Comparative Analysis: Intracellular Precursor Polyproteins of Baboon Endogenous Retroviruses and Human Viral Isolate HL23V

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Intracellular precursor polyproteins of three baboon endogenous retrovirus (BaEV) isolates, m7, 455K, and BILN, were compared with the intracellular proteins of the type C human isolate HL23V by radioimmunoprecipitation, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and tryptic peptide analysis. Human and canine cells infected with m7-BaEV and canine thymus cells infected with BILN-BaEV were characterized by identical precursor polyproteins Pr85^{gag}, Pr70-71^{gag}, Pr65^{gag}, and gPr85^{env}. Canine cells infected with 455K-BaEV consistently showed a slightly different pattern of precursor polyproteins. These included Pr85^{gag}, Pr70^{gag}, Pr67^{gag}, and gPr85^{env}. By tryptic digest mapping of peptides containing [³H]leucine, m7-BaEV and 455K-BaEV were shown to be highly related. By comparison, mapping studies showed that BILN-BaEV was less highly related to m7-BaEV than was 455K-BaEV. Differences in these related BaEV isolates presumably reflected virus-specific differential cleavage of core protein precursors or alterations in polyprotein primary structure or both. Chase-incubated cells infected with BaEV also contained a stable, p28-related polyprotein termed P72^{gag}. This polyprotein migrated upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis slightly slower than the major core protein precursor Pr70-71^{gag} and appeared to arise by posttranslational modification of Pr70-71^{gag}. Immunoprecipitation of extracts of HL23V-infected cells with antisera to simian sarcoma-simian-associated virus proteins and BaEV proteins confirmed that these cells contained two unrelated viral components, one that was similar to m7-BaEV or BILN-BaEV and a second that was related to simian sarcoma-simian-associated virus. Tryptic digest mapping of BaEV and HL23V precursor polyproteins suggested that the BaEV-like component of HL23V was more closely related to m7-BaEV than to 455K-BaEV or BILN-BaEV.

Baboon endogenous type C retroviruses (BaEV) have been isolated from several species of the baboon (*Papio*) genus, including *Papio cynocephalus* (m7-BaEV), *Papio anubis* (455K-BaEV), *Papio hamadryas* (BILN-BaEV), and *Papio papio* (PP-1-BaEV) (4, 13, 28, 29). The m7 and 455K isolates are indistinguishable by nucleic acid hybridization, but partial nucleotide sequence data suggest that they may differ by at least one nucleotide at the 5' end of the genome (14, 28). The other isolates have been shown to be more readily distinguishable by nucleic acid hybridization (28) or by type-specific immunological differences (23, 25, 26). These studies have also demonstrated that, although the various BaEV isolates are closely related, they are distinct from the retroviruses isolated from other primates, including the simian sarcoma virus-gibbon ape leukemia virus

group (SiSV-GaLV) (12-14). A type C retrovirus (HL23V) isolated from cultured leukocytes of a patient with acute myelogenous leukemia has been shown to contain components immunologically related to both SiSV-GaLV and BaEV (9, 11, 18, 19). We report that, analogous to the well-characterized murine and avian type C retrovirus systems (1-3, 7), BaEV viral proteins are made by synthesis and cleavage of precursor polyproteins. In this paper we characterize the virus-specific proteins and intracellular precursor polyproteins of three BaEV isolates by radioimmunoprecipitation assay, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and ion-exchange column chromatography of trypsin-digested proteins. The characteristics of these primate retroviruses are compared with the BaEV-like viral gene products expressed in HL23V-infected cells.

MATERIALS AND METHODS

Cell cultures. The various primate virus isolates were obtained from cultures kindly supplied by Robert C. Gallo, National Institutes of Health, Bethesda, Md., and HEM Research Inc., Rockville, Md. CT/455K-BaEV was originally obtained by cocultivation of canine thymus cells with kidney tissue of *P. anubis* (29). A204/m7-BaEV and CT/m7-BaEV were originally obtained by cocultivation of either human rhabdomyosarcoma cells or canine thymus cells, respectively, with placental tissue from *P. cynocephalus* (4). CT/BILN-BaEV was originally obtained by infection of canine thymus cells with a virus isolated from the tissues of a lymphomatous *P. hamadryas* (13). Simian sarcoma-simian-associated virus [SiSV(SiAV)], originally isolated from a fibrosarcoma of a woolly monkey, was propagated in marmoset cells, 71AP1 (27, 31). The human virus isolate HL23V (9) was grown in either A204 cells or 7573 canine thymus cells. The SiSV-like component of HL23V (9) was grown in normal rat kidney (NRK) cells, which are refractile to infection by the BaEV-like component of HL23V. This SiSV-like virus, termed 23SiSV(SiAV), was then used to infect canine fibroblast CF2th cells.

