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Avian Myeloblastosis Virus Proteins in Leukemic Chicken Myeloblasts

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We have analyzed the avian myeloblastosis virus proteins in two types of leukemic myeloblasts: established myeloblastic cell lines (DU 1765 and DU 11157) and leukemic myeloblasts obtained from the peripheral blood of a leukemic C/E Spafas chicken (no. 21957). Using monospecific antisera for immunoprecipitation and polyacrylamide gel electrophoresis, we have detected *gag* gene-related proteins in the myeloblasts. The DU 1765 and DU 11157 cells contained a p100 protein which possessed antigenic determinants of the viral proteins p27, p19, p15, and p12. The p100 was not found in leukemic myeloblasts from Spafas chickens, and pulse-chase experiments showed that the p100 was not a precursor for the viral proteins. However, the p100 is present in uninfected line 15 chicken embryos. A pr76-like protein was identified in DU 1765 cells but migrated slightly further into gels than the pr76 of Spafas-derived leukemic myeloblasts. The Spafas-derived myeloblasts produced a pr60, whereas the DU 1765 cells contained instead a related protein of 62,000 daltons. Using anti-avian myeloblastosis virus gp85 sera, a glycoprotein of 120,000 daltons (gp120) was detected in all the tested leukemic myeloblasts. The gp120 was also present, in low amounts, in uninfected embryonic spleen and yolk sac cells. The anti-gp85 sera also precipitated a 27,000-dalton protein (h27) in these same cells. Both the gp120 and h27 could not be detected in either uninfected or myeloblastosis-associated virus-infected fibroblasts. Limited peptide hydrolysis revealed that h27 is different from the viral structural protein p27. In conclusion, monospecific antisera for *gag* and *env* gene products of avian myeloblastosis virus did not precipitate any unique or aberrant avian myeloblastosis virus protein from leukemic myeloblasts.

Avian myeloblastosis virus (AMV) has an apparently defective genome and causes acute myeloblastic leukemia in chickens (22). Its two associated helper viruses (myeloblastosis-associated virus [MAV]-1 and MAV-2) also induce neoplasias, nephroblastoma, visceral lymphoid leukosis, and osteogenic osteoblastoma (22, 27). Another defective avian retrovirus, avian erythroblastosis virus (AEV), causes erythroblastic leukemia in chickens. The isolation of mutants temperature sensitive for erythroblastosis suggests that the continued presence of a viral gene product is necessary for the maintenance of the leukemic state in AEV-transformed erythroblasts (12). Thus, by analogy, it may be postulated that AMV contains a leukemogenesis (*luk*) gene whose product is necessary for the initiation or maintenance of acute myeloblastosis, or both. However, temperature-sensitive mutants of AMV have not been isolated. If AMV contains a *luk* gene, it is probably unique and unrelated to transforming sequences in other avian retroviruses. There is no base sequence homology between AMV and complementary DNA representing only the *src* gene of avian sarcoma viruses (25). A complementary DNA probe, specific for AMV, did not hybridize with the viral RNA of three other defective avian transforming retroviruses: avian myelocytomatosis virus (MC29), Mill Hill 2 virus (MH2), and AEV (25). Also, the transforming genomic sequence of AEV appears to be unrelated to that of MC29 or MH2 (4). These viruses contain major deletions in their *gag, pol,* and *env* genes with inserted cellular sequences which are thought to represent the transforming gene (3, 4). This group of defective transforming retroviruses therefore differs from the defective Bryan high-titer Rous sarcoma virus (BH-RSV).

In BH-RSV-transformed cells, the major glycoproteins, gp85 and gp37, are either absent or modified, but all the other viral proteins are present, and the *src* gene is transcribed and translated separately from the viral replication proteins (23). MC29, MH2, and AEV produce, in transformed cells, an aberrant protein containing some *gag*-related proteins unique for each virus. An unprocessed 110,000-dalton protein in MC29-transformed cells contains all or part of p19 and p27, but not p15 nor gp85, and a related 100,000-dalton protein is present in MH2-transformed cells (16). AEV produces a

75,000-dalton protein containing only p10 from the *gag* gene (15). By analogy with these defective transforming avian retroviruses, AMV might be expected to produce an unprocessed, transforming, nonvirion protein containing some *gag* gene products.

In this study we used antisera against either leukemic cells or whole virions or monospecific for each one of the AMV subviral components, in an attempt to identify a specific protein in AMV-transformed myeloblasts. Using immunoprecipitation and polyacrylamide gel electrophoresis, we have not detected in leukemic myeloblasts any aberrant protein resembling that induced by MC29, MH2, or AEV. Thus, either AMV produces a *luk* protein that does not contain any antigen related to the *gag* gene product, as does the *src* gene product of RSV, or it induces leukemia by some other mechanism.

