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Changes in the expression of *N*-acetylglucosaminyltransferase III, IV, V associated with the differentiation of HL-60 cells

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By use of a new assay method based on HPLC, GlcNAc-transferase III, IV and V activities were determined in HL-60 cells, differentiated HL-60 cells and normal myeloid cells. Differentiation along the monocytic lineage with 1α,25-dihydroxyvitamin D_3 resulted in increased GlcNAc-transferase IV and decreased GlcNAc-transferase III activity. Differentiation along the myeloid lineage with retinoic acid resulted in a decrease in GlcNAc-transferase III activity. Although differentiated HL-60 cells show a changed GlcNAc-transferase pattern, they do not resemble normal myeloid cells. Macrophages and granulocytes are characterized by a very low level of GlcNAc-transferase III activity whereas differentiated HL-60 cells still contain this activity. This is the first demonstration of GlcNAc-transferase IV and V activity in a human cell.

N-Acetylglucosaminyltransferase III; *N*-Acetylglucosaminyltransferase IV; *N*-Acetylglucosaminyltransferase V; Differentiation induction; HPLC; Glycoprotein synthesis; (HL-60 cell)

1. INTRODUCTION

Cells of the human promyelocytic leukemic cell-line HL-60 can be induced to undergo myeloid differentiation by retinoic acid [1–4] or DMSO [1–4] and monocytic differentiation by vit D_3 [5] and Ara-C [6]. The asparagine-*N*-linked oligosaccharides found on the cell surface of HL-60 cells differ in size and structure from those found on the cell surface of more differentiated cells [7,8]. Mizoguchi and co-workers [8] suggested that these changes are due to a changed expression of *N*-acetylglucosaminyltransferases, involved in the biosynthesis of tri- and tetraantennary *N*-linked chains, bisected structures and elongated chains. To obtain more insight into the enzymatic basis for the differentiation-dependent structural alterations, we assayed the activities of GlcNAc-transferase III, IV and V (see fig.1) in HL-60 cells and differentiated cells by use of a new method based on HPLC.

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Abbreviations: GlcNAc, *N*-acetylglucosamine; Man, mannose; Ara-C, cytosine-arabinoside; DMSO, dimethyl sulfoxide; vit D_3, 1α,25-dihydroxyvitamin D_3; HPLC, high-performance liquid chromatography; Mes, 2-(*N*-morpholino)ethanesulfonic acid. Enzymes: UDP-GlcNAc:Manβ1→4GlcNAc-R β1→4-*N*-acetylglucosaminyltransferase, GlcNAc-transferase III (EC 2.4.1.144); UDP-GlcNAc:Manα1→3Man-R β1→4-*N*-acetylglucosaminyltransferase, GlcNAc-transferase IV (EC 2.4.1.145); UDP-GlcNAc:Manα1→6Man-R β1→6-*N*-acetylglucosaminyltransferase, GlcNAc-transferase V (EC 2.4.1.155)

2. MATERIALS AND METHODS

Neutrophilic granulocytes were a gift from Dr L. Koenderman (Central Laboratory of the

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Netherlands Red Cross Transfusion Service, Amsterdam, The Netherlands). Human peritoneal macrophages were spun down at low speed from the fluid obtained after peritoneal dialysis. Human promyelocytic HL-60 cells were cultured in the presence or absence of differentiation-inducing agents as described in [9]. Cell pellets were homogenized at 0°C in 10 mM sodium cacodylate, pH 7.0, using a Potter-Elvehjem homogenizer. Protein concentration was determined according to Lowry et al. [10]. Reference oligosaccharides bi, bis, tri and tri′ (see fig.1) were derived by β-galactosidase (jack bean) digestion from the corresponding β4-galactosylated compounds, which were kindly donated by Dr G. Strecker (Université de Lille, Villeneuve d'Ascq, France). UDP-[^{14}C]GlcNAc (149 Ci/mol) was obtained from The Radiochemical Centre, Amersham.