All cells were grown to confluence in minimal Eagle medium containing 10% fetal calf serum and gentamicin at 50 μ g/ml in T-75 flasks. Each infected culture was shown to be capable of producing an infectious virus, as determined by the ability of a filtered growth medium to productively infect canine thymus cells in culture.

Isolation of radioactively labeled intracellular viral proteins. Labeling of cytoplasmic proteins was performed by incubation of cultures for 20 min at 37°C in 10 ml of Earle balanced salt solution supplemented with 2.5 mCi of [³H]leucine (45 Ci/mmol; New England Nuclear Corp., Boston, Mass.). Cultures were either lysed immediately or chase incubated for 60 to 90 min in a complete growth medium without the isotope before lysis. For some experiments, cultures were pulsed for 6 h with [³H]glucosamine (38 Ci/mol; Amersham Corp., Arlington Heights, Ill.) in a complete growth medium and then lysed. The lysis procedure and immunoprecipitation of cytoplasmic viral proteins were performed as described by Arcement et al. (1). Monospecific antisera to BaEV p28, Rauscher murine leukemia virus (RLV) p30, and RLV gp70 or polyspecific antisera to BaEV, SiSV, and GaLV were obtained through the Office of Program Resources and Logistics, Viral Oncology Program, National Institutes of Health. Immunoprecipitation was enhanced by the addition of the Formalin-fixed Cowan I strain of *Staphylococcus aureus* (21). The precipitates were collected by centrifugation, washed three times, and dissolved in sample buffer before electrophoresis. Immunoprecipitated cytoplasmic viral proteins were analyzed by SDS-PAGE on 6 to 12% gradient gels, using the buffer system of Laemmli (16). The gels were subjected to fluorography (5), and the X-ray film was preflashed to obtain a linear response (17).

Peptide mapping. Tryptic digestion of viral proteins and separation of the resultant polypeptide fragments by ion-exchange chromatography on Chromobead type P resin (Technicon Corp., Inc., Tarrytown,

N.Y.) were performed as previously described (1). The two-dimensional analysis of peptides has also been described previously (15). Briefly, dry slab gel bands were digested with trypsin, and the soluble peptides were separated on thin-layer cellulose plates (20 by 20 cm) by electrophoresis in 28% formic acid in the first dimension and by ascending chromatography in the second dimension, using a butanol-pyridine-acetic acid-water (6.2:1:3.3:2.8) buffer system. The spots representing radioactive peptides were detected by fluorography (15).

RESULTS

Immunoprecipitation of BaEV-specific intracellular polyproteins from infected cells. Various cell lines infected with BaEV isolates were compared for the presence of BaEV-related precursor-like polyproteins. Antiserum to m7-BaEV p28 recognized several high-molecular-weight proteins from the m7-BaEV-infected cells (Fig. 1, lanes 1 and 5). These included proteins of approximately 85,000 (Pr85^{gag}), 71,000 (Pr71^{gag}), 70,000 (Pr70^{gag}), and 65,000 (Pr65^{gag}) M_r , and some smaller proteins in the 40,000- to 55,000- M_r range. In pulse-labeled cells infected with m7-BaEV, Pr70^{gag} and Pr71^{gag} always appeared as a closely migrating doublet in gels. Identical profiles of precursor-like proteins were obtained from either canine (Fig. 1, lane 1) or human (Fig. 1, lane 5) cells infected with m7-BaEV or from cells infected with BILN-BaEV (data not shown). Chase incubation of pulse-labeled canine or human cells infected with m7-BaEV (Fig. 1, lanes 2 and 6, respectively) allowed the removal of labeled p28-related precursor polyproteins, the appearance of radioactive p28, and an intensification in the levels of an intermediate-sized protein of approximately 58,000 M_r (P58^{gag}). In addition, a new radiolabeled polyprotein of approximately 72,000 M_r (P72^{gag}) appeared in the cells. Analyses of HL23V-infected, pulse-labeled canine and human cells with anti-BaEV p28 serum (Fig. 1, lanes 3 and 7, respectively) revealed polyprotein profiles that were nearly identical to those of m7-BaEV-infected cells (Fig. 1, lanes 1 and 5). Pulse-labeled HL23V-infected cells consistently contained lower levels of the 40,000- to 50,000- M_r proteins than did cells infected with m7-BaEV or BILN-BaEV. As previously noted for the other BaEV isolates, chase incubation of HL23V-infected cells resulted in the cleavage of the large- M_r precursor-like proteins and the appearance of p28 and two larger proteins, P58^{gag} and P72^{gag} (Fig. 1, lanes 4 and 8). The P72^{gag} band from BaEV- or HL23V-infected cells always migrated slightly slower upon SDS-PAGE than did the 71,000- M_r band in the Pr70-71^{gag} doublet (Fig. 1, lanes 7 and 8). The P72^{gag} band