MATERIALS AND METHODS

Cells and viruses. C/E fertile chicken eggs, negative for gs antigen (gs^-) and for chicken helper factor (chf^-), were obtained from Spafas (Roanoke, Ill.). The preparation of yolk sac and chicken embryonic fibroblast (CEF) cultures followed published techniques (1, 22, 28). Standard AMV, BAI strain A (AMV-S), was a gift from J. W. Beard (Life Sciences, Inc., St. Petersburg, Fla.). MAV-1 and MAV-2 were a gift from C. Moscovici (Veterans Administration Hospital, Gainesville, Fla.). All viruses were subsequently propagated in C/E, gs^-chf^- Spafas chicken cells. Peripheral blood leukemic myeloblasts 21957 were derived by infecting a Spafas C/E chicken with AMV-S. The 21957 cells produced AMV and helper viruses. Two established avian leukemic myeloblast cell lines (DU 11157 and DU 1765) were kindly provided by D. P. Bolognesi of Duke University, Durham, N.C. The DU 11157 cells were established from myeloblasts isolated from a leukemic bird after intravenous inoculation with AMV-S. The DU 1765 cells were isolated from chicken embryo cells after an in vitro infection with AMV-S. By interference assays, both cell lines were reported to be virus producers. However, DU 11157 cells were found to have 10^4-fold less virus than short-term myeloblast cultures. The DU 1765 cells were found to have over 10^6-fold less virus than other leukemic myeloblasts (18). The myeloblast cell lines were grown in roller bottles in Dulbecco-modified Eagle medium (1) supplemented with 10% calf serum and 10% chicken serum.

Antisera. Antisera, made in rabbits against the gp85, p27, p19, p15, and p12 of AMV, were a generous gift from D. P. Bolognesi. In addition, goat anti-AMV p27 serum was obtained through the office of Program Resources and Logistics of the National Cancer Institute. Antisera against leukemic myeloblast proteins were prepared in rabbits by multiple-site intramuscular and subcutaneous injections of DU 1765 cells. Each rabbit received three injections, spaced 2 weeks apart, and was bled 1 week after the last injection. Also, three chickens, which had been injected as 16-day-old embryos with DU 1765 cells and later failed to show leukemia, were hyperimmunized with DU 1765 and DU 11157 cells. Each surviving 6-month-old chicken was injected intramuscularly at multiple sites with 0.3 ml of packed cells. Every 2 weeks, the immunization procedure was repeated for a total of five immunizations, and the chickens were bled 1 week after the last injection. The hamster anti-Schmidt-Ruppin RSV (SR-RSV) antiserum was obtained from tumor-bearing hamsters and sold commercially by Microbiological Associates.

Radioisotope labeling of cells. To label viral and cellular proteins, the cells were first incubated in methionine-free medium for 30 min. Leukemic myeloblasts were labeled with 50 μCi of [^{35}S]methionine (New England Nuclear Corp.) per ml at a cell density of 5×10^6 cells per ml for 4 h and then washed once (150 mM NaCl, 10 mM Tris-hydrochloride, 1 mM EDTA, pH 7.2). The cells were disrupted at 4°C in lysis buffer at a concentration of 5×10^6 cells per ml of lysis buffer (150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulfate [SDS], 10 mM Tris-hydrochloride, pH 7.2). Semiconfluent, logarithmically growing CEF cultures were labeled in a similar manner. For some experiments, cells were labeled with either a ^{3}H-amino acid mixture (200 μCi/ml for 24 h) or with [^{3}H]glucosamine (50 μCi/ml for 18 h).

Immune precipitation. The protein lysates and antisera were centrifuged at $16,000 \times g$ for 10 min. Cell lysate (200 μl) was incubated with the pertinent antiserum (5 μl) for 30 min at 4°C. A 10% suspension (200 μl) of the Cowan I strain of *Staphylococcus aureus* was added and incubated for 30 min to adsorb the immune complexes (17). The *S. aureus* was washed three times with lysis buffer and suspended in 50 μl of sample buffer (0.05 M Tris-hydrochloride, pH 6.8, 1% SDS, 1% β-mercaptoethanol, 10% glycerol, 0.001% phenol red), boiled for 3 min, and centrifuged. The supernatant was electrophoresed in a SDS-polyacrylamide gradient slab gel system.

