

Identification of the Gross Cell Surface Antigen Associated with Murine Leukemia Virus-Infected Cells

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The Gross cell surface antigen (GCSA) is produced by cells that are either exogenously infected with murine leukemia virus (MuLV) or are expressing endogenous MuLV genomes. In immune precipitation assays, GCSA was resolved into two serologically distinct 85,000- and 95,000-dalton viral proteins. These antigenic components are glycosylated forms of the polyprotein precursors of the MuLV internal structural proteins.

Virus-induced murine leukemias can be classified into two groups on the basis of virus-associated cell surface antigens that are detected in the complement-dependent cytotoxicity test (14, 15). The Gross cell surface antigen (GCSA) (15) is produced by cells infected with Gross murine leukemia virus (MuLV), whereas the FMR cell surface antigen (14) is produced by cells infected with the Friend, Moloney, or Rauscher MuLV. GCSA is generally associated with the expression of endogenous MuLV, whereas the FMR antigen is associated with infection by exogenous MuLV.

Antiserum against GCSA is prepared by immunization of C57BL/6 mice with the transplanted AKR spontaneous leukemia K36 (15). Although the C57BL/6 anti-AKR K36 serum is produced by allogeneic immunization, it is cytotoxic for histocompatible C57BL/6 leukemias (e.g., C57BL/6 leukemia E δ G2) that are induced by Gross passage A virus. Tissues from mice of diverse genetic backgrounds can be examined for their GCSA phenotype by absorption of the typing antiserum, with subsequent tests for residual antibody activity against the C57BL/6 E δ G2 leukemia. In such tests, antibody to GCSA is absorbed by leukemias induced by Gross virus (15), by embryo fibroblast cell lines infected with MuLV (13), and by the normal lymphoid tissues of mice of high leukemic strains (AKR, C58, and PL) (15). In contrast, antibody to GCSA is not absorbed by the normal lymphoid tissues of mice of low leukemic strains (C57BL/6, BALB/c, and 129) (15) or by virus-free embryo fibroblast cell lines (13). GCSA also is found in certain leukemias and tumors of mice of low leukemic strains (3, 15), although in these instances, the occurrence of GCSA is correlated with the activation of endogenous MuLV in these tumor cells (3).

Studies with rat antisera (5) indicate that GCSA is antigenically complex, possibly representing as many as three serologically distinct antigens.

In immune electron microscopy, the typing antiserum for GCSA does not stain regions of the C57BL/6 E δ G2 cell surface that are associated with budding virions (1). As a result, it has been suggested that GCSA, although virus associated, is not a structural component of the surface of the virion.

In the studies presented here, we describe (by immune precipitation methods) the immunochemical identification of the GCSA complex. The major components of this complex are two glycosylated precursors (10, 19) of the viral core proteins, which contain antigenic determinants of the MuLV proteins p30, p12, and p10. In addition, some pools of GCSA antiserum contain antibodies against the viral envelope proteins gp70 and p15(E). These findings are in accord with our previous observations (12) that the typing antiserum for GCSA contains antibodies against the viral structural proteins p30, gp70, and p15(E).

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MATERIALS AND METHODS

Cells. The E δ G2 leukemia was originally induced in a C57BL/6 mouse by neonatal inoculation of Gross passage A virus. This leukemia was kindly provided by E. Fleissner, Sloan-Kettering Institute, and has been maintained by serial transplantation of spleen cells in C57BL/6 mice. The E δ G2 leukemia also has been established as a cell line in our laboratory and is grown in RPMI 1640 medium containing 20% fetal calf serum (FCS) and 5×10^{-5} M 2-mercaptoethanol. The AKR K36 leukemia is a long-trans-

planted ascites variant that was derived from a spontaneous AKR leukemia (15).

Antisera. Goat antisera prepared against the purified proteins gp70 (lot 55-167), p30 (lot 55-123) and p12 (lot 55-037) of Rauscher MuLV and p10 (lot 55-465) of AKR MuLV were obtained from the National Cancer Institute, courtesy of R. Wilsnack. Titers of these antisera (50% precipitation of 3×10^3 to 5×10^3 cpm of ^{125}I -labeled proteins) in radioimmunoassays (data provided by R. Wilsnack) were: anti-gp70 (1/6,400), anti-p30 (1/50,000), anti-p12 (1/18,000), and anti-p10 (1/150). Goat antiserum against purified p15 protein of AKR MuLV was kindly provided by E. Fleissner, Sloan-Kettering Institute. The specificity of these antisera with proteins of [^3H]leucine-labeled MuLV has been documented (10); briefly, the goat anti-p15 serum was monospecific for p15 protein; the goat anti-p12 serum co-precipitated p12 and p10 proteins; and the goat anti-gp70 co-precipitated gp70 and p15(E) proteins. The goat anti-p10 serum did not precipitate protein from the [^3H]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 radioimmunoassay (R. Wilsnack, personal communication), although only at low serum dilutions.