FIG. 1. Synthesis and cleavage of BaEV and HL23V precursor polyproteins. Cultures were pulse-labeled for 15 min with [3 H]leucine (lanes 1, 3, 5, 7, and 9) and chase incubated for 1 h (lanes 2, 4, 6, 8, and 10). Cultures labeled included CT/m7-BaEV (lanes 1 and 2), A204/HL23V (lanes 3 and 4), A204/m7-BaEV (lanes 5 and 6), and 7573/HL23V (lanes 7 to 10). Equal portions of cytoplasmic extracts were immunoprecipitated with anti-BaEV p28 (lanes 1 to 8), followed by reimmunoprecipitation with anti-BaEV (lanes 9 and 10). Lane 11 represents a marker of RLV proteins and precursor polyproteins.

appeared to be labeled only in chase-incubated cells (Fig. 1, lanes 2, 4, 6, and 8). None of the cell lines infected with the BaEV isolates or HL23V exhibited a *gag-pol*-like precursor of 180,000 to 200,000 M_r precipitable with anti-BaEV p28 serum (Fig. 1, lanes 1, 3, 5, and 7). If the extracts were reimmunoprecipitated with a polyspecific antiserum to detergent-disrupted m7-BaEV after they were cleared with anti-BaEV p28 serum, the BaEV envelope protein precursors could be visualized free from contamination by core protein-related precursors. When pulse-labeled HL23V-infected cells were thus treated, the precipitates contained only one protein of approximately 85,000 ($gPr85^{env}$) M_r (Fig. 1, lane 9). The same-sized protein could be observed in any of the cell lines infected with the BaEV isolates (data not shown). Chase incubations of up to 90 min resulted in only a slight decrease in size of the envelope protein precursor (Fig. 1, lane 10). The 18,000- M_r protein (p18) precipitated from these pulse-labeled, chase-incubated cells by anti-BaEV serum (Fig. 1, lane 10) is a nonglycosylated BaEV-like envelope protein that shares peptides with $gPr85^{env}$ (data not shown).

Canine cells infected with 455K-BaEV contained a slightly different pattern of p28-related proteins than did cells infected with m7-BaEV, BILN-BaEV, or HL23V (Fig. 2, lanes 2 and 3). Pulse-labeled cells infected with 455K-BaEV

FIG. 2. Intracellular precursor proteins of CT/455K-BaEV versus 7573/HL23V. Cultures were pulse-labeled for 15 min with [3 H]leucine (lanes 1 to 4) and chase incubated for 1 h (lane 5). Extracts of 7573/HL23V (lane 2) and CT/455K-BaEV (lanes 3 to 5) cells were immunoprecipitated with anti-BaEV p28. Lane 1 represents a marker of RLV-specific proteins.

contained several precursor-like proteins precipitable with anti-p28 serum (Fig. 2, lane 3). These included proteins of 85,000 ($Pr85^{eq}$), 70,000 ($Pr70^{eq}$), and 67,000 ($Pr67^{eq}$) M_r , and variable amounts of lower- M_r proteins. Upon chase incubation, these radioactive proteins diminished

in amounts, whereas amounts of labeled p28 increased (Fig. 2, lane 5). Chase-incubated cells infected with 455K-BaEV were also characterized by the presence of P72^{gag} and P58^{gag} (Fig. 2, lane 5).