Polyacrylamide gel electrophoresis. Immune precipitates were analyzed on a 7.5 to 15% linear gradient slab gel apparatus as outlined by Maizel (20). The electrode buffer contained 0.05 M Tris-hydrochloride, 0.384 M glycine, and 0.1% SDS. The stacking gel contained 4% acrylamide, 0.1% bisacrylamide, 0.05 M Tris-hydrochloride (pH 6.8), and 0.1% SDS. The separation gel was a 7.5 to 15% gradient of polyacrylamide (37.5:1, acrylamide-bisacrylamide), 15 cm long. The addition of 0.45% linear polyacrylamide eliminated cracking of gels during drying. Gels were fixed for 20 min in 25% isopropanol-10% acetic acid, stained in 0.01% Coomassie blue G-250 in 12% trichloroacetic acid for 1 h, and destained in 5% acetic acid. The gels were processed for fluorography as described by Bonner and Laskey (6). Exposure times ranged from 24 h to 3 weeks.

Peptide mapping was performed by using the method of limited proteolysis in SDS gels as described by Cleveland and co-workers (9). [^{35}S]methionine-labeled precipitates were run on a 7.5 to 15% gradient polyacrylamide gel as described above. Individual bands were cut from the dried gels and inserted into the slots of a second SDS gel (15% acrylamide). The

wells were half-filled with buffer (15 mM Tris-hydrochloride, pH 6.8, 0.1% SDS, and 1 mM EDTA). After a 1-h soak, 20 μl of buffer containing 20% glycerol and phenol red was added. This was followed immediately with 20 μl of buffer with 10% glycerol containing 0.005 μg of papain. The samples were electrophoresed until the phenol red marker reached the bottom of the stacking gel. The power was turned off for 30 min, after which electrophoresis was continued as normal.

RESULTS

Immunoprecipitation of [^{35}S]methionine-labeled cellular extract with anti-SR-RSV and anti-AMV gp85 sera. CEF or leukemic myeloblasts were labeled with [^{35}S]methionine for 4 h, lysed, and immunoprecipitated with either hamster anti-SR-RSV antiserum or anti-AMV gp85 antiserum. *S. aureus* Cowan I strain was used as an immunoadsorbent (17). The immune precipitate was electrophoresed on a 7.5 to 15% gradient polyacrylamide gel, and the labeled proteins were visualized with fluorography.

The hamster antiserum recognized four major bands in the virus-producing leukemic myeloblasts isolated from bird no. 21957 (Fig. 1, lane 4). These are designated pr76, pr60, p27, p19, and p12/15 (apparent molecular weight × 10^3). The pr76 is the *gag* precursor and contains the virion structural proteins p19, p27, p12, and p15. The pr60 is a cleavage product of the pr76 (29, 30). The same viral proteins are also detectable in MAV-2-infected C/E Spafas CEF (Fig. 1, lane 2). The leukemic myeloblasts of cell line DU 1765, which produce a subgroup B MAV at a low level (18; unpublished data), were similarly analyzed (lane 3). In this gel, the virion structural proteins p27 and p12/15 and the pr60 are not evident. A pr76-like protein is present, but migrates slightly further into the gel than the pr76 from 21957. Additional major protein bands that appear in DU 1765 are p100, p62, and three protein bands between p19 and p15.

The anti-AMV gp85 antiserum did not immunoprecipitate the viral proteins pr76, pr60, p19, or p12/15, but it precipitated from DU 1765 and 21957 cells a protein that migrated where p27 would be (Fig. 1, lanes 7 and 8). Since the DU 1765 cells did not show p27 if treated with the hamster anti-SR-RSV serum (Fig. 1, lane 3), and the anti-gp85 antiserum did not precipitate a 27,000-dalton protein in MAV-infected fibroblasts (Fig. 1, lane 6), this protein (designated h27) does not appear to be the viral p27. In addition to the h27 protein, the anti-gp85 serum precipitated a 120,000-dalton molecule (designated gp120) from DU 1765 and 21957 cells (Fig. 1, lanes 7 and 8). The gp120 was not seen in either uninfected or MAV-2-infected CEF (Fig. 1, lanes 5 and 6).

