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Aleutian Disease Virus, a Parvovirus, Is Proteolytically Degraded During In Vivo Infection in Mink

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The polypeptides of the highly virulent mink-passaged Utah I and the nonvirulent cell culture-adapted ADV-G strain of Aleutian disease virus (ADV) were compared. When CRFK cells infected with either Utah I or ADV-G were analyzed by immunoprecipitation, both viruses induced proteins with molecular weights characteristic of the ADV-G 85,000 (85k)- and 75k-dalton structural proteins (p85 and p75) as well as the 71k-dalton nonvirion protein p71. However, when Utah I, Pullman ADV, and DK ADV (a Danish isolate of ADV) were purified from infected mink, only polypeptides with molecular weights between 27k and 30k could be identified. In addition, trypsin treatment of ADV-G degraded p85 and p75 to smaller antigenic proteins with molecular weights of 24k and 27k, similar to those found for the virulent in vivo viruses. The effect of proteolytic treatment of ADV was then studied in detail. Purification of Utah I ADV from mink organs in the presence of protease inhibitor did not prevent the appearance of the low-molecular-weight proteins and ADV-G proteins were not degraded upon purification from a homogenate of normal mink organs, suggesting that artifactual proteolysis was not occurring. When a serum pool from terminally diseased mink was analyzed by radioimmunoassay for antibody reactivity against trypsinized and nontrypsinized ADV-G, five times higher reactivity was found for the trypsinized ADV-G than for the nontrypsinized ADV-G, an effect which could not be elicited by chymotrypsin or V8 protease treatment, implying that in vivo-produced ADV was being modulated in vivo by trypsin or a trypsin-like enzyme. Trypsinization was shown not to cause a change in ADV virion density, but to decrease the in vitro infectivity of ADV-G for CRFK cells. These studies suggested that during infection of mink ADV proteins are degraded to highly antigenic smaller polypeptides.

Recent studies have conclusively demonstrated that the Aleutian disease virus (ADV) is a nondefective parvovirus (7, 9). Infection with virulent strains of this virus causes a chronic infection with severe hypergammaglobulinemia and fatal immune complex disease (18, 19). Infectious virus and viral antigens have a role in the pathogenesis of the immune complex disease (16, 18), and as a result, study of the virus-specified proteins is of considerable importance.

Several studies on ADV proteins have been reported, but the results are somewhat conflicting. The ADV-G strain, a nonvirulent cell culture-adapted virus (8), has been studied in most detail, and the protein structure of ADV-G shows many similarities with other nondefective parvoviruses (23). This virus has two major structural polypeptides with molecular weights (MWs) of 85,000 (85k) (p85) and 75k (p75), and these proteins show extensive peptide homology, indicating a common genetic origin (9). The polypeptide composition of the virulent Danish ADV isolate (DK ADV) passaged in mink organs has also been reported, and polypeptides with MWs of 86k, 69k, 26k, 19k, and 14k were observed (2). The DK ADV 69k protein was probably analogous to p75 of ADV-G because the calculations were based on an MW of the bovine serum albumin marker protein of 66k for DK ADV and 69k for ADV-G (2). A third study on the mink-passaged, virulent Guelph ADV strain describes four major polypeptides with MWs of 30k, 27k, 21k, and 14k (22). The present study focused on the remarkable differences between in vivo- and in vitro-passaged ADV. We found that trypsin treatment of ADV-G degraded p85 and p75 to polypeptides similar to those seen with in vivo-produced ADVs and greatly reduced infectivity but did not affect the density of the virus in CsCl gradients and enhanced the reactivity of ADV-G as an antigen when used in radioimmunoassay (RIA) against sera from ADV-diseased mink. We concluded that in vivo ADV is exposed to extensive proteolysis, probably as the result of virus processing.

MATERIALS AND METHODS

Animals. For experimental ADV infections, only mink negative for ADV antibody were used.

ADV strains. The Utah I, Pullman, DK, ADV-G, ADV-P, and ferret ADV have all been described previously (1, 8, 11, 17, 20). Intraperitoneal inoculation doses were 10^7 50% infective doses for Utah I, $10^{6.5}$ for Pullman, 0.1 ml of DK viral antigen (1, 2), and various doses of ADV-G (8).

ADV preparation. For immune blotting analyses, in vivo-produced ADV was prepared from mink experimentally infected with Utah I, DK, and Pullman ADV as previously described (4). Briefly, 20% (wt/vol) homogenates of pooled liver, spleen, and mesenteric lymph nodes were freeze-thawed, sonicated, and subjected to Freon extraction and ultracentrifugation for 1 h at 234,000 × *g*. The pellets were resuspended at one-fifth volume and Freon extracted four times before DNase treatment (250 μg/ml in 0.01 M $MgCl_2$ for 1 h at 37°C; P/L Biochemicals, Milwaukee, Wis.) and Sepharose 6B (Pharmacia Fine Chemicals, Uppsala, Sweden) chromatography. This final treatment removes ferritin and DNA from the virus preparation (4). When proteinase inhibitor aprotinin (Trasylol; Mobay Chemical Corp., FBA Pharmaceuticals, New York, N.Y.) was included during the preparation, 10 U was used per ml of buffer.

