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Viral Proteins Expressed on the Surface of Murine Leukemia Cells

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Leukemic cells of AKR mice contain as constituents of their membranes the murine leukemia virus envelope protein gp70 and the precursor polyprotein of the viral internal (core) structural proteins. Both gp70 and the core polyprotein are represented on the cell surface as glycoproteins, as evidenced by incorporation of [^{3}H]glucosamine into their structure and the binding of these proteins to lectins. The glycosylated core polyprotein exists in at least two serologically distinguishable forms: the 95,000-dalton polyprotein reacts with antisera prepared against the viral proteins p30, p12, and p10, whereas the 85,000-dalton polyprotein reacts with antisera prepared against the viral proteins p30 and p12, but not p10. Additional heterogeneity in these cell surface polyproteins has been observed with leukemias induced by exogenous leukemia viruses. Spontaneous leukemia cells of AKR mice invariably express gp70 and the core polyprotein on their cell surface; normal thymocytes of young AKR mice express gp70, but not the core polyprotein on their surface.

The presence of antigens associated with the structural proteins gp70 and p30 of murine leukemia virus (MuLV) on the surface of murine cells has been demonstrated by a wide range of serological (3, 6, 16, 23, 28) and biochemical methods (3, 23, 23a). These antigens appear on the membranes of cells infected with exogenous MuLV (3), as well as on the membranes of cells that are expressing endogenous MuLV genomes (22, 23). The surface localization of these proteins on cells is of considerable biological relevance since, in addition to the functions that these proteins have in the viral replicative cycle, they provide targets for a host immune response (15, 19).

Production of the gp70 protein of endogenous MuLV is under complex genetic control (21): although these proteins are invariably produced in cells that overtly synthesize virions, they may also be produced in certain cells in which viral synthesis is not manifested (2, 21). In the G_{IX} gp70 antigenic system, the expression of a defined subset of gp70 (G_{IX}^+) molecules on the cell surface requires the action of two independent genetic loci (21). Normal lymphoid and leukemic cells of mice from high leukemic strains (AKR and C58) contain G_{IX} gp70 on their membranes; however, the normal lymphoid cells of mice from certain low leukemic strains (129 and CE) also express G_{IX} gp70 on their membrane (21). Furthermore, the expression of G_{IX} gp70 is related to the differentiated state of the cell, since in mice of low leukemic strains this viral antigen appears as a thymocyte-specific alloantigen, whereas in mice of high leukemic strains it is present on a variety of cell types (21).

Antigens associated with the p30 viral core protein have been identified by immunofluorescence (26) and cytotoxicity tests (6) on the surface of murine cells. In one of these studies (6) it was thought that the p30 antigenicity was the result of nonspecific adsorption of the viral core proteins (from extracellular fluids) to the cell surface; however, recent biochemical studies (23a) now indicate that these p30-associated cell surface antigens are the precursor polyprotein (1, 25) of the viral internal (core) structural proteins expressed on the cell surface.

In this report we describe an analysis of viral proteins that are expressed on the surface of murine leukemia cells. By the means of [^{125}I]lactoperoxidase surface labeling, in conjunction with immune precipitation techniques, we have identified two distinct classes of viral antigens that are invariably present on the cell membranes of AKR spontaneous leukemia cells. One class of these antigens is the viral gp70 envelope proteins, whereas the other class is a glycosylated form of the precursor polyprotein of the viral internal structural proteins. We also describe here that the glycosyl-

ated polyproteins exist in at least two distinct forms: a 95,000-dalton species contains antigens associated with the viral proteins p30, p12, and p10, whereas an 85,000-dalton species contains antigens associated with the viral proteins p30 and p12, but not p10. Additional heterogeneity in cell surface polyproteins is observed with leukemias induced by exogenous leukemia viruses.

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MATERIALS AND METHODS

Antisera. Goat antisera prepared against purified proteins gp70 (lot 5S-167; radioimmunoassay [RIA] titer, 6,400), p30 (lot 5S-123; RIA titer, 50,000), and p12 (lot 5S-037; RIA titer, 18,000) of Rauscher MuLV and p10 (lot 5S-465; RIA titer, 150) of AKR MuLV were obtained from the National Cancer Institute, courtesy of R. Wilsnack. An additional goat antiserum prepared against the p15 protein of AKR MuLV was a kind gift of E. Fleissner (Sloan-Kettering Institute, New York).

In preliminary studies these antisera were examined for their range of reactions with Nonidet P-40 (NP-40)-solubilized proteins of [^{3}H]leucine-labeled AKR virus. These results (Fig. 1) can be summarized as follows for each antiserum and its range of specificities: goat anti-p30, monospecific for p30 protein; goat anti-p15, monospecific for p15 protein; goat anti-p12, coprecipitates p12 and p10 proteins; goat anti-gp70, coprecipitates gp70 and p15(E) proteins.