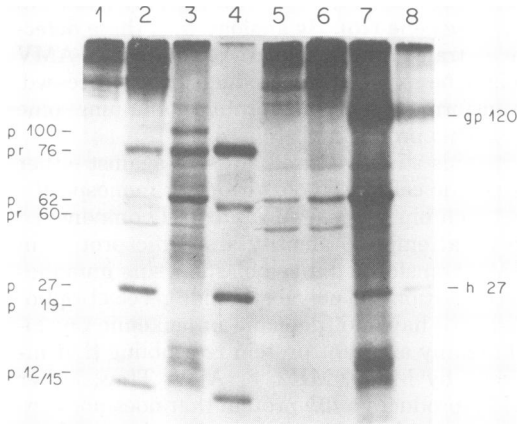

FIG. 1. *[^{35}S]methionine-labeled viral proteins in AMV-transformed myeloblasts precipitated with hamster anti-SR-RSV and rabbit anti-AMV gp85 antisera. The [^{35}S]methionine labeling of the cells, protein extraction, immunoprecipitation, electrophoresis on a 7.5 to 15% gradient polyacrylamide gel, and fluorography were all done as described in the text. Lanes 1 to 4, Hamster anti-SR-RSV antiserum; lanes 5 to 8, rabbit anti-AMV gp85; lanes 1 and 5, uninfected CEF; lanes 2 and 6, MAV-2-infected CEF; lanes 3 and 7, DU 1765 myeloblasts; lanes 4 and 8, leukemic myeloblasts harvested from a Spafas chick, no. 21957, injected with standard AMV.*

To further establish the structural difference between the h27 and p27, limited papain proteolysis and gel electrophoresis were performed. The polypeptide patterns resulting from partial digestion show that h27 and p27 are different proteins (Fig. 2).

The gp120 and h27 were found in two leukemic cell lines (DU 1765 and DU 11157) and in all the Spafas leukemic myeloblasts examined, but not in uninfected or MAV-infected Spafas CEF. Therefore, these proteins are either AMV specific or myeloblast specific. To resolve this problem, we examined uninfected chicken hematopoietic cells. After 15 days of incubation, the spleen of chicken embryos is predominantly myeloid and the yolk sac is the major hematopoietic tissue (24). Immunoprecipitation of [^{35}S]methionine-labeled spleen or yolk sac cells with anti-gp85 serum showed that gp120 and h27 were present in these two tissues (Fig. 3). Therefore, these two molecules appear to be specific to myeloid or at least to hematopoietic cells. After electrophoresis, the gp120 band is broad and diffuse, as is typical of glycoproteins. Consequently, DU 1765 and DU 11157 cells were labeled with [^{3}H]glucosamine. Figure 4 shows

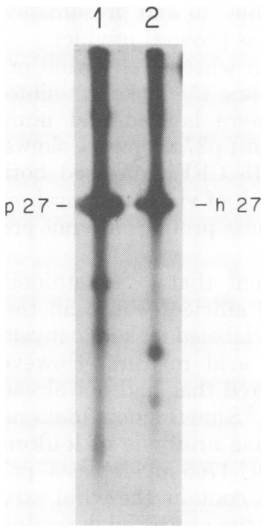

FIG. 2. *Peptide maps of p27 and h27. [^{35}S]methionine-labeled bands of p27 and h27 were cut from dried SDS gels and applied to a second SDS gel (15% acrylamide) in the presence of 0.005 μg of papain. The samples were electrophoresed and processed for fluorography as described in the text. Lane 1, p27; lane 2, h27.*

that gp120 was labeled with [^{3}H]glucosamine but that h27 was not.

Immunoprecipitation of [^{35}S]methionine-labeled cellular extracts with antisera monospecific against AMV structural proteins. As expected, all of the monospecific antisera directed against the *gag* structural proteins precipitated pr76 (Fig. 5). Some of the antisera showed the presence of contaminating antibodies against other *gag* proteins. Both the anti-p19 and anti-p15 sera precipitated p27 (Fig. 5A, lanes 2 and 4). In Fig. 5A (lane 2), the inability of anti-p19 to precipitate pr60 from 21957 leukemic cells is an anomalous result since this antiserum always precipitated pr60 in other experiments. The precipitation of pr60 by anti-p15 serum probably results from the presence of contaminating anti-p27 antibodies (Fig. 5A, lane 4).

A band of 100,000 daltons (p100) was precipitated from DU 1765 myeloblasts by all the anti-*gag* monospecific sera, indicating that it probably contains pr76. However, uninfected Spafas CEF, MAV-2-infected Spafas CEF, and leukemic myeloblasts from our Spafas chickens did not show the p100 band present in DU 1765 leukemic cells which originated in line 15 chickens (17). The p100 was present in the other established line (DU 11157) of leukemic myeloblasts, which also originated in line 15 chickens (18). Up to a 100-fold increase in antisera concentrations did not result in the immunoprecipitation of additional proteins.

