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Expression of the T antigen on a T-lymphoid cell line, SupT1

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We have measured glycosyltransferase activities of SupT1 cells, a T-lymphoid cell line shown to react with autoantibodies in the sera of many HIV patients. Since considerable α-*N*-acetylgalactosaminyl-transferase and β1, 3 galactosyltransferase activities were found in SupT1 cells, at least the O-glycan core 1 structure can probably be synthesized. FACS analysis using an anti-T monoclonal antibody showed expression of the T antigen (Gal β1-3 GalNAc). Glycoproteins with the T antigen were isolated by immunoprecipitation with the anti-T antibody from a SupT1 cell lysate labelled metabolically with ^{3}H-glucosamine and then analysed by SDS-PAGE. It was revealed that the precipitate contained a glycoprotein with a molecular weight corresponding to that of leukosialin. O-glycans were prepared from the immunoprecipitate by alkaline-borohydride treatment and then fractionated on Bio-Gel P-2, GalNAcOH and Gal-GalNAcOH being identified *inter alia*. These results suggest that an anti-T antibody may be included in the autoantibodies found in HIV-1 infected individuals.

Keywords: HIV type 1, leukosialin, O-glycan, carbohydrate antigen, glycosyltransferase

Introduction

T (Thomsen Friedenreich) and Tn antigens are known as representative cancer-associated carbohydrate antigens which are produced on incomplete synthesis of O-glycosidic carbohydrate chains [1]. The cryptic T antigen, which has revealed following sialidase treatment of human blood cells, is reported to have the structure, Galβ1-3GalNAc [2]. Recently, we have found that the Tn antigen has a cluster structure composed of $(GalNAc\text{-}Ser/Thr)_{3\sim4}$ and is expressed on leukosialin, a major membrane glycoprotein in a leukaemic cell line, Jurkat, as well as on cancer cells and ovine submaxillary mucin [3–5]. The carbohydrate structure of Jurkat leukosialin is unique in that most of the carbohydrate chains are truncated in contrast to those of leukosialin from normal leukocytes, the O-glycans of which have more complex structures. Similar experiments revealed that many T-lymphoid cell lines expressed truncated O-glycans [6].

In line with these previous results, we attempted to characterize the O-glycans of leukosialin in SupT1, a T-lymphoid cell line. Many HIV-1 infected individuals produce autoantibodies that bind specifically to the cell surface carbohydrate antigen that is present on leukosialin (CD43) of SupT1 cells [7]. In the present study, we show that the T antigen is expressed on the leukosialin of SupT1 cells.

Materials and methods

^{3}H-Glucosamine-HCl (57.7 Ci mmol^{-1}) and ^{3}H-ENHANCE were purchased from New England Nuclear, Boston. T-lymphoid cell line SupT1 was grown in RPMI 1640 supplemented with 10% calf serum. Monoclonal anti-T antibody 49H.24 (IgM) was kindly provided by Dr B. Longenecker (Department of Immunology, University of Alberta, Edmonton, Alberta, Canada).

Immunofluorescein analysis of the cell surface

For detection of the T antigen, SupT1 cells (1.0×10^6) were incubated for 30 min at 4°C with 49H. 24 (1 μg ml^{-1}) and then with or without rabbit anti-mouse IgM antibody (5 μg ml^{-1}), and finally with FITC-protein A (5 μg ml^{-1}). After each incubation, the cells were extensively washed with PBS. The labelled cells were analysed by flow immunocytofluorometry with a FACS (Becton Dickinson).

Metabolic labelling of cells

SupT1 cells were labelled with ^{3}H-glucosamine (10 μCi ml^{-1}) in RPMI 1640 containing 0.02% glucose for 20 h. The har-

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vested cells were solubilized with 1% Triton X-100, 0.2 M NaCl, 5 mM PMSF in 50 mMTris-HCl buffer, pH 7.4, and then the lysate was centrifuged at 105 000 × **g** for 1 h. The supernatant thus obtained was incubated successively with 49H.24, rabbit anti-mouse IgM antibody and protein A-Sepharose. The immunoprecipitate was extensively washed with the above solution and then subjected to SDS-PAGE using the Laemmli system [8], followed by fluorography.