The goat anti-p10 serum did not demonstrably precipitate p10 protein from [^{3}H]leucine-labeled MuLV; this was presumed to be a result of the low sensitivity of this assay, since anti-p10 serum was reactive with purified ^{125}I-labeled p10 in RIA (R. Wilsnack, personal communication), although only at low serum dilutions.

Cells. Thymoma cells from individual AKR/J mice with primary spontaneous leukemias (SL) were designated by a letter associated with the "SL" abbreviation (AKR SLA, AKR SLB, AKR SLC, etc.). AKR SL cells that were established as cell lines in tissue culture were maintained in RPMI 1640 medium containing 10% fetal calf serum (FCS). Transplanted leukemia cells used in these studies included: AKR K36, derived from an AKR SL; RBL-5, a C57BL/6 leukemia induced by Rauscher MuLV; and E♂G2, a C57BL/6 leukemia induced by Gross passage A virus.

Radioactive labeling of cells and viruses. Leukemic cells from spontaneous thymomas were suspended in phosphate-buffered saline (PBS) by gentle teasing and pipetting. Radiolabeling of cell membranes by the [^{125}I]lactoperoxidase method was performed by the method of Vitetta et al. (24). Briefly, 5 × 10^7 viable cells were suspended in 1 ml of PBS containing 100 μg of lactoperoxidase and 3 mCi of ^{125}I; this reaction mixture was activated by the addition of two pulses of 0.06% H_2O_2 at 5-min intervals. The entire labeling procedure was performed on ice with continuous shaking; the viability of cell suspensions (determined by trypan blue exclusion) before and after radiolabeling was >95%. The labeled cells were then disrupted with gentle vortexing in 0.5% NP-40 for 30 min at 4°C; nonsolubilized cellular structures were removed by centrifugation at 100,000 × *g* for 45 min. The supernatant containing the solubilized membrane proteins was frozen at −20°C in small portions for future analysis.

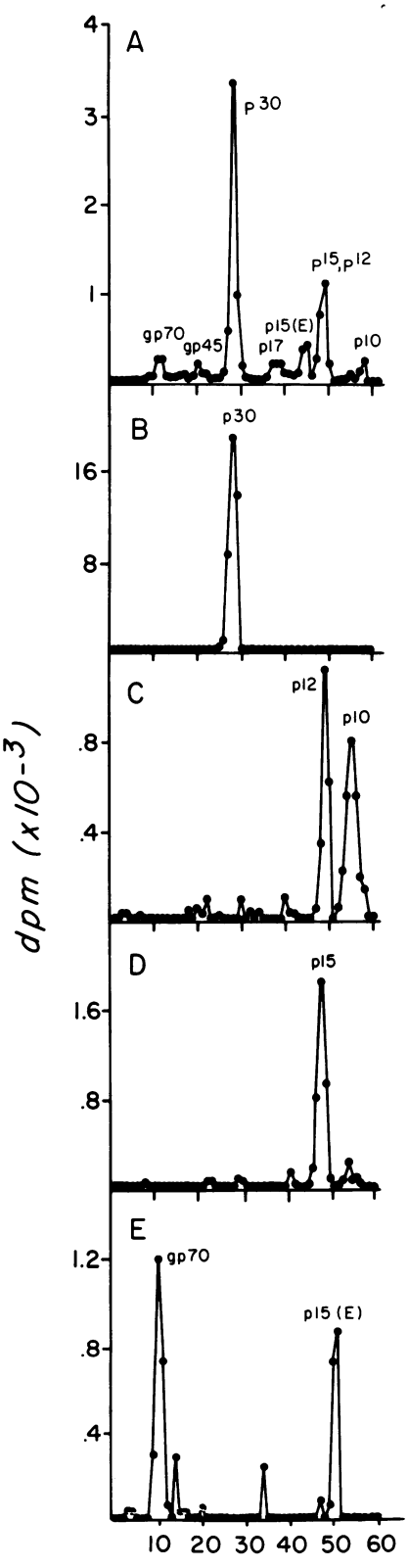
Leukemic cells were radiolabeled with [^{3}H]glucosamine by growth for 12 h in RPMI 1640 medium containing 5 μCi of [^{3}H]glucosamine and 10% FCS per ml.

Proteins of AKR MuLV were radiolabeled by the growth of an AKR mouse embryo fibroblast cell line (which was continuously producing MuLV) overnight in Eagle minimum essential medium containing either [^{3}H]leucine (30 μCi/ml) or [^{14}C]leucine (5 μCi/ml) and 10% FCS. Radiolabeled viruses were isolated by sucrose density gradient centrifugation and then disrupted in 0.5% NP-40 in the same manner as described for the cell membranes.