To determine whether the p100 was virally transmissible, Spafas yolk sac cells were converted with supernatant fluid from DU 11157 leukemic cells which produce low quantities of AMV and subgroup B MAV. The in vitro-transformed myeloblasts were labeled with [^{35}S]methionine, lysed, and immunoprecipitated with hamster anti-SR-RSV serum. They showed a band pattern typical of all the Spafas leukemic myeloblasts we have analyzed, and different from that found in DU 1765 and DU 11157 cells (Fig. 6). The viral proteins p27, p19, p15, and p12 were present, as well as pr76 and pr60. There was a faint band of about 110,000 daltons, but the prominent p100 was missing. Therefore, it appears that the p100 is a protein specific to

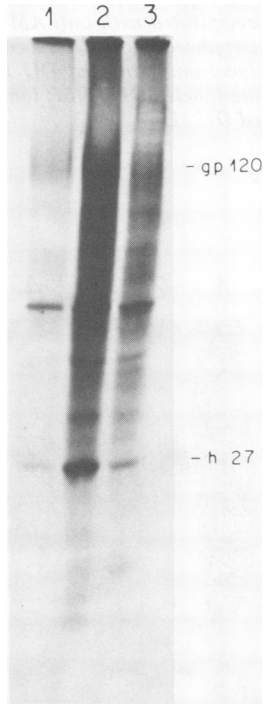

FIG. 3. *Presence of gp120 and h27 in normal hematopoietic cells. Yolk sac cultures from 15-day-old Spafas chicks were transformed in vitro with standard AMV. The leukemic myeloblasts were harvested and labeled with [^{35}S]methionine. Uninfected yolk sac cultures and spleen cells from 15-day-old embryos were also labeled with [^{35}S]methionine. The cells were lysed and immunoprecipitated with anti-AMV gp85 antiserum. The immunoprecipitates were separated on a 7.5 to 15% gradient polyacrylamide gel as previously described. Lane 1, Uninfected yolk sac; lane 2, leukemic myeloblasts; lane 3, uninfected spleen cells.*

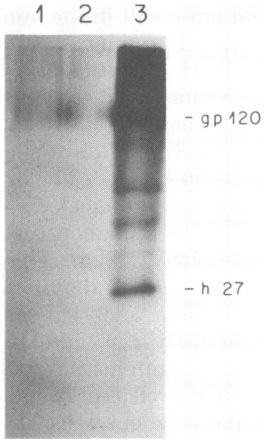

FIG. 4. *Incorporation of [^{3}H]glucosamine in gp120. DU 11157 cells were labeled with [^{35}S]methionine. DU 1765 and DU 11157 cells were labeled with [^{3}H]glucosamine (50 μCi/ml for 18 h). The cell lysates were immunoprecipitated with anti-AMV gp85 antiserum and electrophoresed as described in the text. Lane 1, [^{3}H]glucosamine-labeled DU 1765; lane 2, [^{3}H]glucosamine-labeled DU 11157; lane 3, [^{35}S]methionine-labeled DU 11157.*

chickens of line 15 and presumably represents an endogenous proviral product.

To confirm whether the p100 protein is endogenous in line 15 chickens, uninfected line 15 fibroblasts were labeled and immunoprecipitated with anti-p27. Figure 7 shows that uninfected line 15 CEF expressed both pr76 and p100. The lack of viral structural proteins indicates that these proteins are not processed further.

In the event that [^{35}S]methionine was not incorporated efficiently into all the viral gene products, we labeled leukemic myeloblasts with a ^{3}H-amino acid mixture. However, no new bands appeared that had not already been detected with [^{35}S]methionine (data not shown).

Pulse-chase analysis of leukemic myeloblasts. In DU 1765 myeloblasts, pr76 and p100 appeared to contain the viral structural *gag* proteins, yet the processed derivatives p27, p19, and p12/15 did not appear in the gels of the immunoprecipitates. This probably reflects either the very low release of infectious virus from these cells or the endogenous expression of these proteins by line 15 cells. To investigate