Glycosyltransferase assay

Asialo-ovine submaxillary mucin (asialo-OSM) was used as a substrate for the assaying of α2,6 sialyltransferase and β1,3 galactosyltransferase [9, 10]. Polypeptide N-acetylgalactosaminyltransferase was assayed according to Sugiura et al. [11].

Preparation and fractionation of oligosaccharides

An immunoprecipitate was prepared from a SupT1 cell lysate labelled with ^{3}H-glucosamine and then treated with 0.05 M NaOH, 1 M $NaBH_4$ for 16 h at 45°C according to Carlson [12]. The released oligosaccharides were subjected to fractionation on Bio-Gel P-2. Standards, ^{3}H-labelled GalNAcOH and Galβ1, 3GalNAcOH, were prepared from GalNAc and Galβ1, 3GalNAc by reduction with ^{3}H-$NaBH_4$.

Results and discussion

It has been reported that a selective loss or repression of the O-glycan core 1 β1,3 galactosyltransferase gene causes the exposure of *N*-acetylgalactosamine residues on the cell surface glycoproteins of blood cells of Tn individuals and thus antigenicity is acquired against the truncated O-glycans [13, 14]. We, therefore assayed sialyltransferase and galactosyltransferase activities involved in the synthesis of the core regions of O-glycans. In addition to various T-lymphoid cell lines, LS 180 cells, a human colorectal cancer cell line, were examined as a reference. LS 180 cells are known to produce a large amount of mucin-type glycoproteins.

As shown Fig. 1, polypeptide α-*N*-acetylgalactosaminyltransferase activity of SupT1 cells was comparable to that of Jurkat and Molt 4 cells, both of which express the Tn antigen on leukosialin. In contrast, β1,3 galactosyltransferase activity of SupT1 cells was much higher than that of Jurkat and Molt 4 cells, and comparable to that of LS 180 cells, indicating that the O-glycan core 1 structure, Galβ1, 3GalNAc-Ser/Thr, could be synthesized in SupT1 cells. In addition, α2,6 sialyltransferase activity of SupT1 cells was lower than that of Jurkat and Molt 4 cells, and much lower than that of LS 180 cells, suggesting that sialylated T or Tn antigens are poorly expressed in SupT1 cells.

We then tried to determine which carbohydrate antigens are expressed. SupT1 cells (1×10^6) were incubated with an anti-T antibody (IgM), and then an anti-mouse IgM antibody and FITC-protein A, successively, and examined by FACS. A control experiment was carried out without the secondary antibody. As shown in Fig. 2, it was clear that the T antigen was

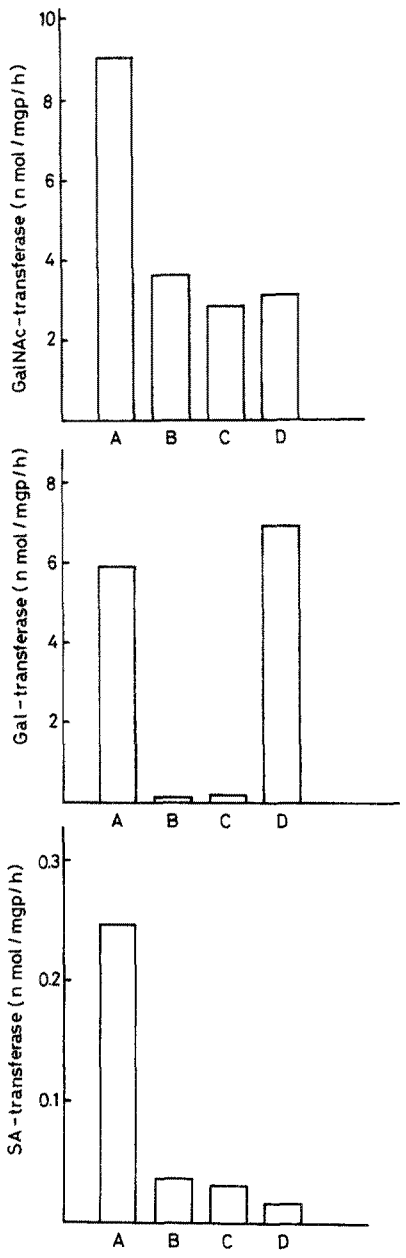

Figure 1. Glycosyltransferase activities of various T-lymphoid cell lines and LS 180 cells. α-*N*-acetylgalactosaminyltransferase, β1,3 galactosyltransferase, and α2,6 sialyltransferase activities were determined as described under Materials and methods. Mean values from duplicate experiments (±5% deviation) were used. A: LS 180, B: Jurkat, C: Molt 4, D: SupT1.