BaEV- and SiSV-like components in HL23V-infected cells. Electrophoretic analyses of immunoprecipitates of extracts from cells infected with HL23V confirmed that these cells contained both a BaEV-like component and an SiSV-like component (Fig. 3). The precursor polyproteins of the two components could be separated readily for analysis by differential immunoprecipitation with the appropriate antiserum. As previously noted (Fig. 1, lane 3), treatment of extracts of pulse-labeled HL23V-infected canine fibroblast cells with antiserum to BaEV p28 precipitated the typical BaEV p28-related polyproteins Pr85^{gag}, Pr70-71^{gag}, and Pr65^{gag} (Fig. 3, lane 1). Likewise, reimmunoprecipitation of extracts with a polyspecific anti-BaEV serum, after clearing them with anti-BaEV p28 serum, revealed the BaEV-like envelope protein precursor gPr85^{env} (Fig. 3, lane 2). Obviously, all BaEV precursors related to BaEV p28 were removed by the first precipitation with anti-BaEV p28. Reimmunoprecipitation of the anti-BaEV p28-cleared cell extract with an antiserum to SiSV p28, however, revealed a new

set of precursor polyproteins that included a minor protein, Pr80^{gag}, and a major protein, Pr60^{gag} (Fig. 3, lane 3). Only trace amounts of these SiSV-like core protein precursors were precipitated by anti-BaEV p28 serum (Fig. 3, lane 1). Immunoprecipitation of extracts of similarly pulse-labeled cells with antiserum to SiSV p28 identified the same two SiSV-like core protein precursor polyproteins, Pr80^{gag} and Pr60^{gag}, but not the BaEV-like precursors (Fig. 3, lane 4). Reimmunoprecipitation of the supernatants of the cell extracts with a polyspecific antiserum to SiSV, after prior clearing with anti-SiSV p28, revealed the presence of a SiSV-like envelope protein precursor, gPr80^{env} (Fig. 3, lane 5). The SiSV-like envelope protein precursor was not precipitable by an antiserum to BaEV proteins (Fig. 3, lane 2). The SiSV-like envelope protein precursor gPr80^{env} migrated significantly faster upon SDS-PAGE than did the analogous BaEV-like precursor gPr85^{env} (Fig. 3, compare lanes 2 and 4). The SiSV-like component of HL23V is infectious for NRK cells to the exclusion of the BaEV-like component. This SiSV-like virus, termed 23SiSV(SiAV), derived from infected NRK cells was used to infect canine fibroblast cells. These cells were pulse-labeled as described above, and cell extracts were treated with an antiserum to SiSV p28 (Fig. 3, lane 6). Super-

FIG. 3. Intracellular precursor proteins of HL23V. Cultures of canine thymus cells infected with HL23V (lanes 1 to 5), or infected with only the SiSV-like component of HL23V (lanes 6 and 7), or marmoset cells infected with SiSV(SiAV) (lanes 8 and 9) were pulse-labeled for 15 min with [³H]leucine. Equal portions of the cytoplasmic extract were immunoprecipitated with anti-BaEV p28 (lane 1), followed by reimmunoprecipitation with either anti-BaEV (lane 2) or anti-SiSV (lanes 3), or with anti-SiSV p28 (lanes 4, 6, and 8), followed by reimmunoprecipitation with anti-SiSV (lanes 5, 7, and 9).

natants from this cleared cell extract were then treated with an antiserum to detergent-disrupted SiSV (Fig. 3, lane 7). Immunoprecipitation resulted in the usual SiSV-like precursors Pr200^{gag-pol}, Pr80^{gag}, and Pr60^{gag} (Fig. 3, lane 6) and gPr80^{env} (Fig. 3, lane 7). Treatment of extracts from these 23SiSV(SiAV)-infected cells with antiserum to BaEV proteins did not reveal any BaEV-like precursor polyproteins (data not shown). Figure 3, lanes 8 and 9, shows the results of similar immunoprecipitations of extracts derived from pulse-labeled marmoset cells (71AP1) infected with SiSV(SiAV).

Labeling of HL23V precursor proteins with [³H]glucosamine. Figure 4 shows the SDS-PAGE profile of HL23V-infected cells that were long term labeled with [³H]glucosamine. No core-related proteins were labeled with glucosamine (Fig. 4, lanes 2 and 3). Both BaEV-like gPr85^{env} (Fig. 4, lane 5) and SiSV-like gPr80^{env} (Fig. 4, lane 6) contained glucosamine. Antiserum to GaLV (Fig. 4, lane 7) also precipitated a SiSV-like glycoprotein, indicative of the high degree of relatedness between GaLV and SiSV. Immunoprecipitation with anti-RLV gp70 (Fig. 4, lane 4) precipitated small amounts of only the SiSV-like envelope protein gPr80^{env}.

