cells per ml, 37°C in 5% CO_2 for 3 h with 2 μl of latex particles (average diameter, 0.8 μm; Difco, Detroit, Mich.) per ml. Then cells were detached with 0.05% trypsin and 0.02% EDTA, collected, fixed with 0.01% glutaraldehyde, and cytocentrifuged onto slides.

Preparation of probes. Northern blot hybridization was performed by using RNA probes radiolabeled with [^{32}P]UTP as previously described (10). For ISH, the probes were radiolabeled with [^{35}S]UTP as previously described (5). The ^{32}P-labeled RNAs had a specific activity of approximately 2.3 × 10^8 cpm/μg, and the ^{35}S-labeled RNAs had a specific activity of approximately 3.0 × 10^8 cpm/μg. Biotinylated RNA probes were prepared with an in vitro transcription kit (Promega, Madison, Wis.) by adding allylamine-UTP (Enzo, New York, N.Y.) instead of [^{35}S]UTP and with subsequent biotinylation with Enzotin (Enzo) as recommended by the manufacturer. A control probe labeled with ^{35}S or biotin was prepared for ISH by using the canine parvovirus genome as templates as previously described (4).

mRNA extraction and Northern blot analysis. mRNA was extracted from mink mesenteric lymph nodes (0.2 to 0.5 g of wet tissue) or CRFK cells by using a Fast Track mRNA preparation kit (Invitrogen, San Diego, Calif.) and quantitated by measuring A_{260}. RNA samples (2.7 to 6.4 μg) were electrophoresed in 1% formaldehyde denaturing agarose gels, Northern blotted as previously described (3), and hybridized with a minus-sense RNA probe.

ISH techniques. Six-micrometer frozen sections of lymph nodes or cytospin specimens (fixed with 0.01% glutaraldehyde before cytocentrifugation) were fixed with 4% paraformaldehyde (PFA) in phosphate-buffered saline at 4°C for 20 min. After being air dried, slides were rewetted with 0.25% Triton X-100 and 0.25% saponin in phosphate-buffered saline. When RNase treatment was required, slides were incubated with 200 μg of RNase A (bovine pancreas; Pharmacia) per ml and 30 U of RNase T_1 (United States Biochemical) per ml in 10 mM Tris pH 7.5–30 mM NaCl at 37°C for 2 h. (This RNase treatment procedure did not per se interfere with subsequent hybridization with RNA probes, because there was no decrease of plus-sense probe signals on ADV-G-infected CRFK cells after RNase treatment [data not shown].) Slides were acetylated twice for 5 min each time with 0.025% acetic anhydride with 0.1 M triethanolamine HCl buffer (pH 8.0), refixed with 4% PFA in phosphate-buffered saline at room temperature for 20 min, and then neutralized with 0.2% glycine in phosphate-buffered saline.

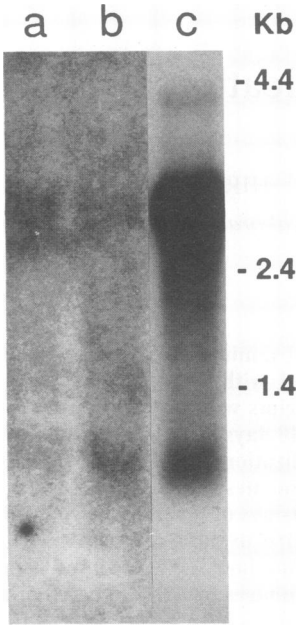

FIG. 1. Northern blot analysis of mRNAs isolated from mesenteric lymph nodes of mink at 10 days p.i. and ADV-G-infected CRFK cells. Poly(A) RNAs were extracted as described in Materials and Methods, and 5-μg (lanes a and b) and 0.1-μg (lane c) samples were electrophoresed in a formaldehyde–1% agarose gel and Northern blotted onto a nylon filter. The filter was probed with an ADV-specific minus-sense RNA probe as described in Materials and Methods. RNA size markers (0.24- to 9.5-kb RNA ladder; GIBCO BRL) are indicated on the right. Lanes: a and b, mRNA from a mink at 10 days p.i. after 5 days of exposure at −70°C; c, mRNA from ADV-G-infected CRFK cells after 3 h of exposure at −70°C.

When slides were hybridized with plus-sense probes to detect viral DNA, slides were denatured with 95% formamide and 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 65°C for 15 min. For detection of viral mRNA, denaturation was omitted.

Next, slides were prehybridized, hybridized with radiolabeled probes at a final concentration of 2 ng/μl, washed, exposed in emulsion, and developed as previously described (5). Finally, the slides were stained with Wright-Giemsa stain for cytospin specimens or with hematoxylin for frozen sections.

When slides were hybridized with biotinylated probes, a final probe concentration of 1 ng/μl was used. After washing, signals were visualized with the DETEC I-*alk* signal-generating system (Enzo) using streptavidin-biotinylated alkaline phosphatase complexes.