Immune precipitations. Immune precipitation reactions generally contained 2 μl of antiserum and 50 μl of radiolabeled cell lysate (10^6 cpm) in a total volume of 250 μl of PBS containing 0.5% NP-40; these conditions varied slightly according to the titer of a particular antiserum and the specific activity of labeling of the membrane preparation. After a 1-h incubation on ice, 200 μl of undiluted antiglobulin (rabbit anti-goat 7*S* gamma globulin, Cappel Laboratories) was added to precipitate the immune complexes. After an additional 2 h of incubation on ice, the precipitates were collected by centrifugation (1,000 × *g* for 20 min) through a cushion of 5% sucrose (in PBS) containing 3% NP-40, followed by three additional washes in PBS with 0.5% NP-40. Centrifugation over the sucrose cushion was particularly effective in the prevention of nonspecific sticking of radiolabeled proteins to the immune precipitates. Precipitates were dissolved at 100°C for 2 min in sample buffer (3% sodium dodecyl sulfate [SDS], 5% 2-mercaptoethanol, and 10% glycerol in 0.06 M Tris-hydrochloride [pH 6.8]) and used for polyacrylamide gel electrophoresis.

SDS-PAGE. Polyacrylamide gel electrophoresis (PAGE) was performed by the method of Laemmli (10) in cylindrical gels (12 cm in length); gels were run at 0.55 mA/tube for 17 h. PAGE of viral proteins was performed in 12.5% gels, whereas PAGE of cellular extracts was performed in 7.5% gels. Samples containing ^{125}I-radiolabeled proteins were coelectrophoresed with ^{131}I-radiolabeled protein markers (phosphorylase a, 94,000 daltons; bovine serum albumin, 68,000 daltons; and the heavy chain of rabbit immunoglobulin, 50,000 daltons). Samples containing ^{3}H-labeled MuLV proteins were coelectrophoresed with ^{14}C-labeled MuLV proteins as markers.

Affinity chromatography. Immunoadsorbant columns were prepared by the covalent linkage of goat antiserum to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Inc.). Lectin columns were prepared by the covalent linkage of ricin



communis (RCA_I) lectin (kindly provided by S. Hakomori, Fred Hutchinson Cancer Research Center) to cyanogen bromide-activated Sepharose 4B. Radiolabeled cell membrane proteins (0.5-ml volume) were chromatographed on 3 ml of immunoadsorbant or lectin columns in PBS containing 0.5% NP-40. In the case of immunoadsorbant columns, proteins that adhered to the column were eluted by 3 M thiocyanate; this eluted fraction was then dialyzed overnight in PBS containing 0.5% NP-40. In the case of lectin columns, bound proteins were eluted by 0.3 M lactose in PBS and 0.5% NP-40.

RESULTS

Detection of viral structural proteins on the surface of leukemia cells. Reactions of anti-MuLV antisera with ^{125}I-labeled membrane proteins of AKR SL are shown in Fig. 2. Four classes of reactions could be distinguished as follows. (i) The anti-p30 and anti-p12 sera, although reacting only with lower-molecular-weight proteins of the virus (see Fig. 1), detected high-molecular-weight proteins (85,000 to 95,000) on the cell surface of AKR SL. No reactions were observed with proteins in the molecular-weight region of 30,000. This pattern of reaction was observed with 12 different primary leukemias. With some leukemias only a single protein of 85,000 was detected, whereas with other leukemias, additional high-molecular-weight (95,000 to 120,000) proteins were also detected. The reactions of anti-p30 and anti-p12 sera with the cell surface proteins were invariably correlated, such that when the anti-p30 serum detected one or multiple proteins, the anti-p12 serum reacted similarly. The anti-p12 serum was slightly less efficient than the anti-p30 serum in the precipitation of these antigens. (ii) The anti-p10 antiserum precipitated a single protein of 95,000 daltons from the cell membrane of certain leukemias (Fig. 3). The presence of this 95,000-dalton antigen on leukemia cells was correlated with the occurrence of an antigen of similar molecular weight that re-

FIG. 1. *PAGE of radioimmune precipitates prepared with goat anti-MuLV antisera and [^{3}H]leucine (NP-40-disrupted) AKR MuLV. (A) Control AKR virus; (B) anti-p30 serum; (C) anti-p12 serum; (D) anti-p15 serum; (E) anti-gp70 serum. PAGE results in (A) to (D) were performed in one experiment; results presented in (E) were from a gel prepared from a separate experiment. PAGE of reactions in (B) to (E) was coelectrophoresed with [^{14}C]leucine AKR MuLV for identification of the precipitated peaks; the ^{14}C data are not graphed in this figure for simplification. Viral proteins p12 and p15 comigrate in the gel system employed for this experiment. In experiments utilizing other isolates of MuLV, it was possible to serologically distinguish the p12 and p15 reactions.*