Tryptic peptide maps of BaEV and HL23V precursor proteins. BaEV- or HL23V-infected cells were pulse-labeled with [³H]leucine and analyzed by SDS-PAGE and

autoradiofluorography. Bands exhibiting radioactivity as detected by fluorography were cut from gels and subjected to tryptic digestion as previously described (1). The resultant polypeptide fragments were separated by ion-exchange chromatography (2, 7).

A comparison of Pr70-71^{gag} from BaEV-infected canine (CT/m7-BaEV) or human (A204/m7-BaEV) cells with Pr70-71^{gag} from HL23V-infected canine cells (7573/HL23V) is shown in Fig. 5. The peptide map of m7-BaEV Pr70-71^{gag}, isolated from either infected canine or human cells, appeared to be identical to the map of the BaEV-like core-related Pr70-71^{gag} seen in HL23V-infected cells. All 21 to 23 leucine-containing peptide peaks seen in m7-BaEV Pr70-71^{gag} were also represented in HL23V Pr70-71^{gag}. Other experiments indicated that Pr70-71^{gag} from cells infected with BILN-BaEV has a peptide map identical to that of m7-BaEV Pr70-71^{gag} (data not shown).

The p28 content of the Pr70-71^{gag} proteins isolated from cells infected with any of the three BaEV isolates or with the HL23V isolate was confirmed by tryptic digest mapping (data not shown). Map peaks numbered 5 to 7, 9, 10, 12 to 15, 17, 18, and 20 were present in both p28 and Pr70-71^{gag} (Fig. 5).

As noted in the SDS-PAGE analysis of precursor proteins in CT/455K-BaEV cells (Fig. 2, lanes 1 and 2), 455K-BaEV exhibited an apparently unique precursor protein cleavage pattern. Figure 6 shows the maps of the leucine-containing peptides characteristic of the three major core protein precursors derived from CT/455K-BaEV cells. A structural relationship of Pr85^{gag}, Pr70^{gag}, and Pr67^{gag} from CT/455K BaEV cells was evident from these mapping studies. Also evident in Fig. 6D was the relatedness of the chase protein P72^{gag} to the gag precursor proteins. In this analysis no new peptides were present in P72^{gag} when it was compared with the pulse-labeled precursors Pr85^{gag}, Pr70^{gag}, and Pr67^{gag}. In fact, P72^{gag} was missing at least one peptide (peak 8) found in the other core protein-related polyproteins.

A very similar relationship existed among the core protein precursors from cells infected with the other BaEV isolates or with HL23V (data not shown). It was apparent from pulse-chase studies (Fig. 1 and 2) that the levels of P72^{gag} in chase-incubated cells correlated well with the levels of Pr70^{gag} and Pr70-71^{gag} in pulse-labeled cells. This result suggested that P72^{gag} may have been derived from posttranslational modification of Pr70^{gag} in 455K-BaEV-infected cells or of Pr70-71^{gag} in cells infected with the other BaEV isolates or with HL23V. It was not apparent from the single-dimensional analysis of [³H]leu-

FIG. 4. Glucosamine labeling of 7573/HL23V. Cells were labeled for 6 h with [³H]glucosamine. Equal portions of cytoplasmic extract were immunoprecipitated with anti-RLV p30 (lane 2), anti-BaEV p28 (lane 3), anti-RLV gp70 (lane 4), anti-BaEV (lane 5), anti-SiSV (lane 6), and anti-GaLV (lane 7). Lane 1 represents a marker of RLV-specific polyproteins.