RESULTS

Northern blot analysis of mRNA from infected mink. To demonstrate ADV mRNA in mink lymph nodes on day 10 p.i. (ADV infected), we performed Northern blot analysis on mRNA extracted from mesenteric lymph nodes from five infected mink. Three of these five mink also showed the

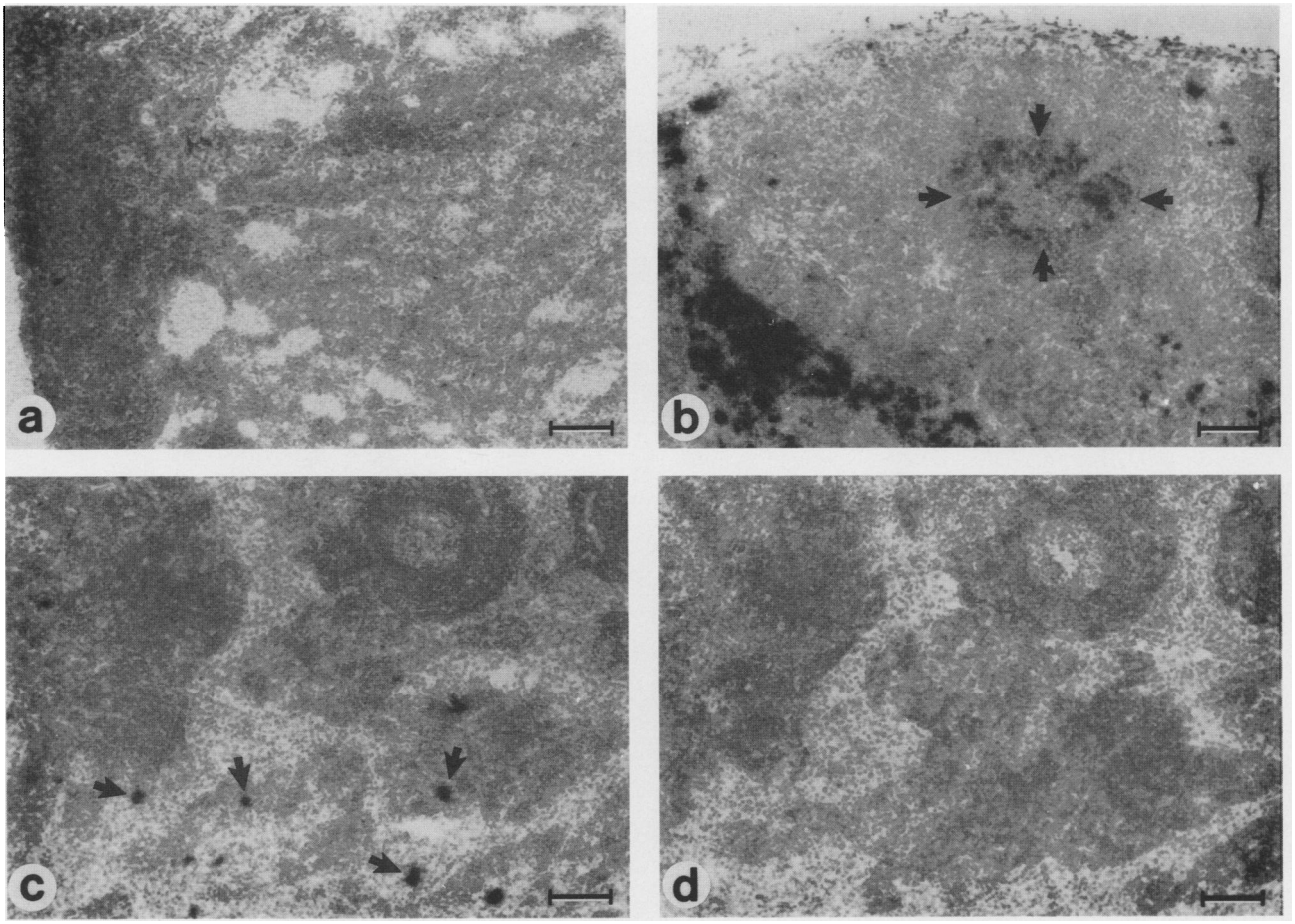

FIG. 2. ISH analysis with radiolabeled probes on PFA-fixed frozen sections of mesenteric lymph nodes from uninfected (control) and infected mink. (a) ISH of a section from an uninfected mink with the ^{35}S-labeled plus-sense probe after 10 days of exposure. No significant signal is visible. Bar, 100 μm. (b) ISH of a section from a mink at day 10 p.i. with the ^{35}S-labeled plus-sense probe after 10 days of exposure. Heavy grain production is visible in the medullary and marginal sinuses, and lighter grain production is visible in the periphery of the germinal center (arrows). Bar, 100 μm. (c) ISH of a section from a mink at day 10 p.i. with the ^{35}S-labeled minus-sense probe without RNase treatment after 10 days of exposure. Scattered but strong signals are visible in the medullary sinus area (arrows). Bar, 100 μm. (d) ISH of a section from a mink at day 10 p.i. with the ^{35}S-labeled minus-sense probe with RNase treatment after 10 days of exposure. No significant signal is visible. Bar, 100 μm.

presence of viral mRNA by ISH (see below). Samples from two of the five mink, both positive for ADV mRNA by ISH, had detectable ADV mRNA on Northern blotting. These two mRNA preparations contained two bands which corresponded to 2.8- and 0.8-kb ADV transcripts (Fig. 1). However, the 4.3-kb transcript could not be clearly detected, although it was readily obvious in mRNA preparation from CRFK cells infected with ADV-G. The mRNA preparations from uninfected mink did not contain ADV mRNA (data not shown). Thus, ADV mRNA was detected in lymph nodes of ADV-infected mink, although not every infected mink was positive. This suggests different levels of ADV infection in individual mink (7).