The GCSA typing antiserum, C57BL/6 anti-AKR K36, was produced in our laboratory by intraperitoneal inoculation of C57BL/6 mice every 2nd week with progressively increasing doses of viable AKR K36 cells. The initial inoculum consisted of 10^4 cells, whereas the final inocula were in excess of 10^8 cells. The serum used in these experiments (pool 2, see Table 1) was collected after the 12th immunization.

Radioactive labeling of cells and viruses. E δ G2 cells from the spleens of C57BL/6 mice were suspended in phosphate-buffered saline (PBS) by gentle teasing and pipetting. Radiolabeling of cell membranes by the ^{125}I -lactoperoxidase method was performed according to Vitetta et al. (20). 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; viability of cell suspensions (determined by trypan blue exclusion) before and after radiolabeling was >95%. The labeled cells were then disrupted with gentle Vortex mixing in 0.5% Nonidet P-40 (NP-40) for 30 min at 4°C; non-solubilized cellular structures were removed by centrifugation at 100,000 $\times g$ for 45 min. The supernatant containing the solubilized membrane proteins was frozen at -20°C in small portions for future analysis.

Immunoglobulin G (IgG), which was carried on the surface of splenic B-lymphocytes, was also radiolabeled by the ^{125}I -lactoperoxidase method; this source of ^{125}I -labeled IgG was removed by passage of the extract through an immunoabsorbant column containing goat anti-mouse IgG serum that was covalently complexed to cyanogen bromide-activated Sepharose 4B. Since E δ G2 maintained in culture did not contain surface IgG, this step was not required for extracts of in vitro-derived cells.

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 [^3H]leucine (30 $\mu\text{Ci/ml}$) or [^{14}C]leucine (5 $\mu\text{Ci/ml}$) 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.

Radioimmune precipitation of MuLV. Radioimmune precipitation (RIP) assays of MuLV were performed according to Ihle et al. (6). Fifty microliters of radiolabeled ([^3H]leucine) AKR MuLV (3,000 dpm) was initially incubated with 200 μl of diluted mouse serum for 1 h at 37°C. Mouse immunoglobulins were then precipitated by the addition of 200 μl of undiluted antiglobulin (goat anti-mouse 7S immunoglobulins; Hyland Laboratories) for 1 h at 37°C and then for 2 h at 4°C. Precipitates were collected by centrifugation at 1,000 $\times g$ for 10 min; residual radioactivity was measured in 350 μl of supernatant.

Immune precipitation of cellular extracts. 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. Following a 1-h incubation on ice, 200 μl of

TABLE 1. Comparison of different pools of GCSA typing antisera for cytotoxicity against C57BL/6 E δ G2 cells and immune precipitation of viral antigens from ^{125}I -labeled C57BL/6 E δ G2 membranes

Antiserum— C57BL/6 anti-AKR pool ^a	Cytotoxic re- action (%) specific ⁵¹ Cr release from E δ G2 cells ^b	Immune precipitation (cpm)	
		¹²⁵ I-labeled gp70 ^c	¹²⁵ I-labeled po- lyprotein ^d
Pool 1	66	1,980	11,953
Pool 2	53	4,964	10,517
Pool 3	39	4,737	9,235
Pool 4	25	1,011	2,858
Pool 5	6	2,713	1,329

^a Pool 1 was kindly provided by E. Stockert, Sloan-Kettering Institute; pools 2 to 5 were each prepared in our laboratory.

^b Cytotoxicity was determined at a 1/20 dilution of antiserum; titration in cytotoxicity tests demonstrated a similar ordering in the potency of these sera.

^c Determined by SDS-PAGE of immune precipitates prepared from a single labeled preparation of C57BL/6 E δ G2 cells.

