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COMPARISON OF THE CARBOHYDRATE PORTION OF MEMBRANE H-2 ALLOANTIGENS ISOLATED FROM SPLEEN CELLS AND TUMOR CELLS

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SUMMARY

[^{3}H]Fucose- and [^{3}H]glucosamine-labeled glycopeptides were obtained from H-2 transplantation alloantigen glycoproteins purified by specific antisera from spleen cells of two strains of inbred mice, C57BL/6 (H-2^b) and BALB/c (H-2^d). Upon Sephadex G-50 column chromatography, these radiolabeled glycopeptides were found to have an elution volume corresponding to a molecular weight of approx. 3300. This value is identical to the molecular size of glycopeptides from H-2 alloantigens isolated from a fibrosarcoma (Meth-A) tumor cell line derived from the BALB/c mouse and a lymphoma (E.L. 4) cell line derived from the C57BL/6 mouse. Upon DEAE-Sephadex column chromatography, the H-2 alloantigen glycopeptides of spleen cells also showed properties almost identical to the glycopeptides of antigen isolated from tumor cells. These findings confirm that the properties of the glycopeptides of H-2 alloantigens from normal spleen and tumor sources were not different by the methods used for comparison.

INTRODUCTION

The rich and varied representation of carbohydrate moieties on the mammalian cell surface is of considerable interest from the viewpoint of the probable role of such compounds in various kinds of cell membrane function such as cell to cell recognition, growth control, *etc.* The possible involvement of carbohydrate alteration in the malignant process has also been suggested by the findings of differences in glycolipid patterns between normal and tumor cells[1-5], and by changes in glycoprotein patterns in cells having undergone viral transformation[6-8]. With respect to the glycoprotein studies, the change of carbohydrate composition as a whole could be explained by a change in the carbohydrate structure of individual membrane glycoproteins, by a change in the population of membrane glycoproteins with unchanged carbohydrate structure[7] or by a combination of these possibilities. Comparative studies of the carbohydrate structure of specific tumor and normal cell membrane glycoproteins are needed in order to resolve the basis of the reported observations.

Membrane located H-2 alloantigens are the major transplantation antigens of mice. The antigenic sites are found on soluble glycoprotein fragments of about 55000

mol.wt. containing 90 % protein and 10 % carbohydrate which can be released from membranes by papain digestion. The carbohydrate is composed of mannose, galactose, fucose, glucosamine and sialic acid[11]. The general properties of the carbohydrate chains have been established by examining the glycopeptides released by pronase digestion of radioactively labeled H-2 antigens from mouse tumor cells[11,12]. It was found that the H-2 glycopeptides from three types of tumor cells (Meth-A, a fibrosarcoma line from BALB/c mice (H-2^d), MTC, a mast cell tumor line from DBA/2 mice (H-2^d,) and E.L. 4, a lymphoma line from C57BL/6 mice (H-2^b) had a unique molecular size (approx. 3300) as compared to the diverse overall size distribution of membrane or cell surface glycopeptides of these tumor cells.

The availability of a method for the purification of the H-2 alloantigens and for the assessment of the Sephadex- G50 and DEAE-Sephadex elution characteristics of their pronase glycopeptides provided a unique opportunity to examine and compare the properties of a specific characterized membrane glycoprotein isolated from normal cells and from tumor cell lines. This paper presents the results of such a study comparing the H-2 glycopeptides from antigen isolated from splenic lymphoid cells of two inbred mouse strains, C57BL/6 (H-2^b) and BALB/c (H-2^d), with the antigen isolated from the E.L. 4 lymphoma cell line derived from the C57BL/6 mouse and the Meth-A fibrosarcoma cell line derived from the BALB/c mouse.

MATERIALS AND METHODS

Carbohydrate labeling of cells

Spleen cells from C57BL/6 or BALB/c mice (Jackson Laboratory) were cultured in an incubator in 10 Falcon plastic tubes (12 mm × 75 mm) (Kramer Scientific Co.). Each tube contained $2 \cdot 10^8$ cells in 1 ml of RPMI-1640 medium (Microbiological Associates) with 10 % fetal calf serum (Grand Island Biological Co.), 20 μg of penicillin and streptomycin, 50 μC of [^{3}H]fucose (4.3 C/mM) or [^{3}H]glucosamine (1.3 C/mM) (New England Nuclear Co.). After a predetermined time (8 h for C57BL/6, 15 h for BALB/c cells) cells were collected by centrifugation at 1000 rev./min for 10 min, washed twice with RPMI-1640 medium. About 1–2 % of the radioactive sugars added in the medium was incorporated into cells.