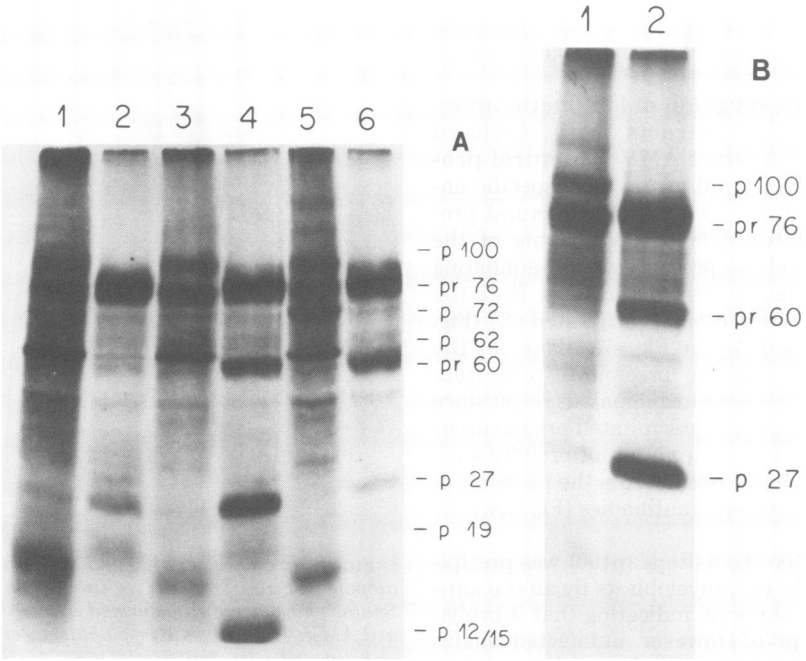

FIG. 5. *[^{35}S]methionine-labeled proteins from 21957 myeloblasts and DU 1765. Cells were labeled with [^{35}S]methionine, immunoprecipitated, and electrophoresed in a 7.5 to 15% gradient polyacrylamide gel as explained in the text. (A) Lanes 1, 3, and 5, [^{35}S]methionine-labeled DU 1765 cells; lanes 2, 4, and 6, [^{35}S]-methionine-labeled myeloblasts from bird no. 21957. For the immunoprecipitations, the following antisera were used: lanes 1 and 2, anti-p19; lanes 3 and 4, anti-p15; lanes 5 and 6, anti-p12. (B) Lane 1, [^{35}S]-methionine-labeled DU 1765 immunoprecipitated with anti-p27; lane 2, [^{35}S]methionine-labeled 21957 immunoprecipitated with anti-p27.*

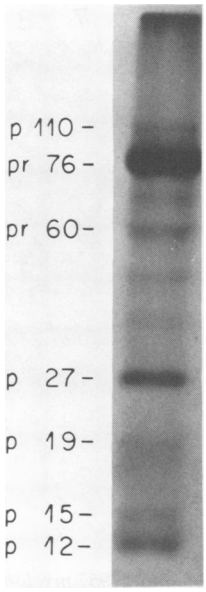

FIG. 6. *[^{35}S]methionine-labeled proteins from myeloblasts converted in vitro by infection of yolk sac cells with virus produced by DU 11157 myeloblasts. The supernatant fluid from a culture of DU 1765 cells was centrifuged for 10 min at 2,000 rpm in an International table-top centrifuge (model CL) and filtered through 0.45-μm filters (Millipore Corp.). Spafas yolk sac cells were infected with this supernatant. The resulting leukemic myeloblasts were harvested 2 weeks later, labeled with [^{35}S]methionine, lysed, and immunoprecipitated with hamster anti-SR-RSV antiserum. Electrophoresis and fluorography were done as described in the text.*

this phenomenon, leukemic myeloblasts were labeled with [^{35}S]methionine for 10 min, chased with cold medium for various time intervals, and immunoprecipitated with anti-p27 serum. The immunoprecipitates were analyzed on polyacrylamide gels (Fig. 8). The amount of radioactivity in both p100 and pr76 declined during the chase period, but the fate of these proteins remains unknown because the radioactivity did not chase into any other protein (Fig. 8, lanes 1 to 4). It is clear, however, that p100 does not chase into pr76, nor vice versa. By contrast, in the high-virus-producing 21957 myeloblasts, radioactivity was chased from pr76 into pr60 and p27 (Fig. 8, lanes 5 to 8). Because of the high initial amounts of radioactivity in the pr76, it is not possible to show a significant reduction of radioactivity in the pr76. This is consistent with published findings (14).

DU 1765 and 21957 Spafas leukemic myeloblasts pulse-chased with [^{35}S]methionine were also immunoprecipitated with anti-gp85 serum (Fig. 9). In both types of leukemic cells, a protein of approximately 100,000 daltons was first labeled, and, with increasing time intervals, the radioactivity appeared to chase into gp120 and into h27. In 21957 myeloblasts, the radioactivity also appeared to chase into a molecule of about 90,000.

DISCUSSION

The avian defective transforming retroviruses MC29, MH2, and AEV share the following characteristics: (i) a large genomic deletion and substitution, (ii) ability to induce neoplasias rapidly in chickens, (iii) transformation of target hematopoietic cells in vitro, (iv) transformation of CEF in vitro, (v) defectiveness for replication, and (vi) production of a large unprocessed protein which contains part of the *gag* structural proteins (4, 15, 16). AMV appears to be defective for replication and converts hematopoietic target cells in vitro, but differs from the MC29, AEV group of retroviruses in several major ways. The AMV DNA genome appears to be only slightly smaller (4.9×10^6 daltons) than the DNA genome (5.3×10^6 daltons) of its two natural helpers, MAV-1 and MAV-2 (2). AMV does not transform fibroblasts in vitro, and, as demonstrated herein, it does not produce a large