ADV-G and [^{35}S]methionine-labeled ADV-G and Utah I preparations were prepared and immunoprecipitated as de-

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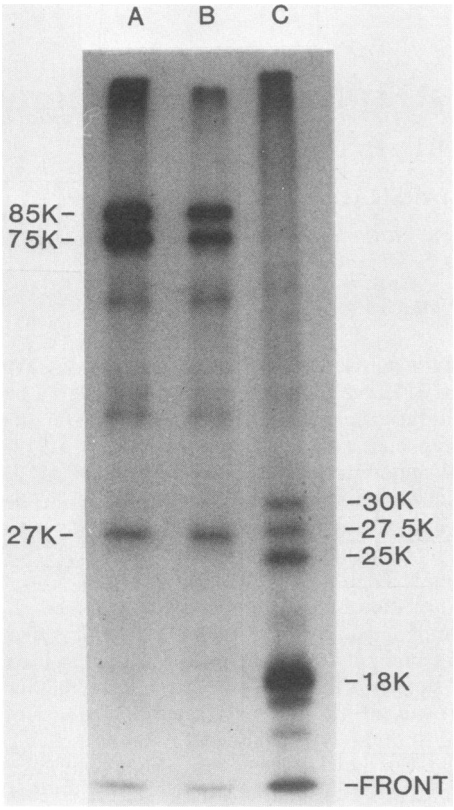

FIG. 1. Comparison of virion-associated proteins of ADV-G and Utah I ADV. Virions of ADV-G or Utah I were purified, respectively, from ADV-G-infected CRFK cells or from mink spleens 10 days after infection with Utah I (10). Peak fractions were labeled with $Na^{125}I$ (9) and analyzed by SDS-PAGE as described previously (8). An autoradiograph of the gel was prepared.

scribed previously (8, 9). CsCl-purified Utah I (10) and ADV-G were purified and radiolabeled with ^{125}I (New England Nuclear Corp., Boston, Mass.) as previously noted (9).

Sera. Blood was taken by cardiac puncture, and serum was isolated by centrifugation for 5 min at 2,500 × *g* after overnight clotting. Serum pools were prepared from 5 to 20 plasmacytotic mink after infection with Pullman and Utah I ADV. A normal serum pool was prepared from 20 mink. The mink antiserum to ADV-P and ferret antiserum to ferret ADV were a gift from D. D. Porter, University of California, Los Angeles.

RIA. ADV antigen and antibody were measured by RIA by using a solid-phase mink antibody to ADV and a ^{125}I-labeled immunoglobulin G (IgG) preparation from the same antiserum (4).

SDS-PAGE and immune blotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described previously (8). The Tween 20 buffer system of Batteiger et al. (6) was used for immune blotting. After transfer of the proteins to nitrocellulose paper the blots were treated with a 1:100 dilution of a serum pool from terminally diseased mink (infected with the Pullman ADV strain) followed by three washes, incubation with 10^6 cpm of ^{125}I-protein A, and finally six washes followed by autoradiography.

Protease digestion. Digestions with trypsin (TRL and TRTPCK; Worthington Diagnostics, Freehold, N.J.), α-chymotrypsin (CDS; Worthington), and V8 protease (Miles Laboratories, Inc., Elkhart, Ind.) were carried out for 1 h at 37°C in a phosphate-saline buffer (pH 7.3). After incubation, soybean trypsin inhibitor (Worthington) was added in twice molar excess over the enzymes. This concentration is adequate for inactivation of both trypsin and α-chymotrypsin (25), but its effectiveness against V8 protease is unknown. However, samples for RIA were diluted into 1% normal serum in RIA buffer, which should be adequate for inactivation of most proteases.

RESULTS

Comparison of virion-associated and nonvirion polypeptides from in vivo- (Utah I) and in vitro-produced (ADV-G) ADV. Previous reports have described virion proteins with different MWs for in vivo- and in vitro-produced ADV (1, 2, 8, 9, 22). Therefore, the virion-associated proteins of CsCl-purified ^{125}I-labeled Utah I and ADV-G were directly compared by SDS-PAGE. Both full (Fig. 1, track A) and empty (Fig. 1, track B) particles of ADV-G contained the same proteins as previously described; the two major proteins were 85k (p85) and 75k daltons (p75) (8, 9). In addition, minor amounts of 60k, 50k, and 27k proteins were seen. Utah I, on the other hand, had no demonstrable large-MW components but had major proteins with MWs of 30k, 27k, 25k, and 18k, similar to those reported for the Guelph (Ontario) and DK strain of ADV (2, 22). This comparison showed that the polypeptides of CsCl-purified ADV-G and in vivo-produced Utah I were clearly different.