ISH of frozen lymph node sections probed with radiolabeled probes. We next examined lymph node sections from mink by ISH with radiolabeled probes (4, 24).

Sections prepared either from two uninfected or six infected mink gave low levels of grains diffusely distributed over the entire slides when they were hybridized with radiolabeled canine parvovirus RNA probes (data not shown). Furthermore, when sections from two control uninfected mink were hybridized with plus- or minus-sense ADV probes, no significant grain production was observed (Fig. 2a). These results suggested that the following positive signals were ADV specific.

When sections from infected mink were hybridized with a radiolabeled plus-sense probe, specific autoradiographic grains were easily identified in all six mink. The signals were located at two histologically distinct sites; one was in the marginal and medullary sinus area, and the other was in the peripheral area of lymph follicles (Fig. 2b), as in previous reports (4, 24). The signals at the former site were stronger than those at the latter.

Sections from the same six infected mink also gave strong signals from scattered cells that were located mainly in medullary sinuses (Fig. 2c) when hybridized with a minus-sense probe. In the lymph follicle area, no signal above the background level was detected. Virtually all of the minus-sense probe signals in medullary sinuses disappeared with RNase treatment before hybridization (Fig. 2d), suggesting that these signals represented ADV mRNA. The numbers of positive cells in medullary sinuses in minus-sense-probed