FIG. 5. Ion-exchange chromatography of trypsin-digested [³H]leucine-labeled Pr70-71^{gag} from CT/m7-BaEV (A), A204/m7-BaEV (B), and 7573/HL23V (C). The peptides are numbered for reference, and a heavy bar indicates the elution of the column with 2 M pyridine acetate (11).

cine-containing tryptic peptides how P72^{gag} might have differed from Pr70^{gag} or Pr70-71^{gag}. To more fully consider the relationship between P72^{gag} and Pr70-71^{gag}, we analyzed the tryptic peptides derived from these polypeptides by a technique which allows the separation of peptides in two dimensions. The results of this analysis (Fig. 7) confirmed our previous observation that the peptide maps of Pr70-71^{gag} and Pr72^{gag} were nearly identical. It was obvious, however,

that Pr70-71^{gag} contained one peptide (Fig. 7A, spot 5a) which was missing from P72^{gag} (Fig. 7B, arrow). Likewise, P72^{gag} contained one peptide (Fig. 7B, spot 5b) which was missing from Pr70-71^{gag} (Fig. 7B, arrow). We presume that spot 5a

FIG. 6. Ion-exchange chromatography of trypsin-digested [³H]leucine-labeled polyproteins from CT/455K-BaEV. Compared are Pr85^{gag} (A), Pr70^{gag} (B), Pr67^{gag} (C), and P72^{gag} (D).

(Fig. 7A) was the same peptide as that labeled peak 8 (Fig. 6B). The peptide in spot 5b (Fig. 7B) was apparently not separated by the one-dimensional analysis (Fig. 6D) and may have been eluting in the void volume. Tryptic maps of P72^{gag} from cells infected with any of the BaEV isolates or with HL23V were nearly identical (data not shown). Likewise, tryptic maps of Pr85^{gag} from m7-BaEV and BILN-BaEV were identical to those of Pr85^{gag} 455K-BaEV (data not shown). The map of 455K-BaEV Pr70^{gag} peptides was similar to the map of m7-BaEV Pr70-71^{gag} (compare Fig. 6B with 5A). The absence of peak 5 in Pr70^{gag} derived from 455K-BaEV-infected cells may have been related to the differences in precursor profiles noted for this isolate in comparison with those for m7-BaEV (Fig. 2).

Figure 8 shows a comparison of the BaEV and HL23V BaEV-like glycoprotein precursor gPr85^{env}. Apparent immediately was the high degree of relatedness to gPr85^{env} from cells infected with the various isolates of BaEV (Fig. 8A to C) and gPr85^{env} from cells infected with the HL23V BaEV-like isolate (Fig. 8D). The gPr85^{env} proteins of m7-BaEV and 455K-BaEV exhibited identical tryptic peptide maps (Fig. 8A and C). The CT/m7-BaEV glycoprotein precursor apparently contained one additional peptide (Fig. 8B, peak 6) not seen in the other BaEV-infected cells. Like A204/m7-BaEV and CT/455K-BaEV, HL23V gPr85^{gag} lacked peptide 6 (Fig. 8D) but contained one additional peak (peak 15). A comparison of the maps of the leucine-containing peptides of gPr85^{env} from cells infected with m7-BaEV or BILN-BaEV is

shown in Fig. 9. The envelope protein precursors of these two BaEV isolates were clearly distinguishable from one another by this mapping procedure. As previously stated, however, the major core protein precursor, Pr70^{gag}, of these two isolates was indistinguishable by this technique (data not shown).

DISCUSSION

Several significant facts pertaining to BaEV replication are apparent from the results presented in this report. First, BaEV core and envelope proteins are synthesized in infected cells by precursor polypeptides, which are apparently analogous to the precursors of murine retroviruses. The immunoprecipitation-SDS-PAGE analyses described are capable of differentiating between the precursor polypeptides of m7-BaEV and 455K-BaEV. We have also used 455K-BaEV obtained from CT/455K-BaEV to infect other canine thymus cells (CF2th). The SDS-PAGE profile of polypeptides obtained from these cells was identical to that obtained from the original CT/455K-BaEV cell culture (data not shown). This result suggests that the minor precursor profile differences between m7-BaEV and 455K-BaEV are reproducible and virus specific. As previously mentioned, peptide mapping studies indicated an extensive structural relationship among 455K-BaEV core protein precursors Pr85^{gag}, Pr70^{gag}, and Pr67^{gag} (Fig. 6). A sequential processing from Pr85^{gag} (Fig. 6A) to Pr70^{gag} (Fig. 6B) and finally to Pr67^{gag} (Fig. 6C) was also suggested by the peptide maps. Processing of Pr85^{gag}, if it actually occurs, apparently results in the loss of peptides 5 and 19 to yield Pr70^{gag},

FIG. 7. Two-dimensional peptide analysis of trypsin-digested [³H]leucine-labeled Pr70-71^{gag} (A) and P72^{gag} (B) from A204/m7-BaEV cells.