Although ADV-G is the only isolate of ADV that grows to

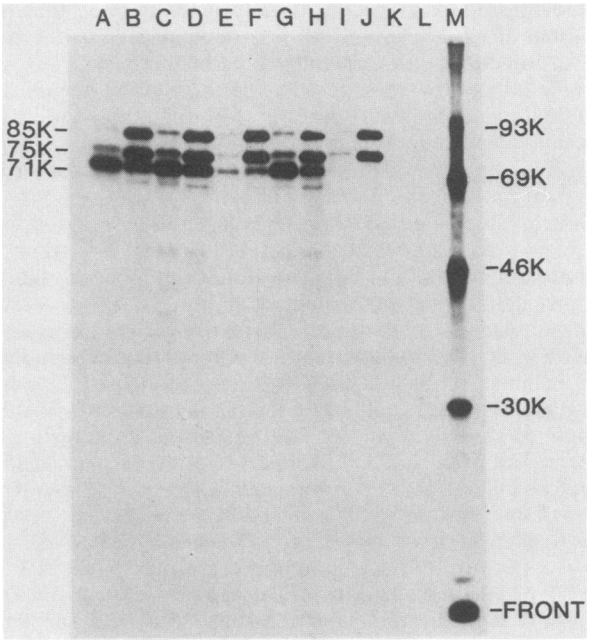

FIG. 2. Immunoprecipitation of [^{35}S]methionine-labeled Utah I ADV (tracks A, C, E, G, I, and K) and ADV-G (tracks B, D, F, H, J, and L). The sera used were as follows: tracks A and B, mink antiserum to Pullman ADV; tracks C and D, mink antiserum to Utah I ADV; tracks E and F, mink antiserum to ADV-G; tracks G and H, mink antiserum to ADV-P; tracks I and J, ferret antiserum to ferret ADV; tracks K and L, normal mink serum. Track M contained MW markers (carbonic anhydrase, 30k; ovalbumin, 46k; bovine serum albumin, 69k; phosphorylase B, 92k).

high titer in cultures of CRFK cells, in vivo-passaged preparations of Utah I induce viral antigen in a small number of infected CRFK cells (8, 12, 17). Since we previously showed that immunoprecipitation of [^{35}S]methionine-labeled infected CRFK cells is a sensitive way to study viral proteins, we infected CRFK cells with ADV-G or Utah I ADV and compared the viral proteins against a panel of mink anti-ADV sera by immunoprecipitation (Fig. 2). Although the relative ratios differed, similar polypeptides were found in the two ADV preparations. For ADV-G, p85 and p75 were detected by all sera, and in addition, all except the ferret anti-ADV sera reacted with the nonvirion p71. For Utah I, on the other hand, the MW of p71 was also 71k, but the two presumed structural proteins had MWs 2 to 3k larger than those of ADV-G. These results indicated that both ADV-G and Utah I directed synthesis of similar large-MW viral proteins in CRFK cells. Furthermore, the small MW proteins found in purified Utah I were not present in detectable quantities.

It was further noted (Fig. 2) that although all serum pools reacted equally with the ADV-G proteins, there was considerable variation exhibited toward the Utah I proteins, and this may have reflected differences in the discrete antigens recognized by these sera.

Comparison of antigenic polypeptides of in vivo- and in vitro-produced ADV by immune blotting. The previous results suggested that although Utah I directed synthesis of antigenic proteins similar to ADV-G in infected CRFK cells, the proteins found in purified Utah I virions were much smaller than those found in ADV-G. For further analysis, we next compared the antigenic reactivity of the proteins of several in vivo ADVs and ADV-G by immune blotting (Fig. 3). Fractions of highly purified Pullman, DK, and Utah I ADV preparations from mink organs were prepared by Sepharose 6B chromatography and resolved by SDS-PAGE together with a similar preparation from normal mink and a sample from ADV-G-infected cells. After SDS-PAGE, the polypeptides were transferred onto nitrocellulose paper and

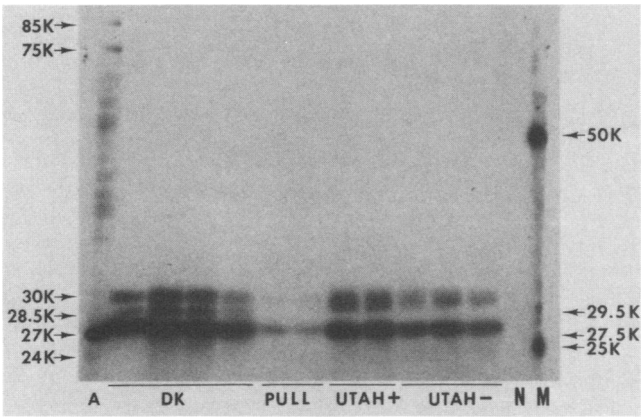

FIG. 3. Analysis of fractions from Sepharose 6B chromatography of different in vivo-produced ADV preparations by SDS-PAGE followed by immune blotting. Track A, an ADV-G preparation; DK tracks, four different fractions from DK ADV purification; Pullman (Pull) tracks, two different fractions from Pullman ADV purification; Utah I$^+$ tracks, two different fractions from Utah I ADV purification prepared in the presence of protease inhibitor (aprotinin); Utah I$^-$ tracks, two different fractions from Utah I ADV purification prepared without protease inhibitor; track N, mock antigen prepared from normal tissues.