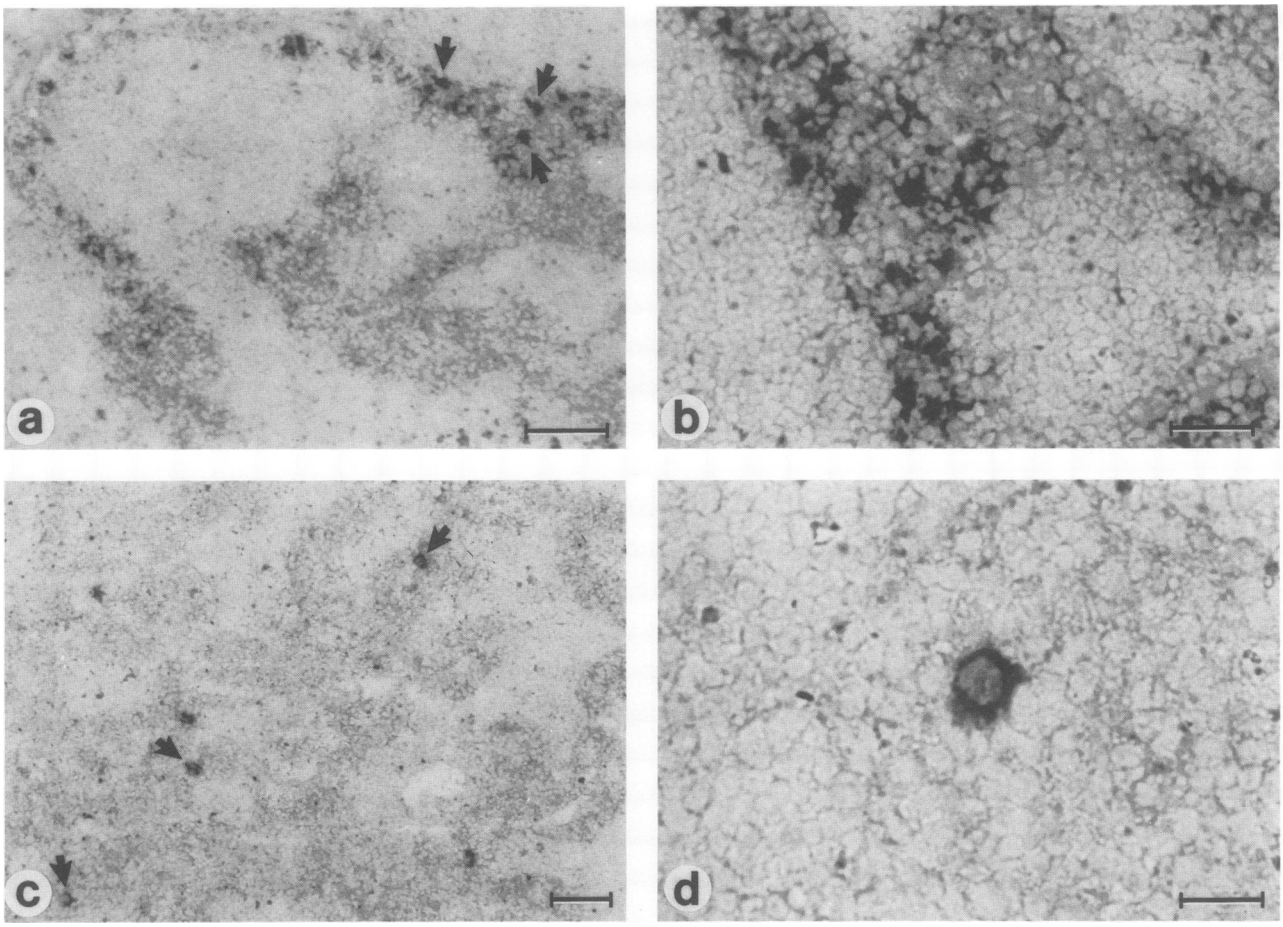

FIG. 3. ISH analysis with biotinylated probes on PFA-fixed frozen sections of mesenteric lymph nodes from infected mink. (a) ISH of a section from a mink at day 10 p.i. with a biotinylated plus-sense probe. In addition to the weak reticular staining in the marginal and medullary sinuses, strong and irregularly shaped staining is occasionally visible in these sinus areas (arrows). Bar, 100 μm. (b) Higher magnification of the medullary sinus area in the section shown in panel a. Bar, 50 μm. (c) ISH of a section from a mink at day 10 p.i. with a biotinylated minus-sense probe without RNase treatment. Scattered positive staining is visible mainly in the medullary sinus area (arrows). Bar, 100 μm. (d) Higher magnification of a positive cell in the section shown in panel c. Positive staining is visible mainly in the irregular cytoplasm. Bar, 25 μm.

sections were much (10- to 100-fold) lower than those in plus-sense-probed sections.

However, in these sections, we could not clearly identify the positive cells, owing to the relatively long track of ^{35}S in the emulsion and the resulting grain scatter.

ISH of frozen lymph node sections probed with biotinylated probes. To define these cells with greater resolution, subjacent sections from the same mink were also hybridized with biotinylated probes. With biotinylated probes, the signal is generated within the cell and scatter of the signal is minimal.

When hybridized with a plus-sense probe, the two control, uninfected mink sections showed weak staining in the reticular network pattern in sinuses (data not shown) that might be caused by nonspecific binding of the biotinylated probe.

Sections from two of the six infected mink exhibited scattered strong staining with a plus-sense probe in sinuses which was readily distinguished from nonspecific reticular staining (Fig. 3a). The ADV-positive cells in these sections had irregular and abundant cytoplasm (Fig. 3b). The numbers of clearly positive cells were much (about 10- to 100-fold) lower than those in sections probed with radiolabeled reagents. In the other four infected mink, no positive signal above background staining could be detected although they were positive with a radiolabeled plus-sense probe. Furthermore, in the region around lymph follicles, no obvious staining was detected with the biotinylated probe in any mink.

Sections from the same six mink were also hybridized with a minus-sense probe. In the two infected mink, which showed positive staining with a biotinylated plus-sense probe, some scattered positive cells were detected, mainly in the medullary sinus (Fig. 3c). These cells were in the same location but lower in number (about 10-fold) than in subjacent sections probed with radiolabeled reagents. In the other four infected mink, we did not detect any obvious signals in the entire sections although they were positive with a radiolabeled minus-sense probe. Hybridization with a higher concentration (more than 1 ng/μl) of biotinylated probes just increased the background staining (data not shown). These results suggest that radiolabeled probes were more sensitive than biotinylated probes.

In sections probed with biotinylated reagents, it was possible to identify positive cells clearly and to see the morphology of these cells. They had relatively large and

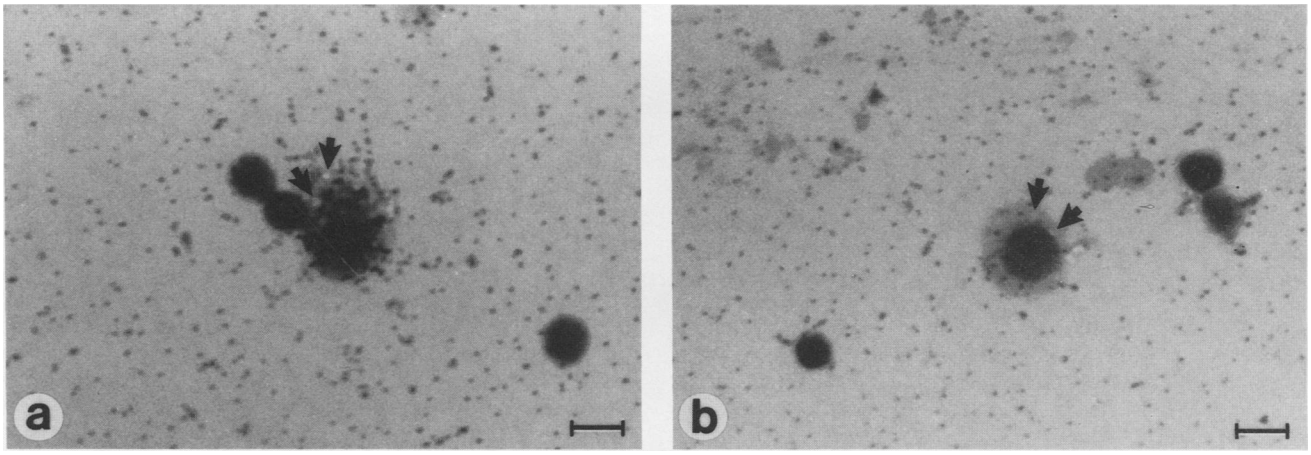

FIG. 4. ISH analysis of glutaraldehyde-PFA-fixed cytospin specimens of mesenteric lymph node cell suspensions of a mink at day 10 p.i. (a) ISH of a cytospin slide with a ^{35}S-labeled plus-sense probe after 5 days of exposure. Significant grain production is visible on mononuclear and cytoplasm-rich cells. Latex particles are visible as white particles (arrows). (b) ISH of a cytospin slide with a ^{35}S-labeled minus-sense probe without RNase treatment after 5 days of exposure. Lighter grain production than that in panel a is visible on the same kind of mononuclear cells. Latex particles are visible as white particles (arrows). Bars, 10 μm.