^d Determined by SDS-PAGE of immune precipitates prepared from a single labeled preparation of C57BL/6 E δ G2 cells. These figures represent the sum of counts per minutes precipitated in both the 85,000- and 95,000-dalton polyprotein peaks; the 85,000-dalton polyprotein was the major species in all precipitates.

undiluted antiglobulin (Rabbit anti-goat 7S immunoglobulin or goat anti-mouse 7S immunoglobulin, Cappel Laboratories) was added to precipitate the immune complexes. After an additional 2-h incubation on ice, the precipitates were collected by centrifugation ($1,000 \times g$ 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 minimizing the nonspecific sticking of radiolabeled proteins to the immune precipitates. In some experiments, the antigen-antibody complexes formed from the incubation of antiserum with the radiolabeled membrane preparation were precipitated by the addition of 3 mg of *Staphylococcus aureus* with a 30-min incubation on ice (8). The *Staphylococcus aureus* precipitates were then washed in the same manner as the antiglobulin precipitates and eluted with sodium dodecyl sulfate (SDS)-containing sample buffer.

Affinity chromatography. Immunoabsorbant columns were prepared by the covalent linkage of goat antiserum to cyanogen bromide-activated Sepharose 4B (Pharmacia). Radiolabeled cell membrane proteins (0.5-ml volume) were chromatographed on 3-ml immunoabsorbant columns in PBS containing 0.5% NP-40. Proteins that adhered to the column were eluted by 3 M thiocyanate; this eluted fraction was then dialyzed overnight into PBS containing 0.5% NP-40.

Complement-dependent cytotoxicity. Assays for complement-dependent cytotoxicity were performed by the ^{51}Cr release method. Briefly, 5×10^6 viable cells in 500 μl of RPMI 1640 medium containing 15% FCS were labeled with 250 μCi of ^{51}Cr by incubation at 37°C for 1 h with frequent, gentle agitation. The assay was performed in the wells of a microtiter plate (Falcon Microtest II); each well of the plate contained (i) 10^4 ^{51}Cr -labeled cells in 50 μl of diluent, (ii) 50 μl of diluted antiserum, and (iii) 50 μl of rabbit serum (diluted 12-fold) as a complement source. The diluent for all procedures was RPMI 1640 medium containing 15% FCS. The plates were incubated at 37°C for 45 min, and the reaction was stopped by the addition of 100 μl of cold diluent to each well. Intact cells were removed from the reaction mixture by centrifugation at 1,000 rpm for 10 min in a refrigerated centrifuge, and a constant volume of each of the supernatants was removed for the determination of the amount of ^{51}Cr released. Calculations were made as follows: % Specific ^{51}Cr release = (experimental release) - (control release) / (maximum release) - (control release).

Maximum ^{51}Cr release was determined by three cycles of freeze-thawing of the radiolabeled cells. Control release was the highest value that was scored in either of the three controls that were performed with each experiment: (i) spontaneous release (no antibody or complement present), (ii) complement release (no antibody present), and (iii) antibody release (no complement present). Results of experiments in which the ^{51}Cr release of any of the controls exceeded 10% of the maximum release value were excluded.

SDS-polyacrylamide gel electrophoresis (PAGE).

PAGE was performed according to Laemmli (11) in cylindrical gels (12 cm in length) or in 1.5-mm slab gels; cylindrical gels were subjected to electrophoresis at 0.55 mA per tube for 17 h, whereas slab gels were subjected to electrophoresis at 100 V for 7 h. PAGE of viral proteins was performed in 12.5% gels. PAGE of cellular extracts was performed in 7.5% gels. Samples containing ^{125}I -labeled proteins were subjected to coelectrophoresis in cylindrical gels with ^{131}I -labeled 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 ^3H -labeled MuLV proteins were subjected to coelectrophoresis with ^{14}C -labeled MuLV as a marker.

RESULTS

To determine the relationship of GCSA to MuLV-coded proteins we utilized two experimental approaches: (i) analysis of the GCSA typing serum (C57BL/6 anti-AKR K36) for antibodies against MuLV proteins, and (ii) analysis of the GCSA typing cell (C57BL/6 E δ G2) for the expression of MuLV proteins on the cell surface.

Precipitation of intact MuLV by GCSA typing serum. RIP assays of intact [^3H]leucine-labeled AKR MuLV with a variety of antiviral sera are shown in Fig. 1.

Goat antiserum prepared against the viral envelope protein gp70 precipitated, to high titer, ^3H -labeled AKR MuLV, whereas goat antisera prepared against the internal viral proteins p30, p15, p12, and p10 had only minimal activity (Fig. 1A).