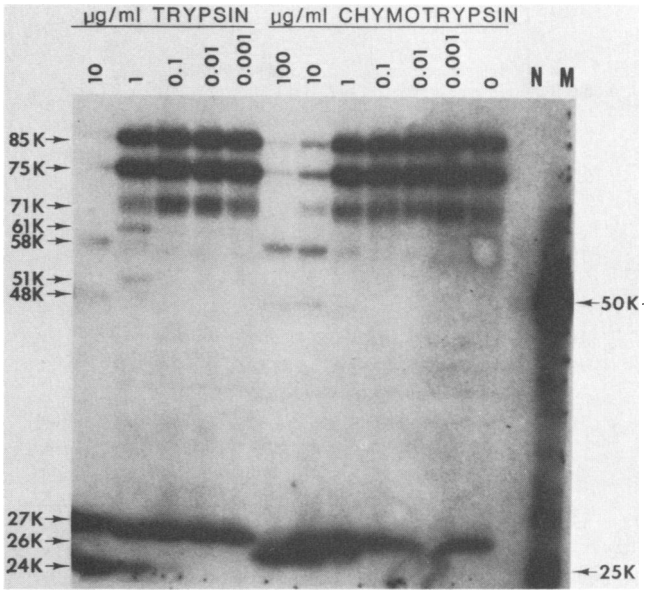

FIG. 4. Polypeptide analyses of protease-digested ADV-G samples by SDS-PAGE followed by immune blotting. Track N contained a lysate of noninfected CRFK cells and track M contained ^{125}I-labeled immunoglobulin heavy (50k) and light (25k) chains. All other tracks contained 50 μl of ADV-G preparation exposed to the indicated protease concentrations for 1 h at 37°C.

treated with a serum pool from ADV-infected mink followed by ^{125}I-labeled staphylococcal protein A. The autoradiograph is shown in Fig. 3. For ADV-G, p85, p75, and a 27k protein (p27) were identified (Fig. 3, lane A). None of the in vivo-produced viruses contained detectable amounts of p85 or p75, but all had antigenically reactive proteins similar in MW to those found in the ^{125}I-labeled Utah I (Fig. 1). A 27.5k- and a 30k-MW polypeptide were observed in all three in vivo-produced viruses (Fig. 3, DK, Pullman, and Utah I). DK and Utah I also contained a 28.5k and a 29.5k protein, respectively. In this experiment, the large-MW polypeptides of DK ADV were not observed (1). These results suggested that the small-MW proteins seen in the preparations were the antigenic components associated with the virus particles. No 25k or 18k polypeptides were detected, suggesting that these proteins may have been non-ADV contaminants of the Utah I ADV analyzed in Fig. 1. Ferritin is a likely contaminant, because ferritin is a major constituent of crude ADV preparations (1), and the major component of human ferritin has an MW of 19k (5).

Effect of proteolytic enzyme treatment on ADV polypeptides and RIA reactivity. The apparent inconsistency of the MWs of the ADV polypeptides found in vitro versus in vivo suggested exposure of in vivo-produced ADV to proteolysis. We therefore tried to mimic this effect by exposing ADV-G to several proteolytic enzymes, including trypsin, α-chymotrypsin, and *Staphylococcus aureus* V8 protease. The protease-digested samples were subjected to SDS-PAGE followed by immune blotting and autoradiography. The results of the trypsin and α-chymotrypsin treatments are displayed in Fig. 4. The ADV-G preparation analyzed gave 85k, 75k, 71k, and 27k polypeptide bands without protease treatment. With increasing concentrations of trypsin, three new immunoreactive bands were observed with MWs 61k, 51k, and 24k. Upon increasing trypsinization, the 61k- and 51k-MW bands disappeared and 58k- and 48k-MW bands appeared; these

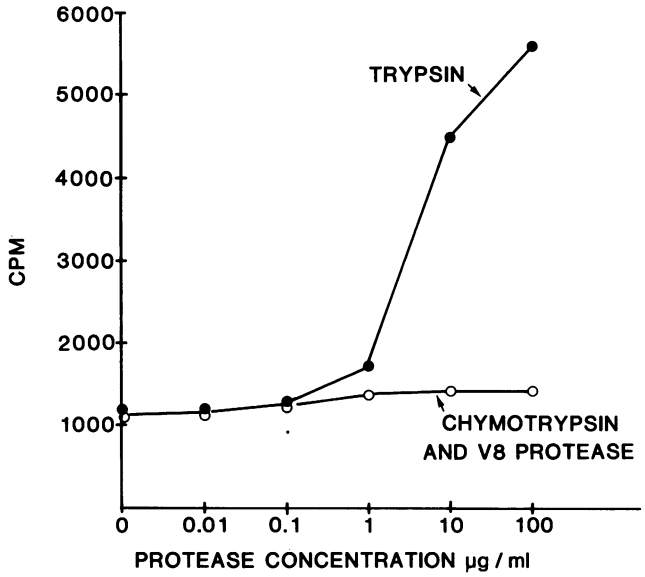

FIG. 5. Influence of protease treatment on ADV-G reactivity in RIA. Samples of ADV-G were treated with protease (trypsin, chymotrypsin, or V8 protease) for 1 h at 37°C in Dulbecco phosphate-buffered saline (ph 7.3). After incubation, soybean trypsin inhibitor was added in molar excess over the enzymes, and the samples were assayed by RIA with mink antibodies against ADV as both solid-phase antibody and ^{125}I-radiolabeled antibody (4).