irregularly shaped cytoplasm and large nuclei and were clearly stained only in the cytoplasm, not in the nuclei (Fig. 3d). Such positive cells were not detected after RNase treatment (data not shown). Because of the location and morphology of these positive cells, we surmised that they were macrophages.

These results, taken together, suggest that cells with macrophagelike morphology in the lymph node medullary sinus area contained ADV DNA and mRNA and must have constituted some of the target cells for ADV replication and gene expression.

ISH of cytospin slides of latex-phagocytized lymph node cells. The results of the previous sections suggest that macrophages are target cells in ADV infection. For a preliminary functional characterization of ADV target cells in mink lymph nodes, lymph node cell suspensions from two infected mink and one uninfected control mink were incubated with latex particles before cytospin preparation and hybridized with radiolabeled probes. In these preparations, 3% of the cells had macrophagelike morphology and almost all of these cells showed phagocytic activity (defined as more than four latex particles in their cytoplasm). No obvious difference in the percentage of macrophagelike cells between infected and uninfected preparations was observed.

In suspensions from infected mink, about 30% of the macrophagelike cells yielded signals when hybridized with a plus-sense probe but only 1% gave signals when hybridized with a minus-sense probe. These positive signals were located mainly in their cytoplasm (Fig. 4a and b). As was observed in the sections, many fewer cells reacted with the minus-sense probe than with the plus-sense probe. No minus-sense probe-positive cells were detected after RNase treatment. Small lymphocytes and blastic cells in these preparations were consistently negative with either probe.

In control preparations from uninfected mink cells, we did not detect signals with either probe (data not shown).

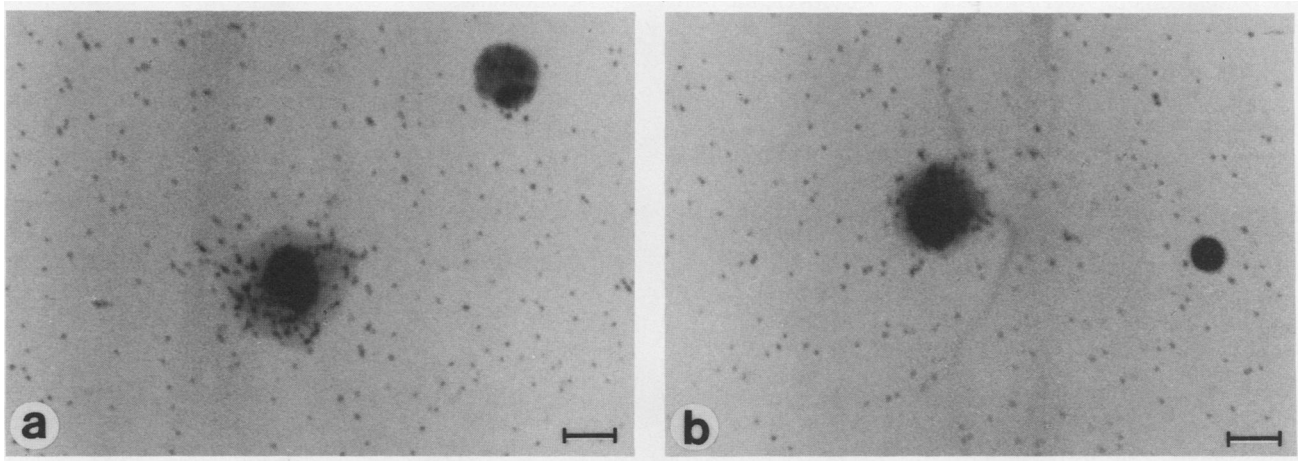

FIG. 5. ISH analysis of glutaraldehyde-PFA-fixed cytospin specimens of peritoneal exudate cells of a mink at day 10 p.i. (a) ISH of a cytospin slide with a ^{35}S-labeled plus-sense probe after 5 days of exposure. Significant signal production is visible mainly in the cytoplasm of the macrophagelike cell. (b) ISH of a cytospin slide with a ^{35}S-labeled minus-sense probe without RNase treatment after 5 days of exposure. Significant signal production is visible mainly in the cytoplasm of the macrophagelike cell. Bars, 10 μm.

These results suggest that a substantial number of phagocytes in suspensions from mink lymph nodes contained ADV DNA and that a much smaller number were engaged in actual ADV gene expression.