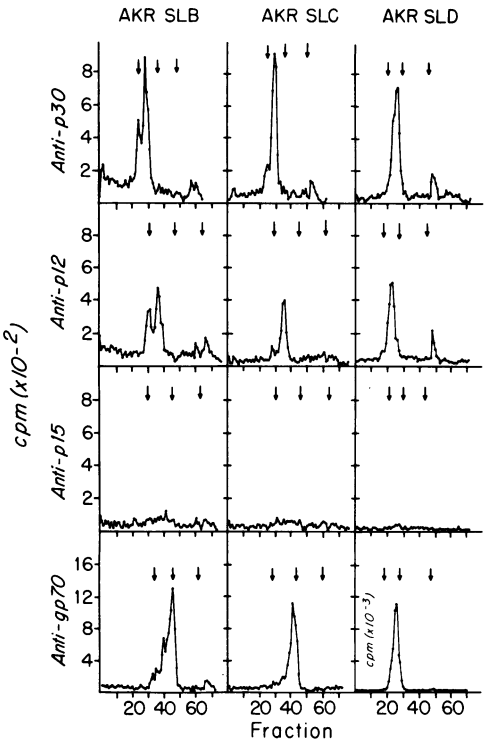


FIG. 2. *RIP of viral antigens from the cell surface of murine leukemic cells. Leukemias AKR SLB, AKR SLC, and AKR SLD were surface labeled by the [^{125}I]lactoperoxidase method. The leukemic cells were then lysed in NP-40 and used for RIP reactions with goat anti-MuLV antisera. Each RIP reaction contained 10^6 cpm of cell surface extract and 2 μl of antiserum in a total volume on 200 μl of PBS (containing 0.5% NP-40). Immune complexes were precipitated by the addition of 200 μl of undiluted rabbit anti-goat IgG, washed four times, and then solubilized in SDS-sample buffer for analysis by PAGE. ^{131}I-labeled markers (phosphorylase a, 94,000 daltons; bovine serum albumin, 68,000 daltons; and immunoglobulin G heavy chain, 50,000 daltons) were included in these gels; the positions of the marker proteins are indicated by the arrows, with the phosphorylase a marker migrating the furthest to the left of the three markers.*

acted with anti-p30 and anti-p12 sera. Anti-p10 serum did not react with cells unless they contained the 95,000-dalton polyprotein. We have concluded that the 95,000-dalton protein contains antigens associated with the viral proteins p30, p12, and p10, whereas the 85,000-dalton protein contains antigens associated with p30 and p12, but not p10. (iii) Anti-p15 serum did not precipitate proteins from most of the AKR cell lysates; with only one of eight AKR cell extracts did we observe (data not shown) the precipitation of an 85,000-dalton protein by the anti-p15 serum. (iv) Reaction of AKR leukemic cell surface antigen preparations with anti-gp70 serum resulted in the precipitation of a single protein, with a molecular weight that varied slightly (68,000 to 75,000) from one cell extract to another (Fig. 2). The protein precipitated by the anti-gp70 serum contained approximately 10 times more ^{125}I radiolabel than did the protein precipitated by the anti-p30 serum.

In specificity controls, antisera prepared against p30 (Fig. 4), p15, p12, and p10 did not react with ^{125}I-labeled cell membrane preparations of normal AKR thymus cells, although the anti-gp70 serum reacted with a single radiolabeled protein of 70,000 daltons in this same antigen preparation.

Reactions of anti-MuLV antisera with ^{125}I-labeled membrane proteins of three long transplanted murine leukemias are shown in Fig. 5. The AKR K36 and C57BL/6 E♂G2 leukemias (both induced by Gross virus) contained viral proteins on their cell surfaces that were analogous to those observed on the surface of AKR SL cells; precipitation of high-molecular-weight antigens (85,000 to 95,000) was observed with anti-p12 and anti-p30 sera, but not with anti-p15 serum. The reaction of cell surface antigen preparations from AKR K36 and C57BL/6 E♂G2 with anti-gp70 serum resulted in the

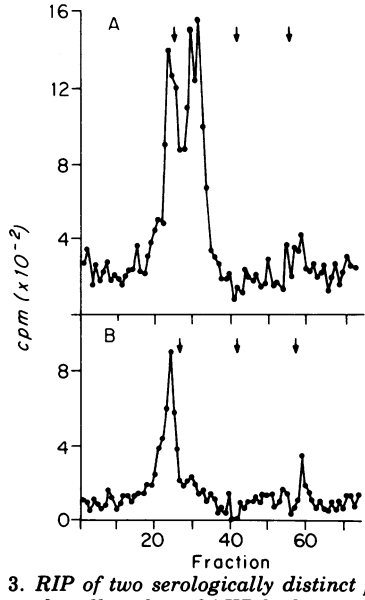


FIG. 3. *RIP of two serologically distinct polyproteins from the cell surface of AKR leukemic cells. ^{125}I-labeled antigen preparations from the AKR SLG leukemia were reacted with antisera prepared against p30 (A) and p10 (B) viral proteins. The migration positions of ^{131}I-labeled molecular-weight markers (see legend to Fig. 2) are indicated by the arrows in each panel.*