bands eventually also disappeared, leaving bands with MWs of 27k and 24k. It seemed likely that these proteins were derived from p85/p75, since trypsin treatment of ADV-G virions containing p85/p75 also yielded proteins with MWs of 60k and 50k (see below and Fig. 7B). α-Chymotrypsin degraded ADV-G in a similar but slightly different pattern (Fig. 4). V8 protease produced a pattern identical to that produced by α-chymotrypsin.

Almost identical results were observed with trypsin or α-chymotrypsin treatment of [^{35}S]methionine-labeled and immunoprecipitated ADV-G (data not shown).

Taken together, these results implied that proteolytic treatment of ADV-G produced antigenic fragments similar in size to those of in vivo-prepared ADV. Two experiments were done in an attempt to exclude artifactual proteolysis. First, Utah I ADV was prepared either in the presence (Fig. 3, Utah$^+$) or absence (Fig. 3, Utah$^-$) of the protease inhibitor aprotinin, and in both cases small-MW proteins were noted. In addition, a reconstitution experiment was performed in which ADV-G was added to a homogenate of normal mink organs before virus preparation and analysis by immune blotting. In this experiment, p85 and p75 were still the major ADV polypeptides detected (data not shown). These observations suggested that significant artifactual proteolysis of ADV was not occurring during the processing of virus from mink organs and that the small-MW peptides observed for the in vivo viruses accurately reflected the composition of the virions at the time of virus preparation.

We also analyzed the effect of protease treatment on ADV-G reactivity in RIA, using portions of the same samples immune blotted in Fig. 4. This was of particular interest since ADV-G reacts only weakly as an antigen in RIA with sera of infected mink (B. Aasted, G. S. Tierney, and M. E. Bloom, Scand. J. Immunol., in press). A marked increase in RIA reactivity was observed with trypsin treatment but not with α-chymotrypsin or V8 protease treatment (Fig. 5). This indicated that trypsin treatment of ADV-G degraded the viral proteins while at the same time the RIA reactivity of the preparation was being enhanced. Trypsin treatment of Utah I ADV did not affect its RIA reactivity (data not shown).

Effect of proteolytic enzymes on ADV infectivity and density in CsCl gradients. When the effect of trypsinization on ADV-G infectivity for CRFK was analyzed, a 2 to 3 $\log_{10}$ decrease in infectivity was observed at trypsin concentrations in excess of 10 µg/ml (Fig. 6). This suggested that extensive proteolysis destroyed much of the infectivity of ADV-G for cell culture. However, when a [^{35}S]methionine-labeled preparation of ADV-G was purified on CsCl gradients either before or after treatment with 2 µg of trypsin per ml, no effect on particle density was noted (Fig. 7A). The peak fractions of the gradients corresponding to the 1.43-g/ml full particles were analyzed by autoradiography of polyacrylamide gels, and it was again apparent that p85 and p75 were the most abundant proteins in the undigested virus (Fig. 7B, lane B). Smaller amounts of proteins with MWs of ca. 60k, 50k, and 30k were also observed (8). The trypsinized ADV-G particles had no detectable p85 or p75 (Fig. 7B, lane C), and tryptic peptides similar to those noted by immune blotting were observed (Fig. 4). A minor protein with an MW of 17k was also seen, but it was not present after another cycle of CsCl centrifugation (data not shown). These experiments indicated that the ADV-G virions maintained particle integrity even after extensive trypsin treatment. Total radioactivity present was similar for the trypsinized and nontrypsinized CsCl ultracentrifugation peaks. This implied that little protein was actually lost from the trypsinized ADV-G particles, thus suggesting that the small-MW proteins were retained in the virus capsid structure. The fact that the total radioactivity of the treated sample was close to that of the untreated also implied that trypsin treatment did not significantly affect recovery of virions from infected cells.

DISCUSSION

This study suggested an explanation for the apparent discrepancy between the small-MW polypeptides found in