ISH of peritoneal exudate cells. We also looked for ADV infection of other phagocytic cells. Cytospin slides were prepared from peritoneal exudate cells of two infected mink and one mink at 10 days after injection with a normal spleen homogenate. In these preparations, about 10% of the total cells had macrophagelike morphology.

In infected mink, about 20% of the macrophagelike cells yielded signals when hybridized with a plus-sense probe but only about 2% gave signals when hybridized with a minus-sense probe (Fig. 5a and b). No minus-sense probe-positive macrophagelike cells were detected after RNase treatment, confirming that this minus-sense probe signal was derived from ADV mRNA.

Almost all of the granulocytes in these preparations showed a low level of grain production when hybridized with either the plus-sense or the minus-sense probe. Since granulocytes in the control preparation also showed the same kind of low-level grain production and these signals were not as RNase sensitive as those in macrophagelike cells (data not shown), these signals were likely derived from nonspecific binding of probes to granulocytes, as reported in other systems (17).

These findings suggest that, as in suspensions of lymph node cells, a small portion of peritoneal macrophages supported ADV mRNA production and that a larger population had ADV DNA.

DISCUSSION

In this study, we showed that a population of cells in ADV-infected mink reacted with a minus-sense probe and that this reaction was RNase sensitive. Therefore, these cells contained ADV mRNA and thus were target cells for ADV infection. On the basis of several criteria, these cells can be tentatively classified as macrophages: (i) location in the medullary sinus in infected mink lymph node tissue sections, (ii) morphology having abundant and irregular cytoplasm, and (iii) phagocytic activity for latex particles.

However, not all phagocytic cells in lymph node cell suspensions or macrophagelike cells in peritoneal exudate cell populations produced ISH signals. Moreover, the number of plus-sense probe signal-producing cells was 10 to 100 times greater than that of minus-sense probe signal-producing cells in all ISH specimens, confirming previous findings that not all cells containing ADV nucleic acids are engaged in ADV gene expression or replication (4). Thus, it may be reasonable to conjecture that only a subpopulation of macrophages is permissive for ADV gene expression after the entry of virions. It was still unclear what conditions or factors determine the permissiveness of macrophages for ADV gene expression.

In our ISH experiments, all of six infected mink showed positive signals with radiolabeled probes. However, with biotinylated probes, we detected clear signals in only two of six infected mink. Consequently, although biotinylated probes were less sensitive than radiolabeled probes, more accurate identification of positive cells was possible with biotinylated reagents. In ^{35}S-labeled probed sections, we could observe just the distribution of autoradiographic grains and could only speculate on positive-cell populations.

We performed Northern blot analysis on poly(A) RNA from three ISH-positive mink. We detected ADV mRNA in two of three mink, which suggests that ISH is more sensitive than Northern blotting for detection of viral mRNA. However, although ISH may be a more sensitive way to detect viral mRNA, it does not define the mRNA species that gives rise to signals. The Northern blot results are important because they can be used to delineate specific viral transcripts. In our present study, the Northern blot results verified that the RNase-sensitive signals generated with a minus-sense probe in ISH were definitely derived from the presence of discrete viral mRNA species.

In our Northern blot studies, we observed the 2.8- and 0.9-kb RNA bands in lymph node mRNA. We were unable to detect the 4.3-kb transcripts in lymph nodes of ADV Utah I-infected mink which were identified in the mRNA of ADV-G-infected CRFK cells (3). Since the abundance of 4.3-kb transcripts in cell culture is lower than that of 2.8-kb transcripts (3), the limit of mRNA preparation and/or sensitivity of Northern blotting might make it difficult to detect the 4.3-kb transcripts in these in vivo preparations. However, it is also possible that the transcription program of ADV Utah I in mink differs from that in the cell culture system. This notion may be suggested by the restricted nature of in vivo infection in adult mink (4).

For this work, we selected PFA as the fixative, which is reported to be superior to other fixatives for detection of the expression of mRNA of cellular genes (21). Also, for hybridization with minus-sense probes for mRNA detection, we omitted the denaturation step to minimize signals from duplex replicative-form DNA. With this method, we could not detect viral mRNA and could detect only viral DNA in the lymph follicle area.

Although we did confirm the presence of ADV mRNA in macrophages located in the medullary sinus area in lymph nodes, some cells located in the lymph follicle area, such as B cells or follicular dendritic cells, might also be target cells for ADV infection, as previously suggested (4, 24). The RNase-sensitive minus-sense signal from cells in the lymph follicle might be below the sensitivity of the method used here. Previously, detection of viral mRNA in lymph follicles by use of periodate-lysine-paraformaldehyde-glutaraldehyde-fixed paraffin sections (4) or acetone-fixed frozen sections (24) was reported. The difference in pretreatment of slides, including fixation, might cause the difference in preservation of nucleic acids. Moreover, in previous methods, denaturation of slides was performed even when hybridization was with a minus-sense probe. This should increase the signals derived from replicative-form DNA and might make it difficult to evaluate the effect of RNase treatment. In fact, in ISH with a minus-sense probe and a cytospin specimen of ADV-G-infected CRFK cells, RNase treatment was less effective on acetone-fixed slides than on PFA-fixed slides (data not shown). In addition, the extent of ADV replication among mink varies (7), possibly because the mink is an outbred experimental animal. This heterogeneity in lymph node samples might also explain different results.