2.1. *Assay of GlcNAc-transferases III, IV and V*

Incubation mixtures contained 12.5 μmol Mes buffer, pH 7.0, 20 μmol GlcNAc, 1.25 μmol $MnCl_2$, 0.5% Triton X-100, 50 nmol oligosaccharide bi, 200 nmol UDP-[^{14}C]GlcNAc (5 Ci/mol) and cell lysate (0.2–1.2 mg protein) in a volume of 100 μl. The mixtures were incubated for 18 h at 37°C. The reaction was stopped by adding 1 ml H_2O and passing the mixture through a column of 1 ml Dowex 1-X8, Ac^--form. The column was washed twice with 0.5 ml H_2O and the combined eluates were treated with $NaBH_4$ for 1 h as described [11], lyophilized and analyzed by HPLC.

2.2. *HPLC*

The lyophilized material was dissolved in 200 μl H_2O. 30 μl of each sample was analyzed by HPLC on a column (4 × 250 mm) of Lichrosorb-NH_2 (Merck, Darmstadt), using a Spectra-Physics SP 8700 delivery system, equipped with a Rheodyne 7105 injection valve. Elution was carried out as indicated in the legend to fig.2. Fractions of 0.6 ml were collected and assayed for radioactivity. UV detection of standards was per-

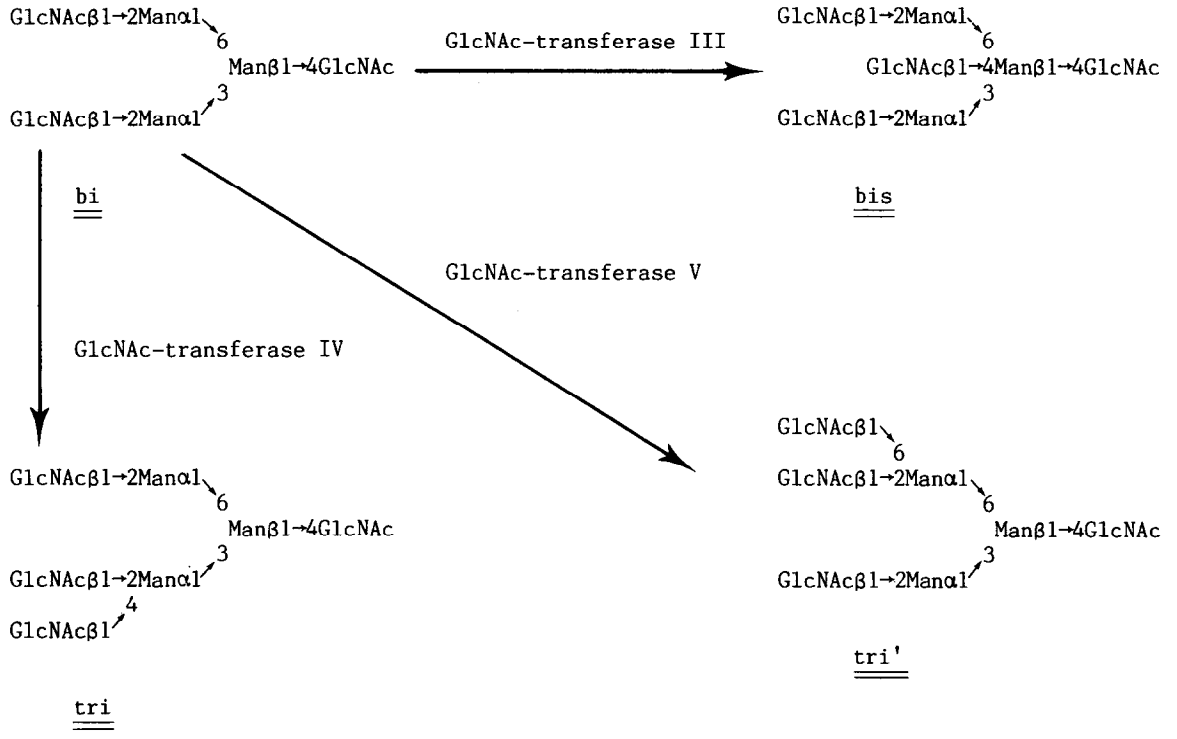

Fig.1. Product oligosaccharide structures (bis), (tri) and (tri′) formed by the action of GlcNAc-transferase III, IV and V on the acceptor oligosaccharide (bi), studied here.

formed with a Hewlett-Packard-1040 A diode array detector, controlled by Hewlett-Packard 85 microcomputer.