The GCSA typing serum, C57BL/6 anti-AKR K36, precipitated to high titer the ^3H -labeled

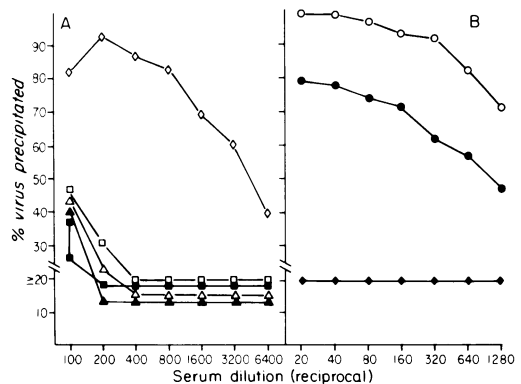


Fig. 1. Precipitation of intact MuLV by goat and mouse antisera. [^3H]leucine-labeled AKR MuLV was reacted in the RIP assay with (A) individual goat antisera prepared against the purified MuLV proteins gp70 (\diamond), p30 (\square), p15 (\triangle), p12 (\blacksquare), and p10 (\blacktriangle), and (B) GCSA typing serum (\circ), normal C57BL/6 serum (\blacklozenge), and normal I strain serum (\bullet).

AKR MuLV (Fig. 1B). In contrast, the sera of normal nonimmunized C57BL/6 mice did not react with the virus (Fig. 1B).

Normal sera from mice of the I strain also precipitated ^3H -labeled AKR MuLV (Fig. 1B). This finding confirms our previous observations (11) that mice of the I strain naturally produce antibodies to their endogenous MuLV.

Precipitation of viral proteins by GCSA typing serum. To determine the protein of the virus to which antibodies in the C57BL/6 anti-AKR K36 serum were directed, the RIP test was performed with detergent-disrupted ^3H -labeled AKR MuLV, and the immune precipitate was examined by PAGE in the presence of SDS.

PAGE of a control preparation of AKR MuLV is shown in Fig. 2A. Eight major viral polypeptides exist in the virion: these are designated gp70, gp45, p30, p17, p15(E), p15, p12, and p10, according to the nomenclature for proteins of the oncornaviruses (2). PAGE of immune precipitates formed between the C57BL/6 anti-AKR K36 serum and the proteins of NP-40-disrupted AKR MuLV (Fig. 2B) demonstrated that the antiviral antibodies present in this serum were directed primarily against the viral proteins p30 and p15(E); however, minor reactions were also observed with gp70, p17, and p15. PAGE of immune precipitates formed between normal sera of C57BL/6 mice and the proteins of NP-40-disrupted AKR MuLV failed to show significant antiviral antibodies in these mice (Fig. 2C).

PAGE of an immune precipitate formed between the normal serum of an I strain mouse with the proteins of NP-40-disrupted AKR MuLV (Fig. 2D) demonstrated antiviral antibodies that were directed primarily against the viral protein p15(E). Analysis of other sera from normal I mice revealed a similar reaction with p15(E), although in some cases these sera also showed an additional reaction with gp70 (11).

The spectrum of anti-MuLV antibodies [precipitating gp70 and p15(E) proteins] in the sera of normal I mice is qualitatively similar to the antiviral antibodies that have been observed in the sera of mice of other inbred strains that develop a natural immune response to MuLV (7, 11). In contrast, antibodies to p30 protein, as observed in the C57BL/6 anti-AKR K36 serum, have not been observed in the sera of normal or immunized mice of a wide variety of genetic backgrounds (7, 11, 12).

Cytotoxic tests with C57BL/6 E δ G2 cells. Cytotoxic assays of ^{51}Cr -labeled C57BL/6 E δ G2 cells with a variety of antiviral sera are shown in Fig. 3.

Goat antisera prepared against the viral pro-

teins gp70, p30, p12, and p10 were strongly cytotoxic for the C57BL/6 E δ G2 cells (Fig. 3A). An antiserum prepared against the p15 protein also was cytotoxic for C57BL/6 E δ G2 but to a