In this study, we detected ADV transcripts in macrophages, which suggests that ADV gene expression occurs in these cells. Generally, tissue-fixed macrophages are considered to be terminally differentiated, noncycling cells. Therefore, the conclusion that macrophages support ADV gene expression may seem inconsistent with the evidence that parvoviruses can initiate productive growth only in cycling cells (14). However, the proliferation of tissue-fixed macrophages in situ has also been reported in some circumstances (25, 35). Furthermore, although productive, permissive in-

fection by parvoviruses requires cycling cells, restricted, low-level infection, as suggested for ADV infection in vivo (4), may not. In addition, our finding that only a small proportion of macrophages supported ADV gene expression suggests that specific intracellular conditions govern the level of replication and gene expression. Nevertheless, although it is still unclear how permissive ADV replication is in these cells, the fact that ADV gene expression occurs in one population of immune cells may provide us with a basis to dissect the immune disorder caused by ADV infection.

The phenotype of AD is characterized by (i) extreme hypergammagloblinemia caused mainly by anti-ADV antibodies (1, 2, 32); (ii) polyclonal antibody production, including anti-DNA antibodies (18, 19); (iii) plasmacytosis (22, 31); (iv) arteritis and fatal immune complex glomerulonephritis (29–31); and (v) prevention of AD lesions by immunosuppressive treatment (12). The sum of these features suggests that ADV infection stimulates some steps in B-cell development and/or maturation on the background of an immune response to ADV. To develop a mechanism by which ADV infection might enhance steps in B-cell development, we can advance several hypotheses. (i) Cytokines that are produced by ADV-infected macrophages might enhance B-cell maturation to antibody-producing cells. (ii) Viral components, such as viral proteins, might directly stimulate B-cell development. (iii) Abnormal antigen presentation by ADV-infected macrophages might disturb subsequent B-cell development.

Macrophages are targets in several other persistent viral infections, such as dengue virus (20) and lactate dehydrogenase virus (11) infections. In these viral infections, antivirus antibodies promote infection of macrophages, that is, antibody-dependent enhancement (11, 20, 33). Neutralizing antibodies to ADV have not been demonstrated in vivo (29). Moreover, the immune complexes containing ADV still maintain infectivity (27). Perhaps in vivo antibody-dependent enhancement plays a role in the pathogenesis of ADV infection, as previously suggested as a hypothesis (29).

ADV infection of macrophages might also contribute to the development of immune complex glomerulonephritis in the later phase of disease. A previous study has indicated that the function of the mononuclear phagocytic system in AD is severely impaired (23). ADV infection might alter macrophage functions, resulting in accelerated deposition of immune complexes.

Clear identification of macrophages as target cells in ADV gene expression provides many suggestions which might be useful in analysis of the immune disorder caused by ADV infection. These ideas may also provide insights into the etiopathogenesis of human immune complex disease and autoimmune diseases.

ACKNOWLEDGMENTS

We thank Bruce Chesebro, Dahn Clemens, John Portis, and Richard Race for critically reviewing the manuscript, Yumi Kanno for secretarial assistance, Robert Evans and Gary Hettrick for preparing the figures, and Jerry Schmidt for invaluable assistance with animal care.