Purification of H-2 alloantigens labeled with [^{3}H]fucose or [^{3}H]glucosamine from spleen cells

Spleen cells from 10 mice cultured with [^{3}H]fucose or [^{3}H]glucosamine were pooled with cells from ten unlabeled spleens as carrier, and the crude membrane fraction was prepared as described previously[9]. About 20 % of the radioactivity present in the cells was recovered in the crude membrane fraction. The carbohydrate labeled H-2 alloantigens were then solubilized from the membrane fraction by papain digestion, partially purified by Sephadex G-150 column chromatography and finally purified by antigen–antibody complex formation using IgG fractions from H-2 alloantiserum by the procedure described for the preparation of carbohydrate labeled H-2 alloantigens from tumor cells[11,12]. As compared to the radioactivity in the membranes, recovery of [^{3}H]fucose in H-2 alloantigen fraction was about 0.3 %, and that of [^{3}H]glucosamine about 0.1 %. This recovery is similar to that observed upon the purification of carbohydrate labeled H-2 alloantigens from tumor cells[11].

The specificity of the antigen–antibody complex formation used as the final

purification procedure was checked as described previously[11,12] and also confirmed from the following evidence. (a) The IgG fraction against H-2^b specificities (H-2 specificities 2,5, 22, 33) complexed only H-2^b materials not H-2^d materials, and *vice versa*. (b) Highly purified H-2 alloantigens from C57BL/6 (H-2^b) and DBA/2 (H-2^d) mice spleens[9] specifically inhibited the complex formation of the labeled antigens of the same H-2 genotype.

Other materials and methods

Tumor cells were prepared in radioactively labeled form as described previously[11,12]. The preparation of the IgG fractions from H-2 alloantiserum, the conditions of pronase digestion, the measurement of radioactivity, and the preparation and properties of [^{14}C]glucosamine-labeled H-2 glycopeptides from EL-4 cells (mol. wt. 3300) were described in previous reports[11,12].

RESULTS

Comparison of H-2 glycopeptides from spleen cells and tumor cells upon Sephadex G-50 column chromatography

The carbohydrate-labeled H-2 alloantigen glycoprotein fragments from spleen cells were reduced to glycopeptides by pronase digestion. The [^{3}H]glycopeptides were then subjected to Sephadex G-50 column chromatography together with an internal standard preparation of [^{14}C]glucosamine labeled H-2 glycopeptides from E.L. 4 tumor cells. A sharp single peak was observed for every H-2 glycopeptide preparation

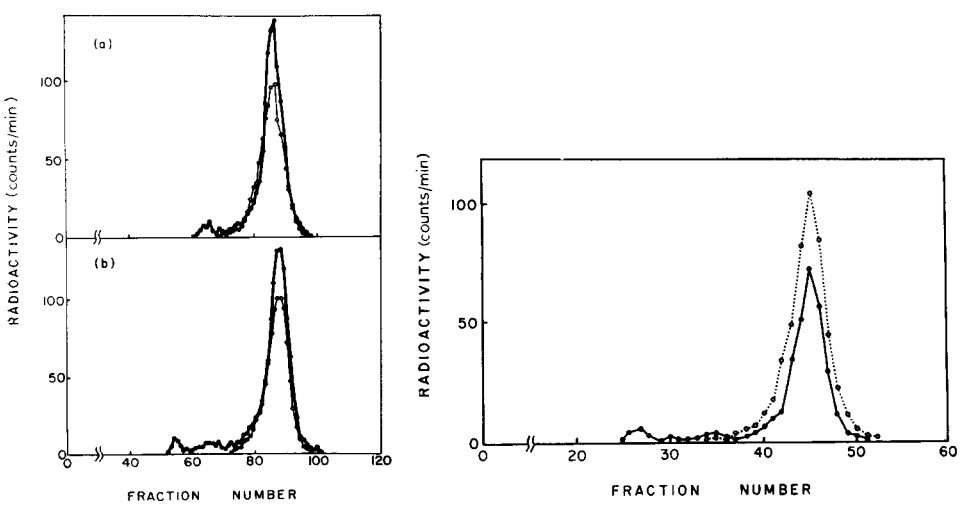

Fig. 1. Sephadex G-50 column chromatography of [^{3}H]fucose-labeled H-2 glycopeptides (●—●) from normal BALB/c cells (H-2^d, Fig. 1a) and normal C57BL/6 cells (H-2^b, Fig. 1b). [^{14}C]glucosamine-labeled H-2 glycopeptides (○—○) from EL-4 tumor cells were also run as internal standards. A column of Sephadex G-50, fine (0.9 cm × 110 cm) was equilibrated and eluted with 0.05 M NaCl, 0.0033 M Tris–HCl (pH 8.4) buffer, and 0.65-ml fractions were collected. Radioactivity was expressed as counts/min per fraction. Void volume of the column corresponded to the Fraction 57.