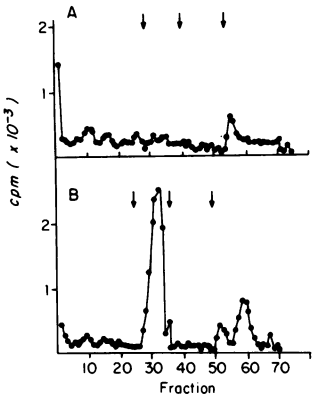


FIG. 4. *RIP of viral antigens from the cell surface of AKR normal thymus cells. ^{125}I-labeled antigen preparations from AKR thymus cells (of an 8-week-old mouse) were reacted with antisera prepared against p30 (A) and gp70 (B). The migration positions of ^{131}I-labeled molecular-weight markers (see the legend to Fig. 2) are indicated by arrows in each panel.*

precipitation of a single protein of approximately 70,000 daltons. An analysis of these same antisera with an ^{125}I-radiolabeled extract prepared from the Rauscher virus-induced C57BL/6 leukemia RLB-5 demonstrated a different pattern of reaction (Fig. 5). Thus, antisera prepared against the MuLV proteins p30 and p12 reacted minimally with this cell line, although an antiserum prepared against p15 reacted with a high-molecular-weight protein of 81,000. This reaction with anti-p15 serum identified an antigen on RBL-5 that differed serologically from the antigen found on the surface of AKR leukemias. As found with other leukemias, the reaction of the cell surface antigen preparation from RLB-5 with anti-gp70 serum resulted in the precipitation of a single protein (75,000 daltons).

Also shown in Fig. 2 through 8 is the precipitation of a 45,000-dalton protein from the surface of murine cells by a variety of antisera. The precipitation of this protein was highly variable from one experiment to another; furthermore, incubation of the radioimmune precipitation (RIP) reactions at 37°C (instead of 4°C) resulted in a large increase in the amount of this material precipitated. This increased precipitation of the 45,000-dalton protein at elevated temperatures was independent of the precipitation of viral proteins; thus, similar amounts of viral proteins were precipitated at 4 and 37°C. On the basis of these findings we have tentatively concluded that the 45,000-dalton protein is actin, which is known to adhere nonspecifically to antigen-antibody complexes (5). In subsequent studies in which we utilized *Staphylococcus aureus* for the collection of immune complexes, we observed a dramatic decrease in the quantity of the 45,000-dalton protein precipitated.

Detection of antigens associated with p30 and p12 proteins on a single polyprotein. The precipitation of an 85,000-dalton protein from the surface of AKR leukemia cells by the anti-p30 and anti-p12 sera suggested that this high-molecular-weight protein might represent the noncleaved polyprotein precursor of the viral core proteins. To demonstrate definitively the presence of p30 and p12 antigenic sites on a single polypeptide, we utilized immunoadsorbant columns to remove selected antigenic specificities from the AKR cell membrane extracts.

Immunoadsorbant columns were prepared by the covalent linkage of goat anti-p30 serum to cyanogen bromide-activated Sepharose 4B. Cell membrane preparations were chromatographed through these columns and then examined for residual antigenic activity with se-

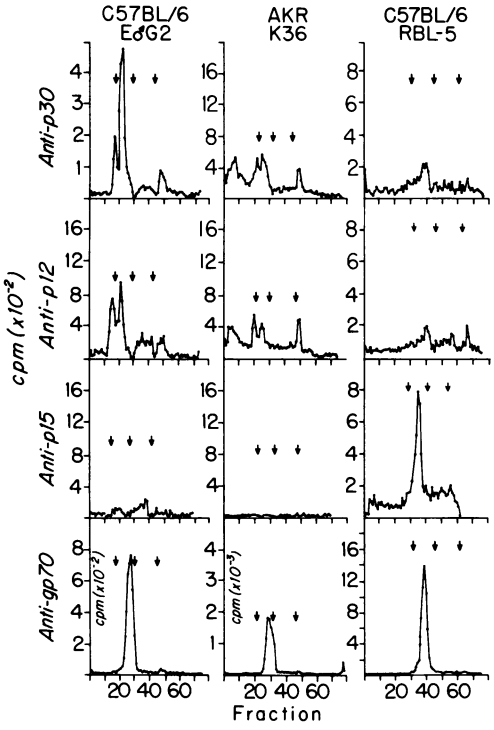


FIG. 5. *RIP of viral antigens from the cell surface of transplanted murine leukemic cells. ^{125}I-labeled antigen preparations from the AKR K36, C57BL/6 E δG2, and C57BL/6 RBL-5 leukemias were reacted with antisera prepared against p30, p15, p12, and gp70 viral proteins. The migration position of ^{131}I-labeled molecular-weight markers (see the legend to Fig. 2) are indicated by the arrows in each panel.*