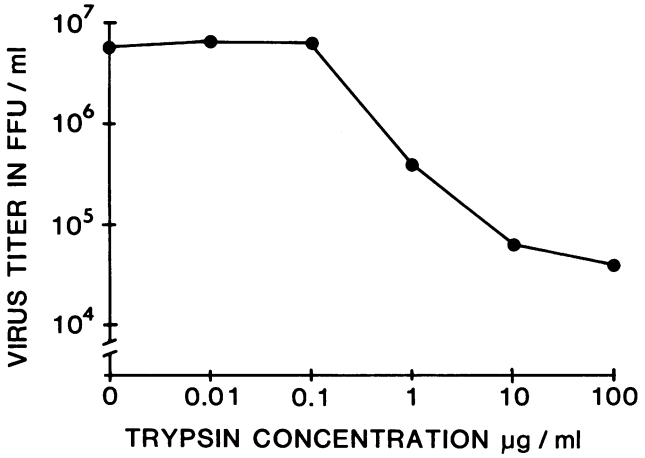

FIG. 6. Effect of trypsin treatment on ADV-G infectivity. A culture of ADV-G-infected CRFK cells was harvested into Dulbecco phosphate-buffered saline, freeze-thawed, and sonicated. Portions were incubated with the indicated concentrations of trypsin for 1 h, and the residual ADV-G infectivity was assayed as described previously (8) after addition of a molar excess of soybean trypsin inhibitor.

in vivo-produced ADV and the larger polypeptides seen in in vitro-produced ADV-G. Parvovirus structural proteins generally are two or three large-MW proteins differing by ca. 10k (23). The smaller of these proteins are all subsets of the largest one, with coterminal carboxyl ends, although the exact genesis of the various polypeptides is not as yet certain (9, 14, 21, 24). The relationships of the structural proteins of ADV-G (p85 and p75) are similar to those of other parvoviruses, but polypeptides with MWs in the range of 20 to 30k, like the ones seen in in vivo-produced ADV, have not been described for the parvovirus family (23), a finding which for some years obfuscated the classification of ADV as a parvovirus. Our results indicated that the small-MW polypeptides found in the in vivo-produced ADV strains were most likely generated from large-MW virion peptides by proteolysis with a trypsin-like enzyme. Our findings also showed that this putative proteolytic processing could be mimicked by trypsinizing ADV-G and that such trypsinized ADV-G still had the same particle integrity as did untreated ADV-G. The densities of untrypsinized and trypsinized ADV-G virus were identical (Fig. 7A), a finding in agreement with that of Paradiso for H1 virus (13). The site of this proteolysis was uncertain but it was unlikely that artifactual proteolysis occurred during isolation from preparations since protease inhibitor did not prevent the generation of the small-MW proteins. Furthermore, normal organ homogenates did not degrade ADV-G proteins when a reconstruction experiment was performed. Viral antigen has been localized to the cytoplasm of phagocytic cells at the height of viral replication in vivo (18). It is plausible that virions are ingested by phagocytes and that the proteolytic enzymes of these cells then degrade the larger-MW virion polypeptides (18). Since ADV is complexed to antibody when isolated from mink organs, this degradation process may depend upon the virus being in immune complexes. In this regard, it was interesting to note that the finding of large-MW ADV proteins in in vivo-produced DK ADV was made in mink given heavy immunosuppressive treatment (1, 2), which suppresses antibody production and thus immune complex formation (which is known in most cases to be a requisite for immune elimination by cells belonging to the reticuloendothelial system).

The trypsin degradation studies of ADV-G (Fig. 4 and 7B) implied that the large-MW proteins of ADV-G were degraded to polypeptides similar in size to those found in in vivo ADV. It will be interesting to compare the ADV-G breakdown products with the proteins of in vivo Utah I ADV by peptide mapping (9) and look for similarities.

Two results from these experiments suggested that the structural polypeptides of ADV-G were different from those

