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NOTES

Two Species of Type C Viral Core Polyprotein on AKR Mouse Leukemia Cells

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Two species of glycosylated type C viral core polyprotein were identified on the surface of AKR spontaneous leukemia cells. One of these cell surface polyproteins was shown by immunoprecipitation to have antigenic determinants of murine leukemia virus p30, p15, p12, and p10; the other had murine leukemia virus p30, p15, and p12, but not p10, determinants. Both species were also expressed on thymocytes from 6-month-old, preleukemic AKR mice.

Recently, a polyprotein species containing antigenic determinants of several murine leukemia virus (MuLV) core proteins was identified on the surface of AKR spontaneous leukemia cells (19). Although this species was not found on thymocytes from 2-month-old AKR mice, its expression is not restricted to leukemic cells, since substantial quantities of polyprotein could be found on thymocytes from 6-month-old mice that were still preleukemic. In this report, we present more detailed studies on the nature of this polyprotein, which reveal that this species is actually a complex of two virus-specified polypeptides, both glycosylated.

Three methods by which this complex can be isotopically labeled are shown in Fig. 1. (i) AKR spontaneous leukemia (SL) cells were labeled with ^{125}I by the lactoperoxidase method (19). After Nonidet P-40 (NP-40) lysis and precipitation with anti-p30 serum, analysis of the immunoprecipitate by sodium dodecyl sulfate-polyacrylamide gradient gel electrophoresis (SDS-PAGE) revealed two components migrating close together in the region of molecular weight >70,000 (Fig. 1A, track 3). These were designated gP95 and gP85 ("gP" = glycosylated polyprotein), since the molecular weights of the two components were estimated by coelectrophoresis with protein markers to be 95,000 and 85,000, respectively. [A tentative molecular weight of 75,000 and the designation p(75) were originally assigned to the complex (19). Since these proteins are derived from the MuLV *gag* gene locus, a more complete designation for these components would be $gP85^{gag}$ and $gP95^{gag}$, as proposed at a recent meeting of workers in this field (NCI Tumor Viral Immunology Workshop, March 1977).] Anti-NTD serum, which has antibodies against a number of MuLV proteins (8, 16, 17), precipitated gp70 in addition to gP95 and gP85 (track 1). As expected, anti-gp70 serum precipitated only gp70 (track 2). (ii) AKR SL cells were labeled with [^3H]-glucosamine in culture for 5 h (19), and a cell lysate was prepared and analyzed as before. The results showed that both gP95 and gP85 could be labeled with glucosamine (Fig. 1B, track 2). Glucosamine-labeled gp70, but not the gP95 or gP85 components, was precipitated by anti-gp70 serum as before (track 1). The precipitation of gP95 by anti-p10 serum will be discussed below (see Fig. 2). (iii) When AKR SL cells were labeled with sodium boro[^3H]-hydride by the galactose oxidase method (7), both gP95 and gP85 were again labeled (Fig. 1C, track 1). The result demonstrated that both components contain galactose as well as glucosamine and confirms the cell surface location of these molecules.

It seemed of interest to determine whether gP95 and gP85 could be distinguished by properties other than their electrophoretic mobilities. Figure 2 shows a comparison of AKR thymocytes and AKR SL cells with respect to the serological specificities of cell surface viral core polyproteins. The thymocytes were taken at 6 months of age, a time when the tissue is, in general, morphologically normal, although it displays an enhanced level of certain MuLV-specific gene products (9, 10, 19). Figure 2A (track 5) shows that both components are present on 6-month-old thymocytes; therefore, neither one is, strictly speaking, leukemia specific. However, in the course of testing lysates