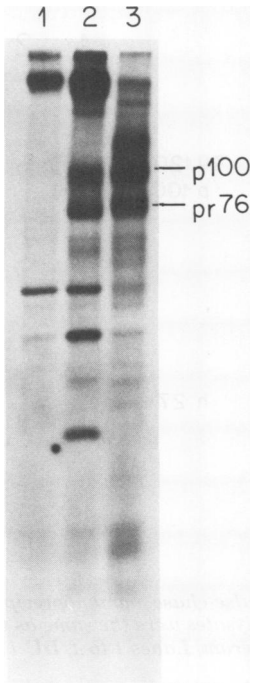

FIG. 7. *[^{35}S]methionine-labeled proteins in line 15 cells. Cells were labeled with [^{35}S]methionine, immunoprecipitated with anti-p27, and electrophoresed in a 7.5 to 15% gradient polyacrylamide gel. Lane 1, Uninfected Spafas fibroblasts; lane 2, uninfected line 15 fibroblasts; lane 3, DU 11157 myeloblasts.*

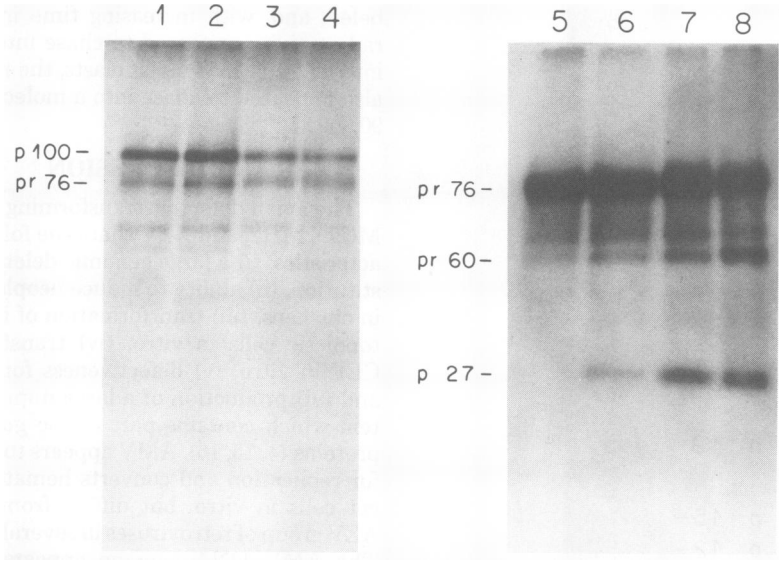

FIG. 8. *Pulse-chase immunoprecipitates with anti-p27 serum. DU 1765 and 21957 myeloblasts were pulse-labeled with [^{35}S]methionine for 10 min (100 μCi/10^7 cells per ml). The radioactive medium was removed and replaced with unlabeled medium. At the completion of the chase times, 5 ml of cold cell wash (150 mM NaCl, 10 mM Tris-hydrochloride, pH 7.2, 1 mM EDTA) was added. The cells were centrifuged, lysed, immunoprecipitated with anti-p27 antiserum, and run on a 7.5 to 15% gradient polyacrylamide gel as described in the text. Chase times: Lanes 1 and 5, 0 min; lanes 2 and 6, 10 min; lanes 3 and 7, 45 min; lanes 4 and 8, 90 min. Lanes 1 to 4, DU 1765 myeloblasts; lanes 5 to 8, 21957 myeloblasts.*

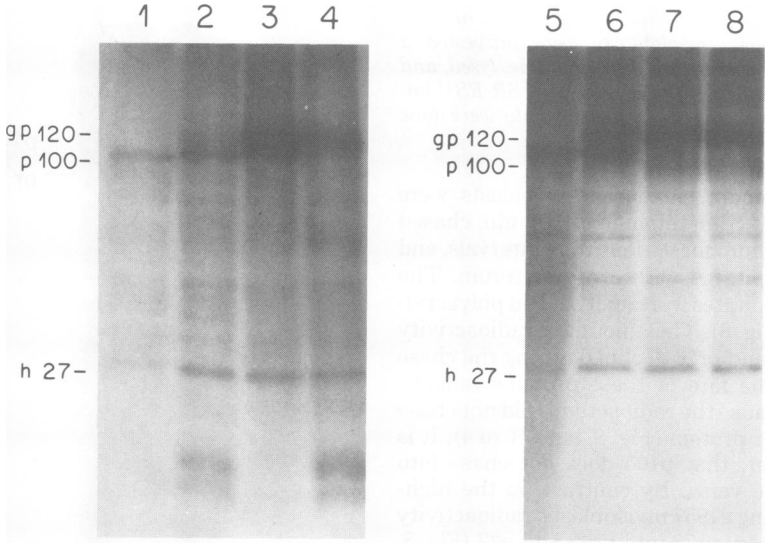

FIG. 9. *Pulse-chase immunoprecipitates with anti-AMV gp85 serum. The [^{35}S]methionine pulse-chase-labeled cell lysates were the same as those used in Fig. 7, but the immunoprecipitates were formed with anti-AMV gp85 serum. Lanes 1 to 4, DU 1765 myeloblasts; lanes 5 to 8, 21957 leukemic myeloblasts.*

aberrant protein that contains *gag*-related structural proteins.

