ABSTRACT

Various T-lymphoid cells were labeled with [3H]glucosamine and then cell lysates were prepared from them. The Tn antigen was immunoprecipitated and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by fluorography. The Tn antigen was found to be expressed on leukosialin, a major glycoprotein of T-lymphoid cells. The carbohydrate moieties of leukosialin were isolated from Jurkat and Molt 4 cells by alkaline borohydride treatment. The leukosialin in both cases predominantly contained single N-acetylgalactosamine residues, consistent with expression of the Tn antigen. Tryptic glycopeptides containing antigenic sites were isolated using an MLS 128 immunoaffinity column and purified by gel filtration and reverse phase column chromatographies. Sequence analyses revealed that all the glycopeptides obtained contained three consecutive residues of N-acetylgalactosamine-Ser/Thr, supporting the idea that the epitopic structure is a cluster of N-acetylgalactosamine-Ser/Thr.

INTRODUCTION

MLS 128, a murine monoclonal antibody, was established by immunizing mice with a human colorectal cancer cell line, LS 180 cells (1). Its binding to mucin glycoproteins containing the Tn antigen was inhibited competitively by NCC-LU-35 (2) and CA 3239 (3), which are known to recognize the Tn antigen. Using MLS 128, we have shown that the epitopic structure of the Tn antigen expressed on T lymphophorin A contains a cluster of three or four consecutive GalNAc-Ser/Thr residues (4).

It has been reported that the Tn antigen is expressed in over 70% of human adenocarcinoma cells (5). The Tn antigen is also expressed on leukosialin, a major membrane glycoprotein, in a leukemic cell line, Jurkat (6). The carbohydrate structure of Jurkat leukosialin is unique in that most of the carbohydrates are truncated in contrast to those of leukosialin from normal leukocytes, of which the O-glycan structures are more complex.

We have investigated expression of the Tn antigen on leukosialin of various T-lymphoid cells differing in the level of differentiation, such as Molt-4, Molt-3, CCRF-CEM, SKW-3, and MT-1 cells and the epitopic structures of the expressed antigens. We have also examined if the expression of the Tn antigen is correlated with the activity of β1,3-galactosyltransferase, which is regarded as a key enzyme for the formation of complex O-glycan structures (7, 8).

Materials and Methods

MLS 128 was prepared as described previously (1). Protein A-Sepharose and Bio-Gel P-2 were from Pharmacia, Uppsala, Sweden, and Bio-Rad, Richmond, VA, respectively. MLS 128-protein A-Sepharose was prepared according to the method of Schneider et al. (9). [3H]Glucosamine-HCl and [3H]Glucosamine were purchased from New England Nuclear, Boston, MA. Jurkat cells were kindly provided by Dr. M. Fukuda (Cancer Research Center, La Jolla, CA). Other T-lymphoid cell lines were provided by the Fujisaki Cell Center, Hayashibara Institute of Biochemistry. They were cultured in RPMI 1640 supplemented with 10% fetal calf serum.

Expression of the Tn Antigen on Various T-Lymphoid Cells. The Tn antigen glycoproteins were isolated by immunoprecipitation and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by fluorography. As shown in Fig. 1, Molt-4 and CCRF-CEM cells gave a discrete band, as Jurkat cells did, but Molt-3, SKW-3, and MT-1 cells did not (data not shown for the latter two cell lines). The molecular weight of leukosialin differed slightly from one cell type to another due to the heterogeneity of the O-glycans.

We determined the activity of β1,3-galactosyltransferase in Jurkat cells and compared it with those in K562 and HL60 cells, which have longer oligosaccharide chains of O-glycans containing the Galβ1→3GalNAc structure. The enzyme activity of Jurkat cells was about one-tenth those of K562 and HL60 cells. Since the activity was not completely deficient, it is uncertain whether all the cells...
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Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of glycoproteins prepared from T-lymphoid cells by immunoprecipitation with MLS 128. T-lymphoid cells (1 × 10^7 cells) were labeled with [3H]glucosamine (10 μCi/ml) metabolically. From each lysate, the Tn antigen glycoproteins were immunoprecipitated with MLS 128 and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by fluorography. Lane A, Jurkat; Lane B, CCRF-CEM; Lane C, Molt-4; Lane D, Molt-3. K, thousands. Ordinate, molecular weight markers.