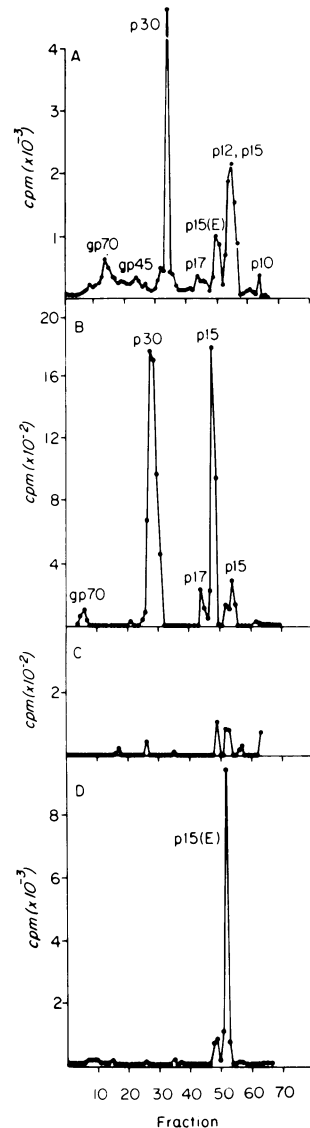


FIG. 2. Precipitation of MuLV proteins by goat and mouse antisera. PAGE of antiglobulin-mediated immune precipitates formed between detergent-disrupted [^3H]leucine-labeled AKR MuLV and (B) GCSA typing serum, (C) C57BL/6 normal serum, and (D) I strain normal serum; (A) Control pattern of AKR virus is also shown. The polyacrylamide gels presented here were derived from several different experiments; therefore, each of the immune precipitates was subjected to coelectrophoresis with [^{14}C]leucine-labeled AKR MuLV for positive identification of the precipitated viral proteins.

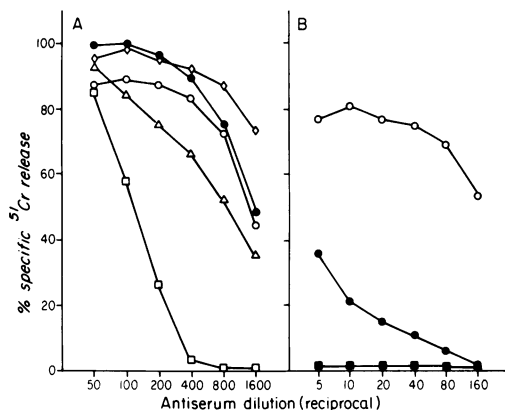


FIG. 3. Cytotoxic assays of *in vivo*-derived C57BL/6 E δ G2 cells with goat and mouse sera. ⁵¹Cr-labeled C57BL/6 E δ G2 cells were tested with (A) individual goat antisera prepared against the purified MuLV proteins gp70 (●), p30 (○), p15 (□), p12 (◇), p10 (△), and (B) GCSA typing serum (○), C57BL/6 normal serum (■), and I strain normal serum (●).

lower titer. In parallel tests, goat antisera to the viral proteins p30, p15, p12, and p10 were not demonstrably cytotoxic for normal C57BL/6 spleen cells. Antiserum prepared against gp70 was weakly cytotoxic (titer, <1/50) for normal C57BL/6 spleen cells; however, this may reflect the low level of gp70 that is known to occur (18) in normal lymphoid tissues of C57BL/6 mice.

The GCSA typing serum, C57BL/6 anti-AKR K36, was strongly cytotoxic for the C57BL/6 E δ G2 cells (Fig. 3B). Sera from normal nonimmunized C57BL/6 mice were not cytotoxic for the C57BL/6 E δ G2 cells (Fig. 3B). Sera from normal I strain mice, although containing higher titers of anti-p15(E) antibody, exhibited minimal cytotoxicity towards the C57BL/6 E δ G2 cells (Fig. 3B). In parallel tests, the GCSA typing serum and normal sera of I strain mice were not cytotoxic for normal C57BL/6 spleen cells.

Precipitation of MuLV antigens from the cell surface of C57BL/6 E δ G2 cells. To identify the MuLV proteins on the surface of C57BL/6 E δ G2 cells to which antibodies in the C57BL/6 anti-AKR K36 serum were directed, the RIP test was performed with detergent-disrupted ¹²⁵I-labeled C57BL/6 E δ G2 membranes, and the immune precipitates were examined by PAGE in the presence of SDS.

PAGE of immune precipitates formed between goat antisera prepared against viral proteins and ¹²⁵I-labeled membrane extracts of C57BL/6 E δ G2 cells is shown in Fig. 4. Three serologically distinct reactions were observed. (i) Anti-gp70 serum precipitated a single 70,000-dalton protein. (ii) Anti-p30 and anti-p12 sera

precipitated two high-molecular-weight (85,000 and 95,000) proteins. We (10) and others (19) have previously demonstrated that these proteins are the glycosylated polyprotein precursors of the internal structural proteins (p30, p15, p12, and p10) of MuLV. (iii) Anti-p10 serum precipitated the higher-molecular-weight (95,000) polyprotein, but did not react with the lower-molecular-weight (85,000) polyprotein.