expressed on SupT1 cells. Although the fluorescence intensities were relatively low, it appears likely that short carbohydrate chains such as the T antigen were not readily accessible to the antibody due, probably, to steric hindrance. Similar results were obtained for the Tn antigen on Jurkat cells (data not shown).

To further confirm the expression of the T antigen and to identify glycoproteins carrying the T antigen, we prepared a SupT1 cell lysate metabolically labelled with ^{3}H-glucosamine.

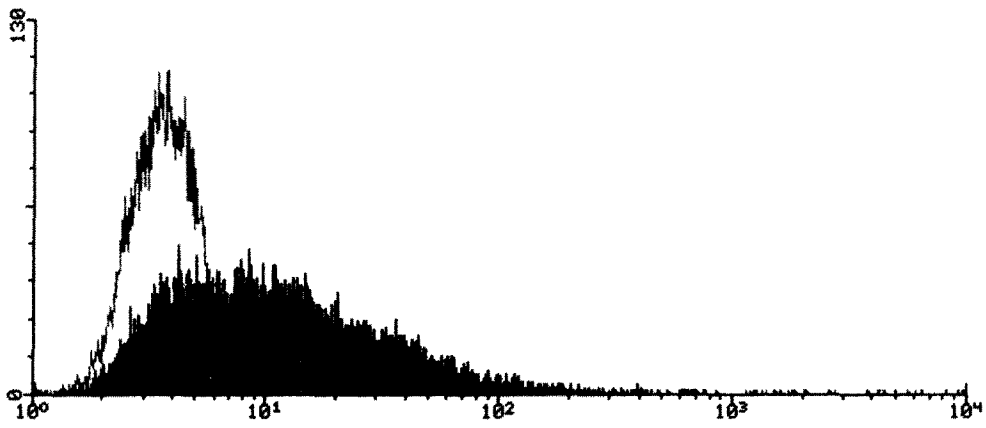

Figure 2. Expression of the T antigen on the cell surface of SupT1 cells. Analysed by FACS, SupT1 cells were incubated with an anti-T antibody (IgM), an anti-mouse IgM antibody and FITC-protein A, successively, as described under Materials and methods. The control experiment is represented in the histogram by the blank space.

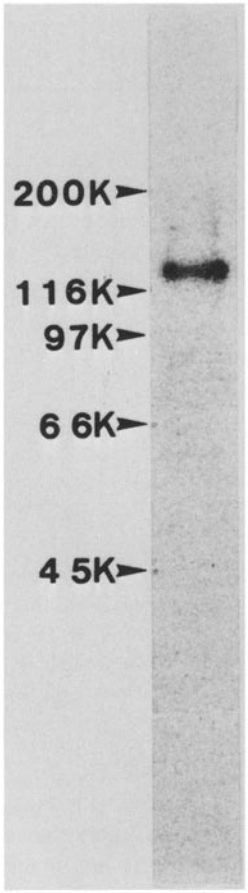

Figure 3. SDS-PAGE of T antigen glycoproteins. SupT1 cells (1×10^7) were labelled with ^{3}H-glucosamine and after which T antigen glycoproteins were immunoprecipitated from a lysate. The glycoproteins were subjected to SDS-PAGE followed by fluorography.