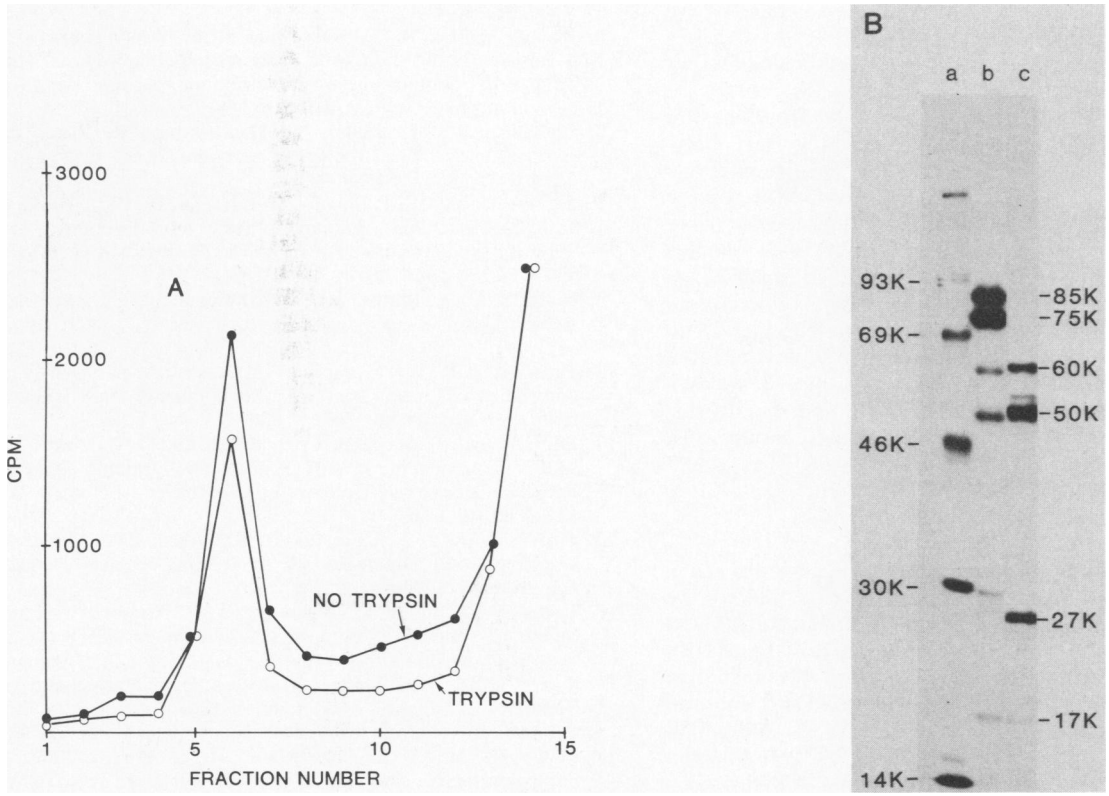

FIG. 7. Isopycnic density gradient analysis of trypsinized ADV-G. Two 150-cm^2 flasks of ADV-G infected CRFK were labeled with 50 μCi of [^{35}S]methionine per ml (New England Nuclear) and harvested at 70 h (8). The cell pellets were collected in 2 ml of 0.01 M Tris (pH 8.0), freeze thawed, sonicated, and divided into two portions. To one portion phenylmethylsulfonyl fluoride (Sigma Chemical Co.) was immediately added to a concentration of 0.001 M (21). The other portion was digested with trypsin (20 μg/ml) for 60 min at 37°C before addition of 1 mM phenylmethylsulfonyl fluoride and 5 μg of soybean trypsin inhibitor per ml. ADV was then purified from both samples by sucrose-CsCl gradients exactly as described previously (8). (A) Fractions of the gradients were analyzed for radioactivity; CPM indicates counts per minute of trichloroacetic acid-insoluble [^{35}S]methionine. (B) Peak fractions corresponding to full virions were subjected to SDS-PAGE and autoradiography (8). Lane A, MW markers (phosphorylase B, 92.5k; bovine serum albumin, 69k; ovalbumin, 46k; carbonic anhydrase, 30k; lysozyme, 14k; lane B, nontrypsinized ADV-G; lane C, trypsinized ADV-G.

of the in vivo ADVs (Utah I, Pullman, DK). First, the structural proteins identified for Utah I ADV by immunoprecipitation of infected CRFK cells were 2 to 3k larger than the 85k and 75k proteins of ADV-G (9), whereas the nonvirion p71s both had the same MW (Fig. 2). Similar differences have been found when immunoprecipitations were performed with anti-p85/p75 monoclonal antibodies (R. E. Race, B. Aasted, B. Chesebro, and M. E. Bloom, manuscript in preparation). Furthermore, when the polypeptides of in vivo-prepared ADVs (Utah I, DK, Pullman) were compared with those of trypsinized ADV-G (Fig. 3 and 4), the MW of the ADV-G proteins again were 2 to 3k smaller. Perhaps this difference reflected a small deletion in the ADV-G structural proteins. Additional work will be required to determine whether this was true and, if so, to localize the deletion on the proteins. Finally, trypsinization of ADV-G greatly reduced the infectivity of ADV-G for CRFK cells (Fig. 6). The absence of large-MW proteins in in vivo ADV virions (Fig. 3) (22) may be the cause for the relatively poor infectivity of these ADVs (12, 17) in vitro. However, such degradation may not be deleterious in vivo since Utah I, Pullman, and DK ADV are highly infectious for mink. Previous work has shown that ADV-G has greatly reduced infectivity for mink (8), but since trypsinization of ADV-G did not enhance the infectivity for mink (M. E. Bloom, unpublished data), it seemed unlikely that the low infectivity for mink of ADV-G was simply related to the size of the virion proteins. It also seems unlikely that the smaller size of the ADV-G proteins was wholly responsible for the low infectivity for mink of ADV-G since in vivo passage of ADV-G restores infectivity for mink (8).

When the polypeptide breakdown pattern from the immunoblots presented in Fig. 4 was compared to RIA analyses performed on the same samples (Fig. 5), a remarkable finding was apparent. Although the ADV-G polypeptide breakdown patterns with trypsin and chymotrypsin were nearly identical, the two protease-treated ADV-G samples behaved very differently in the RIA. Trypsinization of ADV-G increased its reactivity five times in RIA, whereas no change was seen upon chymotrypsinization. This suggested that the proteolytic modification of ADV in vivo is effected by trypsin or a trypsin-like protease and that breakdown polypeptides contain major antigenic determinants. In this regard, it is interesting to note that several monoclonal antibodies reacted only with trypsinized ADV-G polypeptides identical to the 60k, 50k, and 27k proteins seen in Fig. 7B (R. E. Race, B. Aasted, B. Chesebro, and M. E. Bloom, manuscript in preparation).