PAGE of immune precipitates formed between the GCSA typing serum and ¹²⁵I-labeled membrane extracts of C57BL/6 E δ G2 cells are also shown in Fig. 4. This antiserum precipitated three proteins of molecular weights 70,000, 85,000, and 95,000, corresponding in size to the viral envelope protein gp70 and the viral polyprotein precursors that were precipitated by the goat antisera. PAGE of immune precipitates formed between I normal sera and ¹²⁵I-labeled membrane extracts of C57BL/6 E δ G2 demonstrated reactions with a single 70,000-dalton protein. The failure to detect reactions of I normal sera with p15(E) protein on the cell surface was most likely due to the fact that p15(E) is not accessible on the cell surface (or in virions) to iodination by the ¹²⁵I-lactoperoxidase method (unpublished data).

Serological identification of GCSA as the glycosylated polyproteins of MuLV. The precipitation of two proteins of 85,000 and 95,000 daltons from the membrane extract of C57BL/6 E δ G2 cells by GCSA typing serum suggested that GCSA may represent antigenic determinants on the glycosylated polyproteins of MuLV. To definitively demonstrate the presence of p30 antigenic sites on GCSA, we used immunoabsorbant columns to remove selected antigenic specificities from the C57BL/6 E δ G2 extracts.

Immunoabsorbant 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 activity with antisera. Proteins bound to the immunoabsorbant columns were eluted by thiocyanate and examined by immune precipitation for virus-related antigens. The results of these studies with a cell extract from C57BL/6 E δ G2 are shown in Fig. 5.

Before passage through the anti-p30 immunoabsorbant, the C57BL/6 E δ G2 cell extract contained 85,000- and 95,000-dalton antigens that reacted with the anti-p30 serum, and a 70,000-dalton antigen that reacted with the anti-gp70 serum. The GCSA typing serum reacted with 70,000-, 85,000-, and 95,000-dalton species (Fig. 5). After passage through the p30