3. RESULTS

By use of a new assay method based on HPLC, developed as will be described elsewhere, activities of GlcNAc-transferase III, IV and V (fig.1) were determined in normal myeloid cells and differentiated HL-60 cells. Activities of the three transferases could be detected in one assay as illustrated in fig.2. A clear separation between the breakdown products of UDP-[^{14}C]GlcNAc (elution time 0–15 min) and the individual transferase products was obtained.

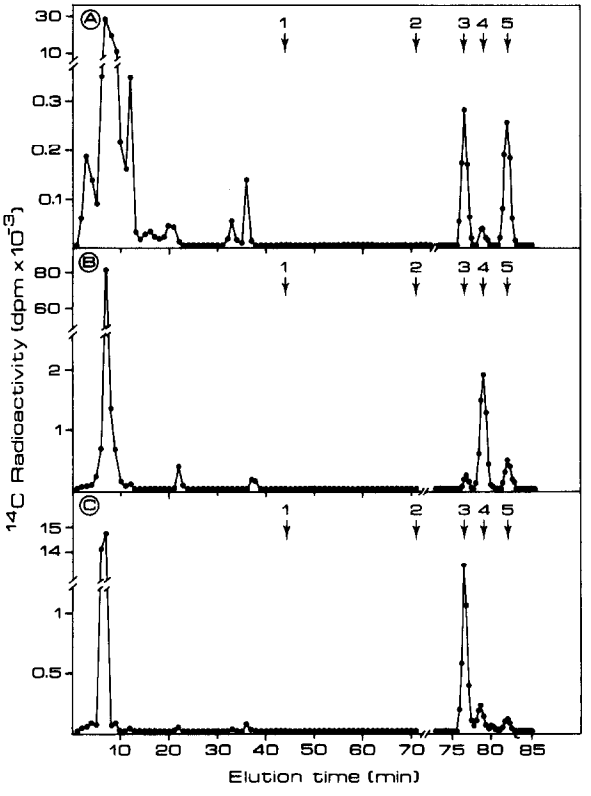

Fig.2. Assay of GlcNAc-transferase III, IV and V activities by HPLC. Analyses were performed on a Lichrosorb-NH_2 column. Elution was carried out at a flow rate of 2 ml·min^{-1}, using an acetonitrile (Lichrosolv grade)/15 mM potassium phosphate (pH 5.2) mixture (4:1, v/v) with a linear gradient, decreasing the acetonitrile concentration by 0.2% min^{-1}. Transferase activities were measured in (A) macrophages, (B) HL-60 cells and (C) vit D_3-induced HL-60 cells. As acceptor oligosaccharide bi was used (fig.1). The arrows indicate the elution position of Galβ1→3GlcNAcβ1→3Galβ1→4Glc (1) and the reduced forms of compounds bi (2), tri (3), bis (4) and tri′ (5).

In macrophages GlcNAc-transferase IV and V activities could be detected (fig.2 and table 1). Only a residual activity of GlcNAc-transferase III was found. No GlcNAc-transferase activities were found in neutrophilic granulocytes (table 1). HL-60 cells appeared to contain a relatively high GlcNAc-transferase III activity in addition to lower activities of GlcNAc-transferase IV and V (table 1). HL-60 cells were also grown in the presence of vit D_3, Ara-C, retinoic acid and DMSO. Induction with Ara-C and DMSO had no significant effect on the activities of each of the GlcNAc-transferases. Comparable activities were found in control HL-60 cells and cells induced with these agents (table 1). Induction with vit D_3, however, resulted in an increase in activity of GlcNAc-transferase IV with a concomitant decrease in GlcNAc-transferase III activity. After

Table 1

GlcNAc-transferase III, IV and V activities in normal myeloid and induced HL-60 cells

Cell	Activity of GlcNAc-transferase (pmol·mg^{-1} protein·h^{-1})		
	III	IV	V
HL-60 cells			
control	162	12	46
+ vit D_3	37	164	22
+ Ara-C	198	10	39
+ retinoic acid	39	16	43
+ DMSO	164	22	41
Normal cells			
macrophages	5	40	42
neutrophilic granulocytes	<1	<1	<1