Table 1

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<tr>
<th>Fraction</th>
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DISCUSSION

The Tn antigen discovered by Dausset et al. (14) was found to be a tumor-associated antigen (15). Recently, we showed that the essential structure of the Tn antigen contained in ovine submaxillary mucin is a cluster of GalNAc-Ser/Thr, i.e., (GalNAc)-Ser-(GalNAc)-Thr-(GalNAc)-Thr (16), and that in Tn glycophorin A comprises a cluster...
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Fig. 3. O-Glycans prepared from Tn antigen glycoproteins by alkaline borohydride treatment. Tn antigen glycoproteins prepared from Jurkat (A) and Molt 4 (B) cells were treated with 0.05 M NaOH and 1 M NaBH₄ at 45°C for 16 h, followed by gel filtration on a Bio-Gel P-2 column (1.0 × 113 cm). Fractions of 1.12 ml were collected and the radioactivity was determined. Arrows 1, 2, and 3, positions of Siaα2→6GalNAc-ol, Galβ1→3GalNAc-ol, and GalNAc-ol, respectively.

Fig. 4. Immunoaffinity chromatography of glycopeptides prepared from leukosialin. A tryptic digest of leukosialin was applied to an MLS 128 immunoaffinity column (0.7 × 11.3 cm). After extensive washing, the column was eluted with 50 mM diethylamine, pH 11.5 (arrow). Fractions of 4.4 ml were collected and the radioactivity was determined.

Fig. 5. Gel filtration of glycopeptides exhibiting Tn antigenicity. Glycopeptides eluted from an MLS 128 immunoaffinity column were fractionated on TSK gels (G3000 and G2500) by HPLC as described under "Materials and Methods." Fractions A, B, and C were obtained.

of three or four GalNAc-Ser/Thr moieties (4). In addition, we found that in Jurkat cells, a T-lymphoid cell line, the Tn antigen was expressed on leukosialin (6). Immunostaining of Jurkat cells with MLS 128 showed that they expressed the Tn antigen homogeneously. Since β-1,3-galactosyltransferase was not completely lost, this fact suggests that some of the GalNAc-Ser/Thr may be galactosylated, yielding longer carbohydrate chains. In fact, it was reported that 17% of O-glycans prepared from Jurkat cell leukosialin are longer carbohydrate chains (12).

Other cell lines such as K562 and HL60 do not exhibit any Tn antigenicity and have high β1,3-galactosyltransferase activity. Cells positive for Tn antigenicity exhibited very low β-1,3-galactosyltransferase activity except for CCRF-CEM cells, which expressed the Tn antigen in spite of the fact that the enzyme activity was at a level comparable to those in K562 and HL60 cells (data not shown). There may be another factor controlling the expression of the Tn antigen.

According to the primary structure of leukosialin deduced from its complementary DNA (13), various cluster structures of GalNAc-Ser/Thr exist in it. Separation of immunoreactive glycopeptides from the tryptic digest of leukosialin from Jurkat cells was not easy, probably due to the similarity of the glycopeptides in their binding properties to the HPLC columns. The amino acid sequences of some glycopeptides (B-1 and C-1) completely coincided with those of the fragments which were expected to be produced from leukosialin on trypsin digestion. These fragments contained cluster structures of GalNAc-Ser/Thr. This glycopeptide is also presumed to contain cluster structures of GalNAc-Ser/Thr, as judged from the primary structure of leukosialin. In addition, the major peak of the C fraction contained the glycopeptide from Leu-209 to Arg-234 contaminated by other glycopeptides (data not shown). These results suggest that the cluster of three or four consecutive GalNAc-Ser/Thr structures is essential for
Leukosialin appears to play an important role in the immune function. It has been shown that leukosialin is absent on cells from patients with Wiskott-Aldrich syndrome characterized by eczema, thrombocytopenia, and profound immunodeficiency (17). Antibodies against leukosialin cause proliferation of peripheral blood T-cells in the presence of monocyes (18). Therefore, leukosialin may be involved in T-cell activation. These truncated O-glycans may influence the cell to cell interactions. Elucidation of the biological significance leukosialin is now in progress.

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Tn Antigen Is Expressed on Leukosialin from T-Lymphoid Cells

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