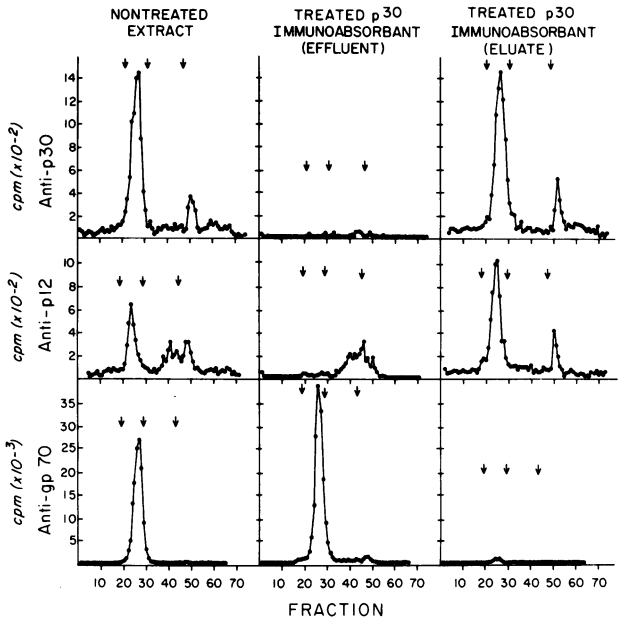


FIG. 6. *Analysis of the cell surface antigens of AKR leukemia cells by the use of immunoadsorbants. A ^{125}I-labeled cell extract from AKR SLF was chromatographed through an anti-p30 immunoadsorbant column (goat anti-p30 serum covalently complexed to cyanogen bromide-activated Sepharose 4B) and then tested by RIP for residual viral antigens. Proteins bound to the immunoadsorbant were eluted by thiocyanate and tested by RIP for viral antigens. ^{131}I-labeled molecular-weight markers (see the legend to Fig. 2) were included with each RIP reaction and are indicated by the arrows in each panel.*

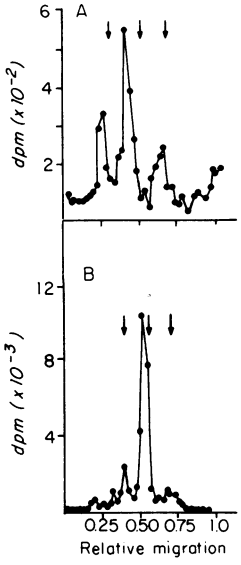


FIG. 7. *RIP of glycoproteins from the surface of leukemia cells. Antigen preparations from [^{3}H]glucosamine-labeled AKR SLF cells were used in immune precipitation assays with antisera prepared against p30 (A) and gp70 (B) viral proteins. ^{131}I-labeled molecular-weight markers (see legend to Fig. 2) were included with each RIP reaction and are indicated by the arrows in each panel.*

lected antisera; proteins bound to the immunoadsorbant columns were eluted by thiocyanate and examined for virus-related antigens by immune precipitation. The results of these studies with a cell extract from an AKR SL are shown in Fig. 6.

The original AKR cell extract contained an 85,000-dalton antigen that reacted with the anti-p30 and anti-p12 sera (Fig. 6). This cell extract also contained a 72,000-dalton antigen that reacted with the anti-gp70 serum. After passage through the p30 immunoadsorbant, the cell extract no longer contained the 85,000-dalton protein that reacted with either the anti-p30 or anti-p12 sera; however, this same extract still reacted with the anti-gp70 serum. Elution of the immunoadsorbant with thiocyanate yielded an 85,000-dalton protein that was immunologically reactive with both the anti-p30 and anti-p12 sera. This same eluted preparation was not reactive with either the anti-p15 or anti-gp70 sera.

We conclude from these studies that the 85,000-dalton antigen is a polyprotein that contains antigenic specificities of both the p30 and p12 proteins of MuLV.

Glycoprotein nature of the cell surface polyprotein. Immune precipitations of cell extracts prepared from [^{3}H]glucosamine-labeled

AKR leukemia cells are shown in Fig. 7. Anti-p30 and anti-p12 sera (but not anti-p15 serum) precipitated glucosamine-containing proteins (of 85,000 and 95,000 daltons) from the cell extract. Anti-gp70 serum precipitated a glucosamine-containing protein of 72,000 daltons.