REFERENCES

1. **Aasted, B., and M. E. Bloom.** 1983. Sensitive radioimmune assay for measuring Aleutian disease virus antigen and antibody. J. Clin. Microbiol. **18**:637–644.
2. **Aasted, B., G. S. Tierney, and M. E. Bloom.** 1984. Analysis of the quantity of antiviral antibodies from mink infected with different Aleutian disease virus strains. Scand. J. Immunol. **19**:395–402.
3. **Alexandersen, S., M. E. Bloom, and S. Perryman.** 1988. Detailed transcription map of Aleutian mink disease parvovirus. J. Virol. **62**:3684–3694.
4. **Alexandersen, S., M. E. Bloom, and J. B. Wolfinbarger.** 1988. Evidence of restricted viral replication in adult mink infected with Aleutian disease of mink parvovirus. J. Virol. **62**:1495–1507.
5. **Alexandersen, S., M. E. Bloom, J. B. Wolfinbarger, and R. E. Race.** 1987. In situ molecular hybridization for detection of Aleutian mink disease parvovirus DNA by using strand-specific probes: identification of target cells for viral replication in cell cultures and in mink kits with virus-induced interstitial pneumonia. J. Virol. **61**:2407–2419.
6. **Bloom, M. E., S. Alexandersen, S. Mori, and J. B. Wolfinbarger.** 1989. Analysis of parvovirus infections using strand-specific hybridization probes. Virus Res. **14**:1–25.
7. **Bloom, M. E., R. E. Race, B. Aasted, and J. B. Wolfinbarger.** 1985. Analysis of Aleutian disease virus infection in vitro and in vivo: demonstration of Aleutian disease virus DNA in tissue of infected mink. J. Virol. **55**:696–703.
8. **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.
9. **Bloom, M. E., R. E. Race, and J. B. Wolfinbarger.** 1980. Characterization of Aleutian disease virus as a parvovirus. J. Virol. **35**:836–843.
10. **Bloom, M. E., R. E. Race, and J. B. Wolfinbarger.** 1987. Analysis of Aleutian disease of mink parvovirus infection using strand-specific hybridization probes. Intervirology **27**:102–111.
11. **Cafruny, W. A., and P. G. W. Plagemann.** 1982. Immune response to lactate dehydrogenase-elevating virus: serologically specific rabbit neutralizing antibody to the virus. Infect. Immun. **37**:1007–1012.
12. **Cheema, A., J. B. Henson, and J. R. Gorham.** 1972. Aleutian disease of mink: prevention of lesions by immunosuppression. Am. J. Pathol. **66**:543–556.
13. **Chesebro, B., M. E. Bloom, W. J. Hadlow, and R. E. Race.** 1975. Purification and ultrastructure of Aleutian disease virus of mink. Nature (London) **254**:456–457.
14. **Cotmore, S. F., and P. Tattersall.** 1987. The autonomously replicating parvoviruses of vertebrates. Adv. Virus Res. **33**:91–174.
15. **Crandell, R. A., C. G. Fabricant, and W. A. Nelson-Rees.** 1973. Development, characterization, and viral susceptibility of a feline (Felis catus) renal cell line (CRFK). In Vitro **9**:176–185.
16. **Eklund, C. M., W. J. Hadlow, R. C. Kennedy, C. C. Boyle, and T. A. Jackson.** 1968. Aleutian disease of mink: properties of the etiologic agent and the host responses. J. Infect. Dis. **118**:510–526.
17. **Haase, A., M. Brahic, L. Stowring, and H. Blum.** 1984. Detection of viral nucleic acids by in situ hybridization. Methods Virol. **VII**:189–226.
18. **Hahn, E. C., and P. S. Hahn.** 1983. Autoimmunity in Aleutian disease: contribution of antiviral and anti-DNA antibody to hypergammaglobulinemia. Infect. Immun. **41**:494–500.
19. **Hahn, E. C., and A. J. Kenyon.** 1980. Anti-deoxyribonucleic acid antibody associated with persistent infection of mink with Aleutian disease virus. Infect. Immun. **29**:452–458.
20. **Halstead, S. B., and E. J. O'Rourke.** 1977. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J. Exp. Med. **146**:201–217.
21. **Lawrence, J. B., and R. H. Singer.** 1985. Quantitative analysis of in situ hybridization methods for the detection of actin gene expression. Nucleic Acids Res. **13**:1777–1799.
22. **Leader, R. W., B. M. Wagner, J. B. Henson, and J. R. Gorham.** 1963. Structural and histochemical observations of liver and kidney in Aleutian disease of mink. Am. J. Pathol. **43**:33–53.
23. **Lodmell, D. L., R. K. Bergman, M. E. Bloom, L. C. Ewalt, W. J. Hadlow, and R. E. Race.** 1990. Impaired phagocytosis by the mononuclear phagocytic system in sapphire mink affected with

Aleutian disease. Proc. Soc. Exp. Biol. Med. **195**:75–78.
24. **Mori, S., J. B. Wolfinbarger, M. Miyazawa, and M. E. Bloom.** 1991. Replication of Aleutian mink disease parvovirus in lymphoid tissues of adult mink: involvement of follicular dendritic cells and macrophages. J. Virol. **65**:952–956.
25. **Parwaresch, M. R., and H. H. Wacker.** 1984. Origin and kinetics of resident tissue macrophages: parabiosis studies with radiolabelled leukocytes. Cell Tissue Kinet. **17**:25–39.
26. **Porter, D. D., F. J. Dixon, and A. E. Larsen.** 1965. Metabolism and function of gamma globulin in Aleutian disease of mink. J. Exp. Med. **121**:889–901.
27. **Porter, D. D., and A. E. Larsen.** 1967. Aleutian disease of mink: infectious virus-antibody complexes in the serum. Proc. Soc. Exp. Biol. Med. **126**:680–682.
28. **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.
29. **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.
30. **Porter, D. D., A. E. Larsen, and H. G. Porter.** 1973. The pathogenesis of Aleutian disease of mink. III. Immune complex arteritis. Am. J. Pathol. **71**:331–344.
31. **Porter, D. D., A. E. Larsen, and H. G. Porter.** 1980. Aleutian disease of mink. Adv. Immunol. **29**:261–286.
32. **Porter, D. D., H. G. Porter, and A. E. Larsen.** 1984. Much of the increased IgG in Aleutian disease of mink is viral antibody. J. Exp. Pathol. **1**:79–88.
33. **Porterfield, J. S.** 1986. Antibody-dependent enhancement of viral infectivity. Adv. Virus Res. **31**:335–355.
34. **Race, R. E., B. Chesebro, M. E. Bloom, B. Aasted, and J. B. Wolfinbarger.** 1986. Monoclonal antibodies against Aleutian disease virus distinguish virus strains and differentiate sites of virus replication from sites of viral antigen sequestration. J. Virol. **57**:285–293.
35. **Sawyer, R. T., P. H. Strausbauch, and A. Volkman.** 1982. Resident macrophage proliferation in mice depleted of blood monocytes by strontium-89. Lab. Invest. **46**:165–170.