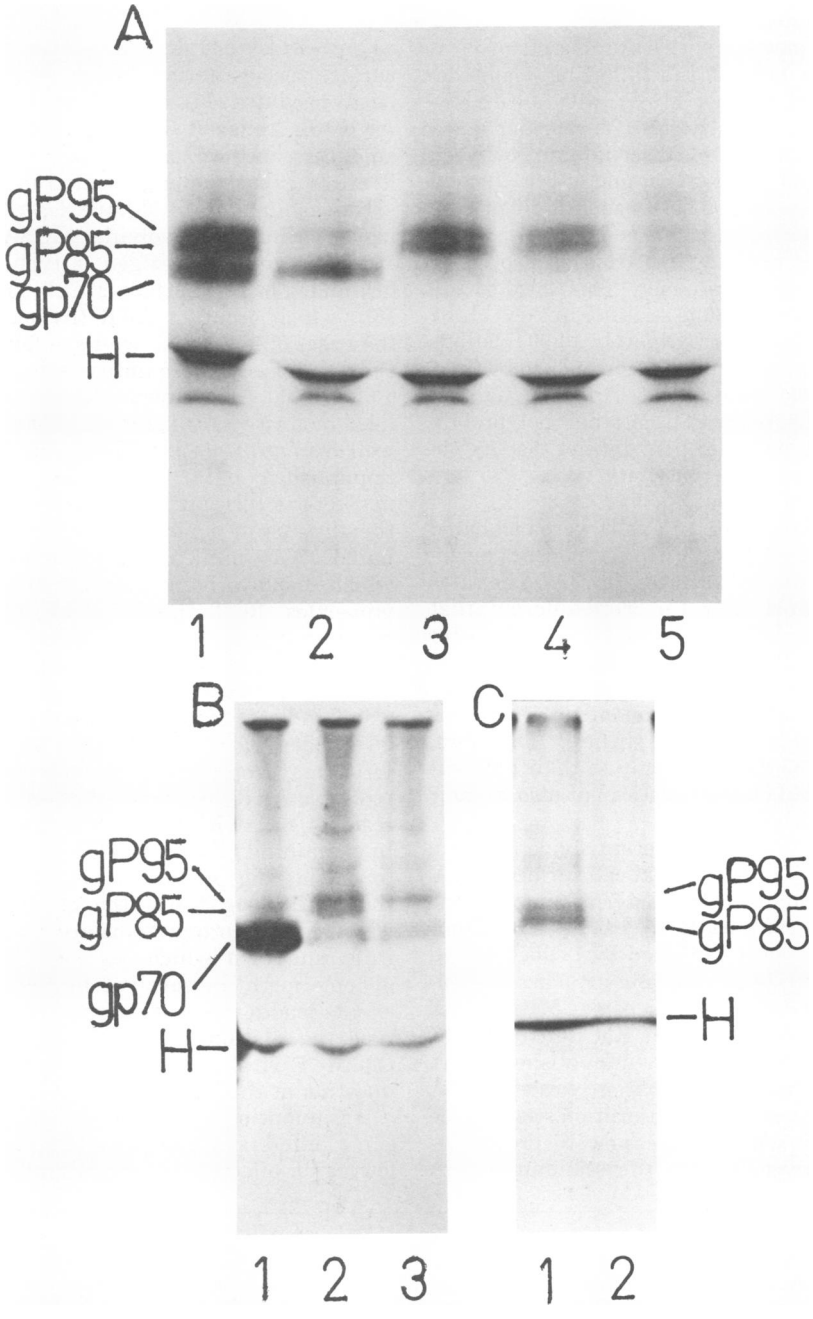

FIG. 1. *Detection of two glycosylated viral polyproteins at the cell surface: three methods of labeling. SDS-PAGE analysis of immunoprecipitates prepared from lysates of: (A) lactoperoxidase- and ^{125}I-labeled AKR SL cells. Antisera were (1) anti-NTD (rat W/Fu × BN F_1 anti-W/FU C58NTD, provided by E. Stockert, Memorial Sloan-Kettering Cancer Center [MSKCC]), (2) anti-gp70 (goat anti-Scripps MuLV gp70, provided by R. Lerner, Scripps Clinic and Research Foundation), (3) anti-Rp30 (rabbit anti-Rauscher-MuLV p30 [reference 19], provided by W. Hardy, Jr., MSKCC, (4) anti-Ap30 (goat anti-AKR-MuLV p30, provided by R. Wilsnack, Huntingdon Research Center), and (5) normal rabbit serum; (B) precursor [^{3}H]glucosamine-labeled AKR SL cells. Antisera included: (1) anti-gp70, (2) anti-Rp30, and (3) anti-Ap10 (goat anti-AKR-MuLV p10, provided by R. Wilsnack); (C) galactose oxidase- and sodium boro[^{3}H]hydride-labeled AKR SL cells.*

of radioiodinated AKR SL cells and of 6-month-old AKR thymocytes with a number of antiviral antisera, we noticed the following phenomenon. Antisera against MuLV p10 (anti-AKR-MuLV p10, anti-Friend-MuLV p10, and anti-BALB V2 p10) could discriminate between gP95 and gP85 (tracks 2, 3, and 4; Fig. 2A and B): the gP95 species on both 6-month-old preleukemic thymocytes and SL cells were reactive with antisera made against this viral protein; the gP85 species were not. This lack of p10 determinants on gP85 is striking in view of the size difference of approximately 10,000 daltons between the two components, as judged by their electrophoretic migrations. Antisera against viral proteins representing other polyprotein regions (p30, p15, and p12) did not display obvious differences in reactivity with gP95 and gP85 (data not shown).