The glycoprotein nature of the polyprotein also was confirmed by lectin binding studies. Lectin columns were prepared by the covalent linkage of ricin communis (RCA_I) lectin to cyanogen bromide-activated Sepharose 4B. A ^{125}I-labeled cell membrane preparation from an AKR leukemia was chromatographed through a lectin column and then examined for residual activity with anti-p30 and anti-gp70 sera (Fig. 8). Glycoproteins bound to the lectin column were eluted in 0.4 M lactose and were also examined by immune precipitation for p30- and gp70-associated antigens (Fig. 8).

Prior to passage through the lectin column, the cell extract contained antigens that reacted with the anti-p30 (85,000 daltons) and anti-gp70 (72,000 daltons) sera. After passage through the column, the cell extract no longer reacted with either the anti-p30 or anti-gp70 serum. Elution of the lectin column with 0.3 M lactose yielded antigens of 85,000 and 72,000 daltons that reacted with anti-p30 and anti-gp70 sera, respectively.

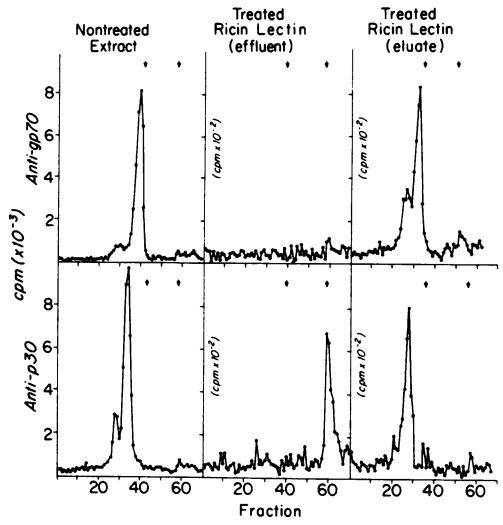


FIG. 8. *Analysis of the cell surface antigens of AKR leukemia cells by the use of lectins. A ^{125}I-labeled antigen preparation from the AKR SLF leukemia was chromatographed through a lectin column (ricin communis [RCA_I] lectin covalently complexed to cyanogen bromide-activated Sepharose 4B) and then tested by RIP for residual viral antigens. Proteins bound to the lectin column were eluted with 0.4 M lactose and tested by RIP for viral antigens. The migration positions of ^{131}I-labeled molecular-weight markers (bovine serum albumin, 68,000; heavy-chain immunoglobulin G, 50,000) are indicated by the arrows in each panel.*

DISCUSSION

Antisera prepared against MuLV identify on the surface of murine leukemia cells the viral envelope protein gp70 and a polyprotein of the viral core proteins. The evidence that these antigens are located on the cell surface includes the following: (i) these proteins are radiolabeled by the [^{125}I]lactoperoxidase method, whereas p30, the major intracellular core protein, is not labeled by the same procedure {In other studies, we have found that immune precipitates formed between anti-p30 serum and NP-40 lysates of metabolically labeled ([^3H]leucine) AKR SL cells contain predominantly the p30 and Pr65 proteins. The 85,000- and 95,000-dalton glycosylated polyproteins comprise only a minor part of the cellular proteins that react with anti-p30 serum.}; and (ii) these proteins can be visually identified on the surface of cells by immunofluorescence tests with viable leukemia cells (unpublished data). Furthermore, we have found that these proteins are glycoproteins, as shown by their glucosamine content and their binding to lectins. These findings confirm those of Tung et al. (23a) in a similar cell system.

The identity of these viral antigens has been determined by immunoprecipitation with antisera that were prepared against isolated viral proteins. In the case of the viral envelope antigen, antisera prepared against gp70 precipitated a single protein from the cell surface, which varied in molecular weight from 69,000 to 75,000, depending upon the cell used as the source of antigen. On the other hand, the presence of the core polyprotein on the cell surface was demonstrated by the reaction of high-molecular-weight membrane proteins (85,000 and 95,000) with antisera that were prepared against serologically unrelated (low-molecular-weight) virion core proteins. The precise molecular weights of these polyproteins is still open to question since the carbohydrate moieties of these proteins may influence their electrophoretic behavior in SDS-PAGE.

On the basis of serological data we have concluded that a 95,000-dalton polyprotein on the cell surface contains antigenic determinants of the MuLV proteins p30, p12, and p10, whereas an 85,000-dalton polyprotein contains antigenic determinants of p30 and p12, but not p10. Although it is tempting to speculate that the 85,000-dalton polyprotein is a cleavage product (minus p10) of the 95,000-dalton polyprotein,

definitive conclusions will require peptide mapping.

In precipitation reactions with membrane antigens of most AKR leukemias, we have not observed precipitation of the 85,000- or 95,000-dalton polyproteins with antiserum prepared against p15. These results differ from those of Tung et al. (23a), who observed the low-level precipitation of the glycosylated polyprotein by anti-p15 serum. Differences in our findings may reflect the conditions used for the preparation of cell extracts and RIP assays; in particular, assays performed by Tung et al. were conducted at 37°C, whereas our analysis was performed at 4°C. The failure to obtain reactions with anti-p15 serum may reflect conformational states of the polyprotein in NP-40 rather than the absolute peptide composition of the molecule.