We have attempted to identify specific AMV proteins in leukemic myeloblasts by using immunoprecipitation by various viral antisera. Two types of leukemic myeloblasts were analyzed: established myeloblastic cell lines DU 1765 and DU 11157, and leukemic myeloblasts obtained from the peripheral blood of a leukemic C/E Spafas chicken (no. 21957). DU 1765 myeloblasts produced low levels of subgroup B MAV only, and DU 11157 cells produced low levels of

both a subgroup B MAV and AMV. The 21957 cells produced both AMV and helper viruses. Several proteins immunoprecipitated by AMV antisera deserved further analysis. The anti-AMV gp85 serum precipitated a glycoprotein of about 120,000 daltons from all the myeloblasts tested. However, this glycoprotein was also present in low concentration in uninfected chicken embryonic spleen and yolk sac cells and may be identical to the gp125 found by Collins and coworkers in normal chicken cells (10). Bosch and his co-workers also reported finding in uninfected chicken cells a 135,000-dalton glycoprotein related to the viral gp85 (7). A second protein, h27, was also immunoprecipitated by anti-AMV gp85 serum from leukemic myeloblasts but not from uninfected or MAV-infected CEF. This h27 was also detected at a lower concentration in normal chicken embryonic spleen and yolk sac cells. Peptide mapping revealed that h27 is not related to the viral structural protein p27. Pulse-chase experiments also indicated that the gp120 is neither a precursor nor a derivative of the h27 protein. Instead, it appears that a 100,000-dalton molecule may be the precursor of the gp120.

Proteins immunoprecipitated from the established lines of leukemic myeloblasts originating from line 15 chickens differed in several ways from proteins of peripheral blood leukemic myeloblasts generated in this laboratory. Both DU 1765 and DU 11157 cells contained a p100 protein which possessed antigenic determinants of the viral proteins p27, p19, p15, and p12 but was absent in myeloblasts from our Spafas chickens. However, pulse-chase experiments indicated that the p100 was not a precursor of the viral structural proteins. The p100 immunoprecipitated by anti-p27 serum appears to be related to or identical with the p120 reported by Eisenman to represent endogenous provirus expression (11). This was further substantiated by the absence of p100 in Spafas yolk sac myeloblasts converted with virus produced by DU 11157 cells and the presence of p100 in line 15 CEF. This indicates that the p100 in DU 1765 and DU 11157 cells is a product of line 15 chicken cells and does not have an AMV origin. Immunoprecipitation by anti-SR-RSV or anti-AMV gp85 sera also revealed a p62 and three proteins between p19 and p15 in DU 1765 cells. These proteins were never observed in our 21957 or other leukemic myeloblasts nor in our CEF. Thus they appear to be of host origin, peculiar to the DU 1765 cells. The DU 1765 cells showed another unique characteristic as compared to recently induced Spafas leukemic myeloblasts, such as 21957: the pr76 of DU 1765 migrated slightly further in gels than the pr76 of Spafas-derived leukemic myeloblasts.

If AMV were analogous to the other acute avian retroviruses, an aberrant protein containing some of the *gag* proteins should have been present in leukemic cells. In addition, R-loop and Southern blot analyses with a λ-proviral hybrid containing the AMV provirus have indicated that AMV contains a cellular substitution in the region normally occupied by the envelope gene. This substitution does not appear to extend into the *gag* gene (L. M. Souza, J. N. Strommer, R. L. Hillyard, M. C. Komarumy, and M. A., Baluda, Proc. Natl. Acad. Sci. U.S.A., in press). Therefore, if there is an AMV gene product responsible for leukemogenesis, either it is antigenically unrelated to the viral *gag* proteins or it migrates with other viral gene products in polyacrylamide gels. Also, when using antisera from chickens and rabbits immunized with DU 1765 cells, no other AMV-specific protein could be detected. AMV could therefore resemble the defective BH-RSV in genomic structure expression. If the AMV *luk* gene product is analogous to the RSV *src* protein, then only animals that are leukemic or have had spontaneous leukemia regression may contain the appropriate antibodies for its detection (8, 26).

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