When radioiodinated SL cells were incubated at 37°C, both gP95 and gP85 components disappeared from the cell surface (Fig. 3A). The rates of disappearance, however, were different: that of gP95, especially in the first 2 h, was faster than that of gP85 (compare with Fig. 1A). gP95 was readily detectable in the medium (Fig. 3B), suggesting that this component, at least, was exfoliated from the cell surface. Therefore, gP95 and gP85 differ not only in their physical and serological characteristics but also in their physiological behavior.

As mentioned above, recent evidence from our laboratory indicates that cell surface MuLV core polyproteins carry determinants of the Gross cell surface antigen (GCSA) (15). Similar data have been obtained by Ledbetter and Nowinski (12). Both gP95 and gP85 are precipitable by the GCSA typing serum, C57BL/6 anti-AKR spontaneous leukemia K36 (anti-K36) (J.-S. Tung and E. Fleissner, unpublished data); thus, both components apparently carry GCSA determinants, although absorption studies (see reference 15) will be necessary to prove this point unequivocally. On the standard GCSA typing cell, E♂ G2 and anti-K36 serum, as well as anti-MuLV p30 sera, also detect a larger cell surface species, termed p(150), which may not be glycosylated (15). If this species is present on AKR SL cells, it is in considerably lower amounts, since we have not been able to detect it in the experiments presented in this report.

Kawashima et al. (9) have described physiological changes involving GCSA and the MuLV-specific G_{IX} antigen (a type-specific determinant of cell surface MuLV gp70 [13, 17, 18]), which occur in AKR thymocytes before the onset of leukemia. We have found that the observed rise of these antigens in preleukemic 6-month-old AKR thymocytes is closely paralleled by a rise in cell surface gP95 and gP85, as well as in gp70 (19; J.-S. Tung and E. Fleissner, unpublished data). The increase in these markers is correlated with the appearance in the thymus of a novel type of MuLV, designated MCF (mink cell focus inducing) virus, which displays both ecotropic and xenotropic properties (10; J. Hartley et al., in press). By means of the antisera at our disposal we have not as yet succeeded in making a serological distinction between gP95 and gP85 which would indicate that one or other of these species is encoded by the MCF viral genome. MCF virus may represent a recombinant between ecotropic AKR virus and another viral genome resident in AKR (J. Hartley et al., in press). In examining gP95 and gP85, we are restricted to considering products or partial products of the MuLV *gag* locus, the genetic region in which MuLV core proteins are specified. Since it is unknown which regions of the MuLV genome might be involved in recombinational events leading to generation of the MCF genotype, the relationship of gP95 or gP85 or both to the MCF viral genome must remain an open question at this time.

It is tempting to relate the physical and antigenic differences between gP95 and gP85 (allowing for effects of glycosylation) to the differ-

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Antisera were (1) anti-Rp30 and (2) normal rabbit serum. Labeled AKR SL cells were lysed with 0.5% NP-40, cell lysates were reacted with antiserum and subsequently with anti-immunoglobulin serum, and the immunoprecipitates were washed, denatured in a mixture of 0.1% SDS, 8 M urea, and 0.1 M Tris (pH 8.6) with 2% 2-mercaptoethanol (vol/vol) by boiling for 2 min, and applied to 5 to 17% gradient slab gels for electrophoresis at 24 mA per slab for 3 to 4 h (11). The gel slabs were fixed in trichloroacetic acid and washed with 7% acetic acid. Gels containing ^{125}I-labeled samples were dried and exposed to X-ray film (Kodak XR-2) directly. Gels containing ^{3}H-labeled samples were subjected to dimethyl sulfoxide and 2,5-diphenyloxazole treatment (4) and then dried and exposed to X-ray film. Radioactive bands identified were gP95, gP85, gp70, and H; the last appears to represent trapping of radioactivity by the heavy chain of immunoglobulin molecules. The electrophoretic mobilities of sodium boro[^{3}H]hydride-labeled gP95 and gP85 components were consistently faster than mobilities of the same components labeled with either ^{125}I or [^{3}H]glucosamine. This increase in mobilities may be due to neuraminidase treatment, which is a necessary step in galactose oxidase labeling. Protein markers phosphorylase a, bovine serum albumin, and human immunoglobulin G were used to estimate molecular weights.