FIG. 8. Ion-exchange chromatography of trypsin-digested [^3H]leucine-labeled gPr85^{env} from A204/m7-BaEV (A), CT/m7-BaEV (B), CT/455K-BaEV (C), and A204/HL23V (D).

which subsequently loses peptide 9 to yield Pr67^{gag}. Alternatively, it is possible that Pr85^{gag} is not a direct precursor to either Pr70^{gag} or

FIG. 9. Ion-exchange chromatography of trypsin-digested [^3H]leucine-labeled gPr85^{env} from A204/m7-BaEV (—) versus CT/BILN-BaEV (---).

Pr67^{gag}. These related polyproteins actually may be synthesized from distinct but related mRNA's.

Tryptic digest analyses of leucine-containing peptides of SDS-PAGE-purified BaEV-specific precursors suggest a high degree of relatedness among the three BaEV isolates, especially in the core protein-related gene products. Similar analyses of the envelope gene products indicate near identity for m7-BaEV and 455K-BaEV, but clearly distinguish these isolates from BILN-BaEV. These observations are in agreement with previously published estimates of the relationship among various BaEV isolates based on immunological and nucleic acid hybridization data (9, 18, 19, 22, 24). Second, it is interesting that none of the cell lines, when they were examined with anti-BaEV p28 or anti-BaEV serum, expressed an obvious gag-pol common precursor analogous to Pr200^{gag-pol} observed in murine retrovirus-infected cells (1, 2, 15). Several possibilities exist to explain the apparent absence of this expected precursor. It is, of course, possible that BaEV are unique among retroviruses and do not require synthesis and cleavage of a core protein polymerase read-through translation product for synthesis of their polymerase. The absence of this common precursor may indicate the existence of a unique subset mRNA in BaEV-infected cells that codes solely for BaEV polymerase. It seems more likely, however, that the BaEV core protein polymerase precursor is synthesized in these cells but at levels below the current level of detection of our techniques. This would require a significant reduction in polymerase synthesis in comparison with that needed for other retrovirus systems (e.g., murine or avian retroviruses) in which

Pr200^{gag-pol} is easily identified by these procedures. A third possibility is that antisera to BaEV core proteins do not immunoprecipitate the BaEV core protein polymerase precursor polyprotein because of the alteration of core protein antigenic sites in the common precursor. Further studies with antisera to BaEV polymerase will be required to explain the apparent absence of Pr200^{gag-pol} in BaEV-infected cells.

A third interesting observation pertaining to BaEV replication is the presence of a 72,000-*M_r*, p28-related polyprotein (P72^{gag}) in extracts of pulse-labeled, chase-incubated BaEV-infected cells. P72^{gag} migrates upon SDS-PAGE slightly, but consistently, more slowly than does pulse-labeled precursor Pr70-71^{gag}. This relatively stable polyprotein is not labeled during short pulse-labeling and is, therefore, apparently not a primary product of BaEV mRNA translation. Synthesis of P72^{gag} presumably involves posttranslational modification of another core protein precursor. As we have already mentioned, the amount of P72^{gag} in chase-incubated cells correlates relatively well with the amount of Pr70^{gag} or Pr70-71^{gag} labeled during pulses. This correlation suggests that it is Pr70^{gag} or Pr70-71^{gag} that is a precursor to P72^{gag}. This interpretation is favored by the results of peptide mapping experiments (Fig. 6 and 7). The most plausible interpretation of these results is that peptide 5a (Fig. 7A) in Pr70-71^{gag} is altered by posttranslational modification to become peptide 5b in Pr72^{gag} (Fig. 7B). We could postulate that the alteration of this peptide is by glycosylation. Glycosylation is known to result in the retardation of protein migration upon SDS-PAGE, and glycosylated peptides often migrate in the void volume upon column chromatography or near the origin upon two-dimensional analysis (L. J. Arcement, R. B. Arlinghaus, and R. B. Naso, unpublished data). Also, glycosylated core protein precursors have been observed in the murine retroviral systems (6, 8, 30). Unlike the glycosylated murine retrovirus core protein precursor gPr80^{gag}, P72^{gag} is an apparently stable intracellular protein. Murine gPr80^{gag} is synthesized in pulse-labeled cells and is chased into gPr93-95^{gag} on the cell surface (30) and to a lower-*M_r* precursor, Pr45^{gag}, containing p15, pp12, and the so-called leader sequence (R. B. Naso and R. B. Arlinghaus, manuscript in preparation). Furthermore, if P72^{gag} is glycosylated, it does not contain enough glucosamine to label with [³H]glucosamine (Fig. 4, lane 3). Similar labeling experiments with [³H]mannose are in progress. The relatively large intracellular amounts of such a stable core protein precursor undoubtedly relate to the rather poor produc-

tion of BaEV particles by most infected cells studied in laboratories. As such, the presence of P72^{gag} may represent the functioning of a post-translational control mechanism in BaEV replication.