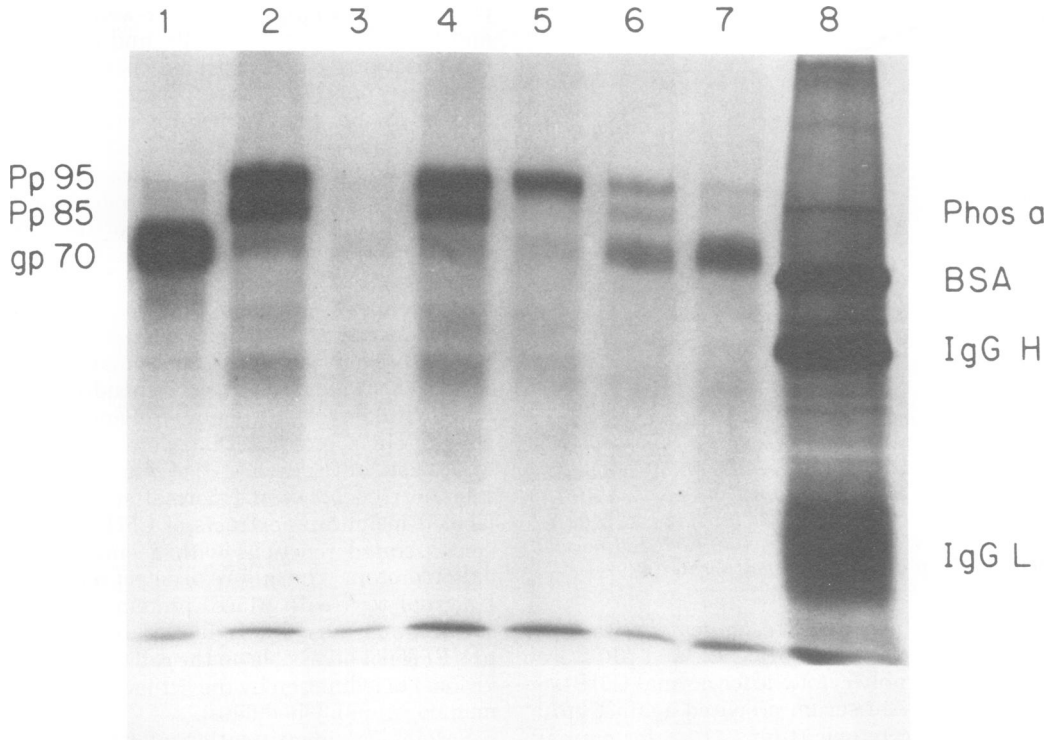


FIG. 4. Immune precipitation of MuLV proteins from the membrane of C57BL/6 E δ G2 cells. PAGE of RIP (*Staphylococcus aureus*) reactions formed between detergent-disrupted ¹²⁵I-lactoperoxidase-labeled *in vitro*-derived C57BL/6 E δ G2 cells and (1) anti-gp70 serum; (2) anti-p30 serum; (3) anti-p15 serum; (4) anti-p12 serum; (5) anti-p10 serum; (6) GCSA typing serum; (7) normal I strain serum; and (8) molecular weight markers (phosphorylase a [Phos a], 94,000; bovine serum albumin [BSA], 68,000; IgG, heavy chain, 50,000; IgG, light chain, 25,000). Pp85 and Pp95 refer to the migration positions of the 85,000- and 95,000-dalton polyproteins, respectively.

immunoabsorbant, the cell extract no longer contained the 85,000- and 95,000-dalton antigens that were immunologically reactive with both the anti-p30 and GCSA typing serum. However, the 70,000-dalton antigen that was reactive with the anti-gp70 and the GCSA typing serum was still present. Elution of the adsorbed proteins with thiocyanate yielded the 85,000- and 95,000-dalton proteins that were immunologically reactive with both the anti-p30 and GCSA typing serum. Therefore, we have concluded that GCSA and p30 protein share common antigenic determinants.

In a comparison (Table 1) of several pools of C57BL/6 anti-AKR K36 sera, it was found that the cytotoxicity of these sera for the C57BL/6 E δ G2 cell was correlated with their reactions with the 85,000- and 95,000-dalton cell surface antigens. The cytotoxic activity of these antisera was unrelated to their reactions with the 70,000-dalton cell surface antigen.

DISCUSSION

The studies presented here demonstrate that GCSA is a complex composed of several MuLV-

coded proteins. The major components of GCSA are the 85,000- and 95,000-dalton glycosylated polyproteins that contain antigenic determinants of the viral internal structural proteins. This is demonstrated by the following: (i) the GCSA typing serum predominantly precipitates 85,000- and 95,000-dalton proteins from ¹²⁵I-labeled C57BL/6 E δ G2 membrane preparations, (ii) the 85,000- and 95,000-dalton proteins that react with the GCSA typing serum can be selectively removed from the C57BL/6 E δ G2 extract by an anti-p30 immunoabsorbant, and (iii) the cytotoxic activity of GCSA antisera is correlated with the reaction of these sera with the 85,000- and 95,000-dalton proteins.

These findings are in agreement with those of Snyder et al. (16), who independently demonstrated that the major component of the GCSA reaction is the glycosylated polyprotein precursor to the MuLV internal structural proteins. In addition to using immune precipitation methods, their studies also included the demonstration that the isolated viral protein p30 was capable of absorbing the majority of cytotoxic activity of GCSA typing serum. Although our