Fig. 2. Sephadex G-50 column chromatography of [^{3}H]glucosamine-labeled H-2 glycopeptides (●—●) from normal BALB/c (H-2^d) cells and [^{14}C]glucosamine-labeled H-2 glycopeptides (○—○) from EL-4 tumor cells (H-2^b). Conditions were same as for Fig. 1, except 1.3-ml fractions were collected.

from spleen cells so far examined, *i.e.*, [^{3}H]fucose- or [^{3}H]glucosamine-labeled H-2 glycopeptides from BALB/c (H-2^d, Figs. 1a and 2) and [^{3}H]fucose labeled H-2 glycopeptides from C57BL/6 (H-2^b, Fig. 1b). In all cases, the profile of the H-2 glycopeptide pattern from spleen derived H-2 alloantigen coincided with the profile of the internal standard from tumor cell H-2 alloantigen. Differences of 200 mol. wt. would have been detectable under the chromatographic conditions used[11].

As already reported, the chromatographic patterns on Sephadex G-50 column chromatography of the H-2 glycopeptides from the E.L. 4 or Meth-A cells show a single sharp peak corresponding to molecular weight of 3300 calculated from glycopeptide markers[11]. In view of the complete coincidence of the pattern for the labeled glycopeptides from spleen cells of the two strains examined in the present experiments to the pattern of the internal standard from E.L. 4 cells, we can conclude that the glycopeptides from H-2 glycoproteins of normal spleen cells and tumor cells are identical in molecular weight.

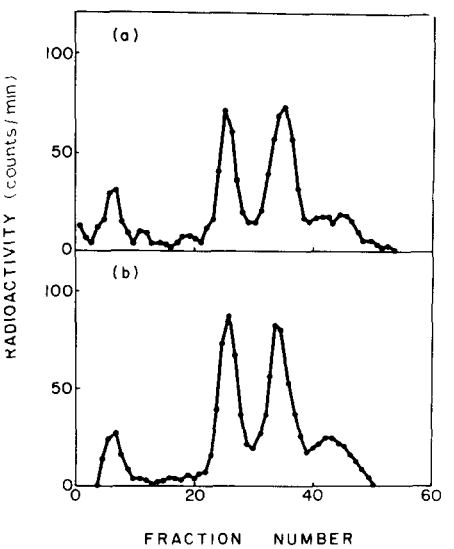

Fig. 3. DEAE-Sephadex A-25 column chromatography of [^{3}H]fucose-labeled H-2 glycopeptides from normal BALB/c (H-2^d) spleen cells (a) and Meth-A tumor cells (H-2^d) (b). The glycopeptide solution was desalted on a column of Sephadex G-15 equilibrated with 0.01 M Tris–HCl buffer (pH 8.4) and then applied to a column of DEAE-Sephadex (0.9 cm × 10 cm) equilibrated with 0.01 M Tris-HCl buffer (pH 8.4). Elution was begun with 15 ml of the same buffer, and then a linear gradient elution was initiated. The mixing chamber contained 100 ml of the starting buffer, and the reservoir contained 100 ml of 0.3 M NaCl, 0.01 M Tris–HCl buffer (pH 8.4). 2 ml per fraction was collected.

Comparison of H-2 glycopeptides from spleen cells and tumor cells by DEAE-Sephadex column chromatography

The H-2 glycopeptide fractions from E.L. 4 cells or Meth-A cells were previously separated into two major fractions upon DEAE-Sephadex A-25 column chromatography[11]. We examined the [^{3}H]fucose-labeled H-2 glycopeptides from BALB/c spleens by DEAE-Sephadex column chromatography and also found two major peaks (Fig. 3a). The elution profile appeared essentially identical to the profile of [^{3}H]fucose-labeled H-2 glycopeptides from Meth-A cells, shown in Fig. 3b for comparison.

DISCUSSION

The present findings of essentially coincident patterns for the H-2 glycopeptides from spleen cells and tumor cells upon Sephadex G-50 and DEAE-Sephadex chromatography strongly suggests that the carbohydrate moieties of the antigens are almost identical whether derived from normal lymphoid spleen cells or tumor cells derived from the same inbred mouse line. The overall similarity in content of neutral sugar, glucosamine and sialic acid between both tumor and normal cells has already been reported[10]. The present report also supports and extends our previous conclusions that the H-2 alloantigens from tumor cells and normal cells are very alike in overall biochemical properties[10] and that, further, the carbohydrate moieties of the antigens from cells of different H-2 genotypes[11] are also extremely similar.

While differences in sequence or linkage within the carbohydrate chain of the H-2 antigen of normal spleen lymphoid cells and the derived tumor cells might go undetected by our chromatographic methods, our detection system would have detected differences of greater than 200 in molecular weight. Our results thus show that for at least one specific membrane glycoprotein, there is no difference in the glycopeptide size between the product from normal and tumor sources. Hence, this suggests that the disorder in malignant cells accounting for changes in the overall glycopeptide pattern on Sephadex chromatography[6–8] must be selective and not a general change found in all glycoproteins.

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