Finally, this study has provided some interesting facts concerning the BaEV-like component of the human isolate retrovirus HL23V and its relationship to true BaEV isolates. It is obvious from the results described that HL23V contains two components, one related to BaEV and the other related to SiSV(SiAV). This finding is certainly in agreement with the results of other analytical procedures (9, 19). It is also apparent that antisera to BaEV and SiSV(SiAV) proteins, such as those obtained from the Office of Program Resources and Logistics of the National Institutes of Health, are capable, when used in radioimmunoprecipitation-SDS-PAGE analyses, of distinguishing between the precursor proteins of these two components in HL23V-infected cells. This fact is substantiated by the tryptic digest results presented here. It has recently been reported that the genome of viruses isolated from HL23V-infected canine fibroblasts (A7573) was similar by oligonucleotide mapping to the genome of SiAV (24). This report, however, was unable to detect a species of RNA from a virus released from these cells that was similar to BaEV, suggesting that the BaEV-like component of HL23V released from infected A7573 cells comprises less than 10% of the total virus (24). The intracellular gene products resulting from translation of the BaEV component RNA, however, can be observed readily by radioimmunoprecipitation of cell extracts, as reported here. It is interesting to postulate that the low extracellular level of the BaEV-like component of HL23V relates to the synthesis of stable P72^{gag} in these cells. It has also been reported that HL23V derived from early passages of the human peripheral leukocytes and obtained by cocultivation with human (NC37) cells or infection of bat (CCL88) cells (10) contained a virus component that was identical to the San Francisco isolate of GaLV (24). We have not observed this component in A7573 cells infected with virus derived from a later passage of the human leukocytes. It appears that a GaLV-like virus is the predominant species of SiSV-GaLV-like virus isolated from early passage HL23 leukocytes, whereas an SiSV-like virus is the predominant species of virus isolated from later passage leukocytes (24).

We have demonstrated that the intracellular proteins of the BaEV-like component of HL23V are identical in size to the proteins of m7-BaEV. By tryptic peptide mapping, Pr70^{gag} from

HL23V is identical to Pr70^{gag} from human or canine cells infected with m7-BaEV. Comparisons of precursor polyprotein antigenic similarities, molecular weights, and peptide content illustrate that the BaEV-like viral component of HL23V is more related to m7-BaEV than to 455K-BaEV. Similarly, antibodies raised against m7-BaEV reverse transcriptase cross-react more significantly with HL23V than with 455K-BaEV (23). The envelope precursor of HL23V, gPr85^{env}, contains glucosamine and is nearly identical to both the m7-BaEV and the 455K-BaEV envelope precursors. Although the human isolate HL23V is shown to contain a component that is substantially related to BaEV, conventional liquid hybridization studies indicate that HL23V is not a human endogenous virus (22). These results suggest that the BaEV-like component of HL23V represents a horizontal cross-species infection of human cells with m7-BaEV.

The SiSV-like intracellular viral component of HL23V was isolated by immunoprecipitation with sera directed against SiSV(SiAV) proteins. The SiSV-like proteins of HL23V are distinguishable from the BaEV-like component of HL23V by analysis of intracellular precursor profiles (Fig. 3) and by tryptic digest analyses (data not shown). The HL23V SiSV-related viral component is replicated by synthesis and cleavage of a major core protein precursor, Pr60^{gag}, minor core protein precursors, Pr200^{gag-pol} and Pr80^{gag}, and an envelope protein precursor, gPr80^{env}. A more complete characterization of the precursor polyproteins of the SiSV(SiAV)-like component of HL23V and their relationships to true SiSV(SiAV), GaLV, and another human isolate, HEL-12V (20), will be presented later (S. A. Whiteley, C. A. Edbauer, and R. B. Naso, manuscript in preparation).

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