HL-60 cells were grown for 4 days in the presence of 10^{-7} M vit D_3, Ara-C or retinoic acid or 1.25% DMSO [9]. Cell lysates were assayed for the different GlcNAc-transferase activities using oligosaccharide bi as an acceptor. Enzyme activities were assayed as described in section 2 and analyzed by HPLC as described in the legend to fig.1

induction with retinoic acid a reduction of GlcNAc-transferase III activity was seen, whereas the activities of GlcNAc-transferase IV and V remained essentially unchanged (table 1). Different ratios of GlcNAc-transferase activities in cells stimulated by vit D_3 and retinoic acid compared to the other cells are the result of these changes.

4. DISCUSSION

The demonstration that differentiation of HL-60 cells is accompanied by changes in size and structure of their oligosaccharide chains [7,8] prompted us to study the activity levels of GlcNAc-transferase III, IV and V (fig.1) in these cells. Based on the occurrence of bisected structures in HL-60 cells it was suggested that the expression of GlcNAc-transferase III is unique for cells belonging to the myeloid lineage [8]. In accord with the structures we were able to detect relatively high GlcNAc-transferase III activity in the cell lysates. This enzyme was first described in hen oviduct [12] and plays a key role in the synthesis of bisected *N*-glycans [13].

Upon induction of monocytic differentiation of HL-60 cells with vit D_3 and myeloid differentiation with retinoic acid a dramatic decrease in the activity of GlcNAc-transferase III was encountered. A still lower GlcNAc-transferase III activity was found in normal human macrophages, whereas the enzyme activity in granulocytes was below detection. The latter observation, which is consistent with the absence of bisected *N*-linked glycans on normal granulocytes [14,15], shows that the expression of GlcNAc-transferase III is not a general feature of myeloid cells as was suggested by Mizoguchi et al. [8]. Rather, it might be characteristic for a certain stage of cell maturation or, alternatively, it might be associated with the malignant character of the HL-60 cells.

Earlier bisected structures have been reported to occur in hepatomas, but to be absent in normal liver tissue [16]. In accord with this structural evidence expression of GlcNAc-transferase III was seen in a pre-cancerous stage of liver carcinogenesis [17]. This is yet another example where malignant transformation of cells is accompanied by a strong expression of this key glycosyltransferase.

Induction of myeloid differentiation of HL-60 cells by DMSO did not result in gross changes in the activities of GlcNAc-transferases III, IV and V. This would suggest that the oligosaccharide chains of HL-60 cells before and after induction with this agent will not show major differences. Indeed, it has been reported that the oligosaccharide pattern of DMSO-induced cells is very similar to that of HL-60 cells [8].

This result might seem to conflict with the decrease of GlcNAc-transferase III activity upon induction of myeloid differentiation of HL-60 cells by retinoic acid. It is, however, known that DMSO and retinoic acid act in a different way [18]. The first agent acts on less mature cells than the second inducer. Moreover, the morphological changes accompanying the differentiation process occur much faster under the influence of retinoic acid. Therefore, more pronounced changes in the levels of GlcNAc-transferases and consequently in the structures of cell surface oligosaccharides must be expected upon induction with retinoic acid.

Although induction of myeloid differentiation of HL-60 cells did not result in gross changes in the activities of GlcNAc-transferase IV and V, a greatly increased activity of GlcNAc-transferase IV was encountered upon vit D_3-induced monocytic differentiation. Normal macrophages also expressed a higher level of GlcNAc-transferase IV activity than uninduced HL-60 cells. Along with the decreased activity of GlcNAc-transferase III in vit D_3-induced cells and macrophages, these results suggest that monocytic differentiation of HL-60 cells is characterized by a shift from bisected oligosaccharide structures to tri- and tetraantennary glycans of the non-bisected type.

No GlcNAc-transferase IV and V activity could be detected in granulocytes. Yet tri- and tetraantennary structures occur on these cells [14,15]. A possible explanation is that these structures are formed at certain precursor stages, before the GlcNAc-transferase activities are lost during subsequent maturation. Further work is required to verify this possibility.

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