In most cases the glycosylated core polyprotein was found on the surface of AKR leukemias as a single, 85,000-dalton species. In some AKR leukemias, additional higher-molecular-weight species (95,000 to 120,000) were identified. Heterogeneity of the core polyprotein was also observed in leukemias induced by exogenous leukemia viruses. Thus, we found that a leukemia (RBL-5) induced by Rauscher MuLV contained a polyprotein that reacted strongly with anti-p15 serum, but weakly with anti-p30 and anti-p12 sera. The heterogeneity of the glycosylated polyproteins on the cell surface may reflect: (i) differences in the peptide composition of the polyproteins (possibly due to different degrees of proteolytic cleavage), (ii) differences in the conformational states of the polyproteins (as a reflection of minor sequence differences), and (iii) differences in the glycosylation of the individual polyproteins.

In recent studies, Smart and Hogg (20) described the occurrence of several unique glycoproteins on the surface of cells infected with MuLV and MSV (MuLV). These proteins, identified by lectin chromatography, migrated in SDS-PAGE, with apparent molecular weights of 70,000 (corresponding to gp70), 85,000, and 95,000. Although these authors did not attempt a serological identification of these proteins, it is likely from our studies that the 85,000- and 95,000-dalton proteins represent the glycosylated core polyproteins. In contrast to the apparent similarity of these findings is the observation by Smart and Hogg (20) that the 85,000- and 95,000-dalton proteins did not bind to a ricin lectin, whereas in our studies these proteins did bind to ricin. This may be explained by differences in the cell lines examined, since the carbohydrate content of glycoproteins may vary according to the cell of origin.

The function that the viral envelope protein and core polyproteins play on the cell surface is open to conjecture. Presumably, the envelope protein gp70 is localized at the membrane for incorporation into progency virus (3); however, this protein also may occur on the cell surface at sites that are not associated with budding virions (2, 23). Pulse-chase studies of MuLV-infected cells indicate that the viral envelope proteins gp70 and p15(E) are derived by proteolytic cleavage of a cytoplasmic polyprotein precursor (4, 12) of 85,000 daltons, whereas the viral internal structural proteins (p30, p15, p12, p10) are derived by proteolytic cleavage of a cytoplasmic polyprotein (1, 11) of 65,000 daltons. Results presented here suggest that, in addition to the biosynthetic pathways involved in the generation of the viral internal structural proteins, the 65,000-dalton polyprotein (or a variant of this molecule) is glycosylated and inserted into the membrane. The function of the glycosylated core protein is unknown. Furthermore, whether this protein enters progeny virus also has yet to be determined. Nevertheless, the presence of this protein on the cell surface provides sites for immunological recognition by the host of MuLV-producing cells.

In this regard, we have recently described (15) that antisera prepared in C57BL/6 and BALB/c mice against the AKR leukemia K36 react with the viral proteins gp70, p30, and p15(E). Immune reponse of the mouse to gp70 and p15(E) is a well-documented event (7–9, 13–15) and, as such, this aspect of the finding was not unexpected; however, the production of antibody against p30 protein was restricted to immunizations against the K36 leukemia (15). It appears, therefore, that mice are immunologically nonresponsive to the monomer of the p30 protein, but are capable of an immune response to antigenic determinants of p30 that are constituents of the core polyprotein. This may be the result of a hapten-carrier relationship between the p30 antigenic determinants and the core polyprotein.

Immunization of mice or rats of inbred strains with leukemic cells has led to the definition of several distinct cell surface antigenic systems associated with MuLV: thus, (i) the G (Gross) cell surface antigen (GCSA) (18) was identified by immunization of C57BL/6 mice with the AKR K36 leukemia, (ii) the FMR antigen (15) was identified by immunization of mice with leukemias induced by Friend, Moloney, or Rauscher viruses, (iii) the X.1 antigen (19) was identified by immunization of genetically defined F_1 hybrid mice with radiation-induced leukemias of BALB/c mice, and (iv) the G_{IX} antigen (21) was identified by immunization of

W/Fu rats with syngenic rat leukemias induced by wild-type MuLV. More recently, the G_{1X} and X.1 antigens have been identified as type-specific antigenic determinants of the gp70 viral envelope protein (16, 22, 23). The detection of anti-p30 antibodies in the reference typing antiserum (C57BL/6 anti-AKR K36) for the GCSA system (15) led us to investigate the relationship between GCSA and the glycosylated core polyprotein that occurs on the membrane of murine leukemias. We have found that GCSA is, in fact, an antigenic determinant of the glycosylated core polyproteins (Ledbetter and Nowinski, submitted for publication).

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