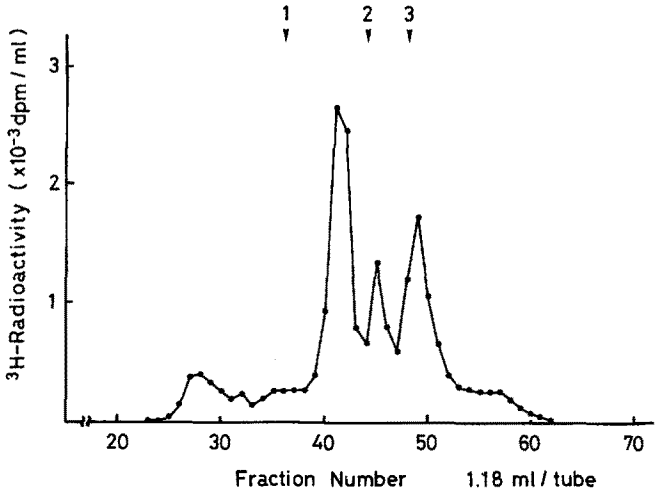

Figure 4. Gel filtration of O-glycans released from the T antigen glycoproteins by alkaline borohydride treatment. Immunopurified T antigen glycoproteins were treated with 0.05 M NaOH and 1 M $NaBH_4$ at 45°C for 16 h, and then applied to a column of Bio-Gel P-2 (1.0 × 113 cm). Fractions of 1.18 ml were collected and the radioactivity was determined. 1) SAα2,6 GalNAcOH; 2) Galβ1,3 GalNAcOH; 3) GalNAcOH.

The T antigen glycoproteins were immunoprecipitated with an antibody and then subjected to SDS-PAGE. Fluorography revealed a single band corresponding to a molecular weight of about 120 kDa, which apparently coincided with the molecular weight of leukosialin expressed on Jurkat cells (Fig. 3).

Further experiments were carried out to confirm the presence of Gal-GalNAc in the immunoprecipitated glycoprotein. The immunoprecipitate was treated with 0.05 M NaOH and 1 M

$NaBH_4$ at 45°C for 16 h to release O-glycans. The oligosaccharides were then fractionated on Bio-Gel P-2, as shown in Fig. 4. Most of the oligosaccharides consisted of small carbohydrate chains such as GalNAcOH and Gal-GalNAcOH. GalNAcOH and Gal-GalNAcOH were further identified by paper chromatography (data not shown). The largest peak, material eluted in front of Gal-GalNAc, could not be identified, but its estimated molecular weight corresponded to that of a non-sialylated trisaccharide. In the case of Jurkat and Molt 4 cells, GalNAcOH was predominant, which is consistent with the fact that β1,3 galactosyltransferase is present in SupT1 cells, but only in trace amounts in Jurkat and Molt 4 cells. The presence of a considerable amount of unsubstituted GalNAc in SupT1 cells, in spite of the presence of β1,3 galactosyltransferase, suggests that the synthesis of oligosaccharide chains may not be absolutely dependent on the occurrence of competent glycosyltransferases. This has often been observed for many cancer cells, such as LS 180 cells, producing mucin-type glycoproteins. In these cells, all glycosyltransferases necessary for the synthesis of O-glycans are present, yet truncated O-glycans are abundant.

The presence of unsubstituted GalNAc residues on SupT1 cells suggests that the Tn antigen may also be expressed, this being consistent with the finding of Hansen *et al.* [15]. Thus an immunoprecipitate was prepared from metabolically labelled SupT1 cells using the monoclonal antibody, MLS 128, which recognizes the Tn antigen, and then analysed by SDS-PAGE. Fluorography revealed, however, a single discrete band at a position corresponding to a slightly higher molecular weight than that of the T antigen glycoprotein.

Since the essential structure of the Tn antigen is a $(GalNAc\text{-}Ser/Thr)_{3\text{-}4}$ cluster [3–5], the GalNAc residues present in the T antigen glycoprotein should be linked to the polypeptide chain in a scattered fashion. This and the present findings suggest that a part of the synthetic pathway for O-glycans is defective in T cells infected with HIV, so that truncated O-glycans such as the T and Tn antigens are produced. It is important to investigate the biological roles of glycoproteins with truncated O-glycans. For instance, leukosialin appears to play an important role in the immune system [16, 17], regulating T cell adhesion [18]. Since normal leukosialin is a heavily sialylated glycoprotein, unusually glycosylated leukosialin may have different biological properties which affect cell behaviour.

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