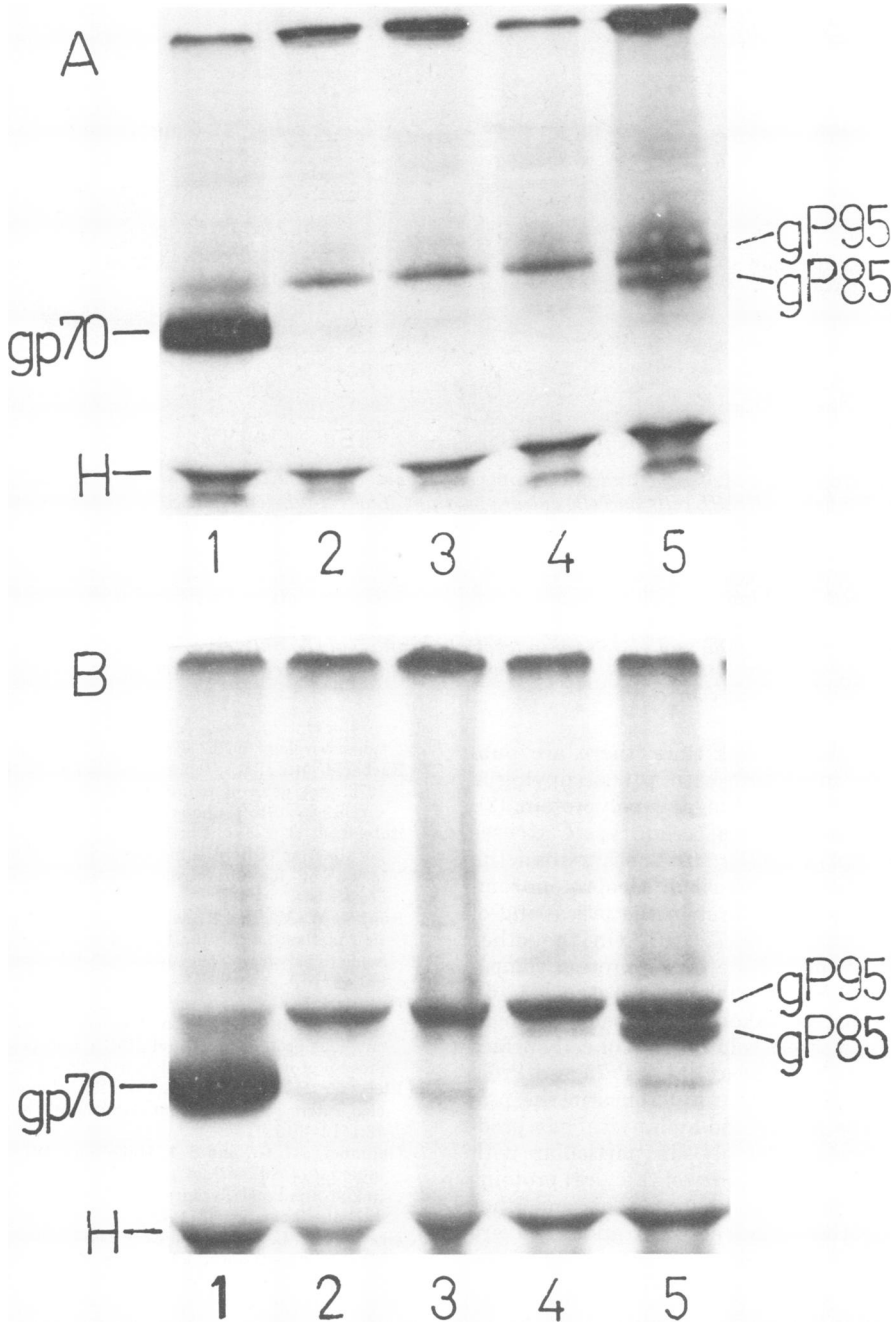

FIG. 2. *Discrimination of gP95 and gP85 by anti-MuLV p10 sera. SDS-PAGE analysis of immunoprecipitates from ^{125}I-labeled cell lysates of (A) thymocytes of 6-month-old, preleukemic AKR mice and (B) AKR SL cells. Antisera were (1) anti-gp70, (2) anti-BV2 p10 (goat anti-BALB virus 2 p10, provided by R. Wilsnack), (3) anti-Ap10, (4) anti-Fp10 (rabbit anti-Friend-MuLV p10, provided by D. Bolognesi, Duke University Medical Center), and (5) anti-Rp30. For detailed methods, see legend to Fig. 1.*

ence in size reported for two intracellular MuLV *gag* products, Pr75 and Pr65, in infected tissue culture cells (1, 5, 14, 20). Since Pr75 is considered a precursor to Pr65, such a relationship would depend on the p10 protein being cleaved first from the larger precursor in the process of virion assembly. Direct biochemical or serological evidence for the latter conclusion

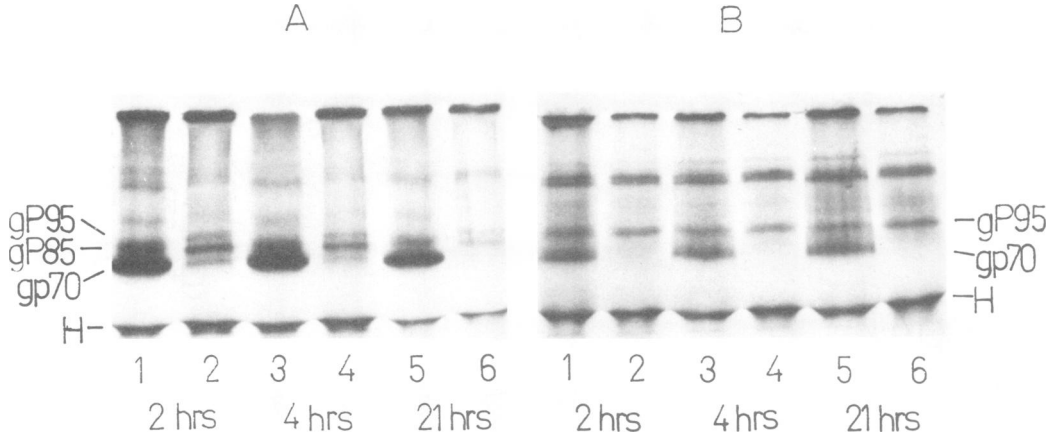

FIG. 3. *Differential loss of gP95 and gP85 from cell surfaces. SDS-PAGE analysis of immunoprecipitates from (A) ^{125}I-labeled AKR SL cells and (B) culture medium. The ^{125}I-labeled AKR SL cells were incubated in RPMI 1640 medium with 20% fetal calf serum at 37°C in 5% CO_2. At different time intervals, cells were pelleted gently and washed three times with phosphate-buffered saline. The lysed cells and the media were subjected to immunoprecipitation and SDS-PAGE as before. Times for which the cells and medium were incubated together are indicated on the figure. For both gels, tracks 1, 3, and 5 represent precipitations with anti-gp70 serum, and tracks 2, 4, and 6 represent precipitations with anti-Rp30 serum. The bands with mobilities near those of gP85 and gP95 seen in tracks 1, 3, and 5 of (A) and (B), respectively, are nonspecific since these are observed with normal goat serum. The high-molecular-weight doublet in (B) can also be observed in normal serum controls.*

is not available at this time; there are published data consistent with p10 occupying a terminal position in the *gag* polyprotein (2). Evidence from studies of avian type C viruses indicates that a protein (p15) other than the major component of the ribonucleoprotein complex (p12) is split off first in the processing of the *gag* precursor, Pr76 (21). On the other hand, MuLV p10 is the major protein component of the MuLV ribonucleoprotein (3, 6). Thus, it must be taken into consideration in postulating a direct relationship of cell surface gP95 and gP85 to intracellular Pr75 and Pr65, that this would imply a difference in the processing of the MuLV and avian viral *gag* products for virion assembly—in particular with respect to the functionality of the viral proteins that are the first to be split off. This and other models for the origin of gP95 and gP85 are currently being tested.

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