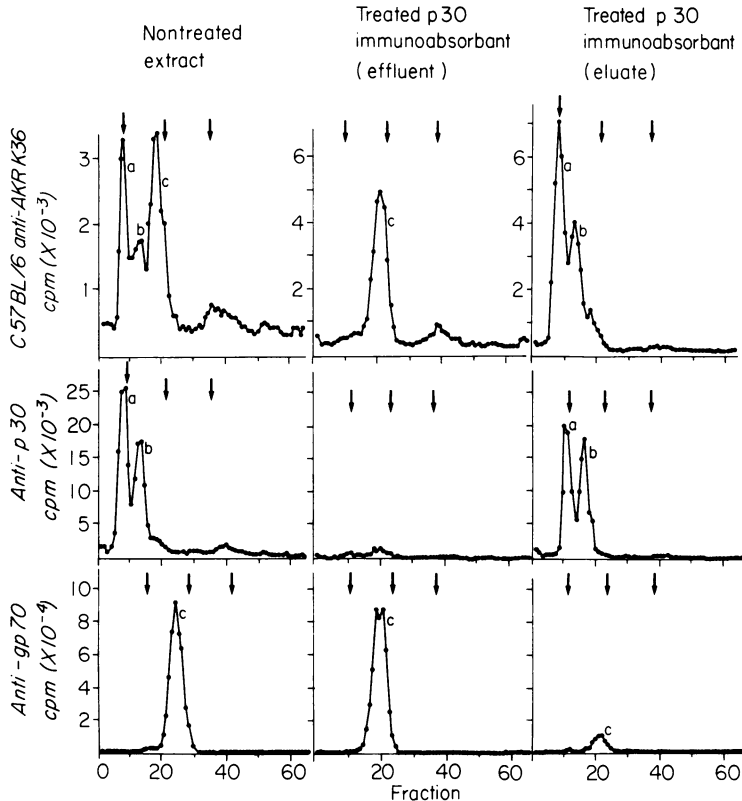


FIG. 5. Identification of GCSA as an antigenic determinant of the glycosylated polyproteins of MuLV. A ^{125}I -lactoperoxidase-labeled extract of *in vitro*-derived C57BL/6 E δ G2 cells was chromatographed through an anti-p30 immunoabsorbant column (goat anti-p30 serum covalently bound to cyanogen bromide-activated Sepharose 4B) and then tested for residual antigens by PAGE of *Staphylococcus aureus*-mediated precipitates with anti-gp70, anti-p30, and GCSA typing sera. Proteins bound to the immunoabsorbant were eluted by thiocyanate and also tested for viral antigens with anti-gp70, anti-p30, and GCSA typing sera. Peaks designated by lowercase letters had molecular weights of (a) 95,000; (b) 85,000; and (c) 70,000. Each of the immune precipitates was subjected to coelectrophoresis with ^{131}I -labeled molecular weight markers (phosphorylase a, 94,000; bovine serum albumin, 68,000; IgG heavy chain, 50,000). The migration of the marker proteins is indicated by the arrows, with the phosphorylase a marker to the left.

studies of GCSA are generally in accord, a discrepancy has occurred concerning the presence of antigens associated with p15 protein in the glycosylated polyprotein. Snyder et al. (16) reported that anti-p15 serum precipitated the 85,000-dalton polyprotein, but we have not been able to confirm this observation. We have observed, however, that anti-p15 serum is cytotoxic for E δ G2 cells. Differences in our findings may reflect the conditions used for the preparation of cell extracts and RIP assays. The failure to obtain precipitation reactions with anti-p15 serum may reflect minor conformational variations of the polyprotein in NP-40-containing solutions, rather than the absolute peptide composition of the molecule. These questions will be resolved by comparative peptide mapping of GCSA and the viral proteins.

We have also found that antibodies against

the gp70 and p15(E) viral proteins are present in some pools of GCSA typing antisera. These GCSA antisera precipitate (i) intact MuLV, (ii) gp70 and p15(E) proteins from detergent-disrupted MuLV, and (iii) a 70,000-dalton protein from the cell surface of C57BL/6 E δ G2. However, it should be stressed that the presence of antibody to gp70 and p15(E) in GCSA typing serum varies considerably from one pool of serum to another. In a comparison of several pools of C57BL/6 anti-AKR K36 serum (Table 1), we observed that the cytotoxic titer of these sera for C57BL/6 E δ G2 was closely related to the precipitation titer of these sera for the 85,000- and 95,000-dalton polyproteins. Furthermore, we have found that normal sera of I strain mice, which contain high titers of antibody against p15(E), were only minimally cytotoxic for C57BL/6 E δ G2.

The immune response of mice to the glycosylated polyprotein appears to be restricted to animals that have been immunized with leukemia cells. In previous studies (12) we examined a panel of sera from C57BL/6 mice that were immunized with a variety of cellular antigens for antibodies against MuLV. With the exception of the GCSA typing serum, these sera either (i) failed to contain demonstrable antiviral antibodies, or (ii) contained antibodies exclusively against the MuLV proteins p15(E) and gp70. The presence of high titers of anti-p30 antibody in the GCSA typing sera led us to hypothesize that the AKR K36 cells contained on their surface a unique configuration of the p30 antigenic determinants. From the studies described here, we now attribute the anti-p30 antibodies in the GCSA typing serum to immunization by the glycosylated polyproteins of MuLV that occur as integral components of the cell surface of the AKR K36 cell. Recently, Stephenson et al. (17) demonstrated by radioimmunoassay that mice of low leukemic strains can be immunized with purified preparations of p30 protein. However, it appears that the titers of anti-p30 antibodies induced by purified p30 protein are significantly lower than those obtained by immunization with cells that contain the glycosylated polyproteins as surface antigens.

The identification of GCSA as a complex of glycosylated virus-coded proteins that are expressed on the cell surface may relate to the observations of Knight et al. (9), who found that purified p30 protein blocked the killing of MuLV-infected leukemia cells by cytotoxic lymphocytes. The isolation of GCSA (by the combined use of lectin and immunoadsorbent affinity chromatography) will allow us to determine the role of these antigens in cell-mediated immunity to leukemia.

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