Finally, several lines of evidence have suggested that the small-MW breakdown products may have a role in the pathogenesis of Aleutian disease. First, although some of the immune complexes in Aleutian disease are infectious (16), the majority are too small (9 to 25S) (15) to contain intact virions (100 to 120S). Second, antibody to ADV structural proteins in mink sera is highly reactive with the small-MW proteins found in Utah I or in trypsinized ADV-G (Fig. 3 and 4). Consequently, we suggest that these small-MW proteins may be viral antigens found in some of the immune complexes. Further analysis of the viral polypeptides and antibodies found deposited in kidneys will provide interesting information on this aspect of Aleutian disease.

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LITERATURE CITED

1. **Aasted, B.** 1980. Purification and characterization of Aleutian disease virus. Acta Pathol. Microbiol. Scand. **88**:323–328.
2. **Aasted, B.** 1980. Purification and characterization of Aleutian disease virus, p. 1-10. *In* Proceedings of the Second International Scientific Congress in Fur Animal Production, Section 5, Vedbaek, Copenhagen, Denmark.
3. **Aasted, B., and B. Avery.** 1983. An easy method for comparing antibody affinities to related antigens. Acta Pathol. Microbiol. Immunol. Scand. **91**:65–67.
4. **Aasted, B., and M. E. Bloom.** 1983. Sensitive radioimmune assay for measuring Aleutian disease virus antigen and antibody. J. Clin. Microbiol. **18**:637–644.
5. **Arosio, P., T. G. Adelman, and J. W. Drysdale.** 1978. On ferritin heterogeneity. Further evidence for heteropolymers. J. Biol. Chem. **253**:4451–4458.
6. **Batteiger, B., W. J. Newhall V, and R. B. Jones.** 1982. The use of Tween 20 as a blocking agent in the immunological detection of proteins transferred to nitrocellulose membranes. J. Immunol. Methods **55**:297–307.
7. **Bloom, M. E., L. W. Mayer, and C. F. Garon.** 1983. Characterization of the Aleutian disease virus genome and its intracellular forms. J. Virol. **45**:977–984.
8. **Bloom, M. E., R. E. Race, and J. B. Wolfinbarger.** 1980. Characterization of Aleutian disease virus as a parvovirus. J. Virol. **35**:836–843.
9. **Bloom, M. E., R. E. Race, and J. B. Wolfinbarger.** 1982. Identification of a nonvirion protein of Aleutian disease virus: mink with Aleutian disease have antibody to both virion and nonvirion proteins. J. Virol. **43**:608–616.
10. **Chesebro, B., M. Bloom, W. Hadlow, and R. Race.** 1975. Purification and ultrastructure of Aleutian disease virus of mink. Nature (London) **254**:456–457.
11. **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.
12. **Hahn, E. C., L. Ramos, and A. J. Kenyon.** 1977. Expression of Aleutian mink disease antigen in cell culture. Infect. Immun. **15**:204–211.
13. **Paradiso, P. R.** 1981. Infectious process of the parvovirus H-1: correlation of protein content, particle density, and viral infectivity. J. Virol. **39**:800–807.
14. **Pintel, D., D. Dadachanji, C. R. Astell, and D. C. Ward.** 1983. The genome of minute virus of mice, an autonomous parvovirus, encodes two overlapping transcription units. Nucleic Acids Res. **11**:1019–1038.
15. **Porter, D. D., F. J. Dixon, and A. E. Larsen.** 1965. Metabolism and function of gammaglobulin in Aleutian disease of mink. J. Exp. Med. **121**:889–900.
16. **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.
17. **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.
18. **Porter, D. D., A. E. Larsen, and H. G. Porter.** 1969. The pathogenesis of Aleutian disease of mink. I. In vivo viral replication and the host antibody response to viral antigen. J. Exp. Med. **130**:575–593.
19. **Porter, D. D., A. E. Larsen, and H. G. Porter.** 1980. Aleutian disease of mink. Adv. Immunol. **29**:261–286.
20. **Porter, H. G., D. D. Porter, and A. E. Larsen.** 1982. Aleutian disease in ferrets. Infect. Immun. **36**:379–386.
21. **Rhode, S. L., III, and P. R. Paradiso.** 1983. Parvovirus genome: nucleotide sequence of H-1 and mapping of its genes by hybrid-arrested translation. J. Virol. **45**:173–184.
22. **Shahrabadi, M. S., H. J. Cho, and R. G. Marusyk.** 1977.

Characterization of the protein and nucleic acid of Aleutian disease virus. J. Virol. **23**:353–362.

23. **Tattersall, P.** 1978. Parvovirus protein structure and virion maturation, p. 53–72. *In* D. C. Ward and P. Tattersall (ed.), Replication of mammalian parvoviruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Tattersall, P., A. J. Shatkin, and D. C. Ward.** 1977. Sequence homology between the structural polypeptides of minute virus of mice. J. Mol. Biol. **111**:375–394.
25. **Wu, F. C., and M. Laskowski.** 1955. Action of the naturally occurring trypsin inhibitors against chymotrypsins α and β. J. Biol. Chem. **213**:609–619.