Glycoconjugate J (1988) 5: 271-281

Development of Distinct Clonal Patterns of Carbohydrate-binding Activity in Human Promyelocytic HL 60 Cells and Histiocytic U 937 Cells During DMSO- or PMA-Induced Differentiation

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Received March 22/June 30, 1988.

Key words: HL 60 and U 937 cells, phagocytosis, IgG, complement, glycolipid, liposome binding, fluorescence microscopy

Increased ability to recognize carbohydrate structures on particles was observed in promyelocytic HL 60 cells and histiocytic U 937 cells during differentiation induced *in vitro* with dimethylsulfoxide (DMSO) or phorbol myristate acetate (PMA). The size of the cells and increased capacity to bind and ingest IgG- or complement-coated yeast particles were used as indicators of phagocytic maturation. Carbohydrate affinities were assessed by the binding of glycolipid-containing liposomes displaying mannose, galactose, lactose, *N*-acetylgalactosamine, fucose, inositol, or ganglioside residues. With DMSO, HL 60 cells showed greater affinity for mannose and ganglioside residues, and with PMA also for fucosyl ligands. U 937 cells displayed a slightly different pattern; mannose binding was present before induction and by DMSO affinity was clearly augmented for galactose, fucose, ganglioside and inositol residues. With PMA these effects were smaller except for increased binding of lactosyl liposomes.

Subclones of cells derived from U 937 (Cl 1, Cl 2 and Cl 3) appeared more mature already in the absence of inducing agent, and the lectin activity was barely affected by DMSO or PMA. Incidentally, Cl 1 lacked mannose affinity, which was fully expressed in Cl 2. With respect to inositol and ganglioside residues the reverse pattern was observed.

In conclusion, DMSO- or PMA-mediated maturation in HL 60 and U 937 cells is accompanied by increased carbohydrate binding similar to what has been found in mature macrophages and granulocytes, indicating that these cellular systems can be used for further assessment of the molecular origin of lectin-like membrane components in phagocytic cells.

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Cell-cell recognition employs specific protein-protein and protein-carbohydrate interactions, as well as non-specific hydrophobic and ionic forces. The recognition is reciprocal in the sense that lectin-like substances may occur either on the bacterial [1-3] or on the mammalian cell membrane [4, 5], or on both, thereby promoting cell-to-cell contact [5, 6].

Different glycoconjugates have come into focus as receptor molecules for bacterial fimbriae (pili) which bind specifically to different short oligosaccharides [2]. Membrane glycoconjugates have also been identified as important recognition markers of malignant transformation [5-8].

The role of mammalian lectins is particularly well-studied in different liver cells whereby they facilitate the blood clearance of different molecules and particles which display mannosyl, galactosyl or fucosyl residues [9-11]. Lectin-like activity has been found also on intestinal cells [12].

HL 60 cells [13] develop some characteristics of polymorphonuclear leukocytes (PMNL), e.g. morphology and phagocytic capacity [14, 15], during DMSO-induction *in vitro*, although they are deficient in the content and the release of some granulae enzymes [16]. When PMA is used instead of DMSO they are assumed to undergo macrophage maturation [17, 18]. In both cases, there are specific changes in the cell surface glycoprotein patterns [19, 20]. The histiocytic cell line U 937 [21] also matures in the presence of DMSO and PMA, which is also accompanied by altered cell surface characteristics [22, 23].

The aims of the present study were to assess: (1) whether specific carbohydrate binding (lectin activity) could be induced *in vitro* with DMSO or PMA in HL 60 and U 937 cells, similar to what has been found in macrophages and granulocytes. The binding of fluorescent glycolipid-containing liposomes was used to measure lectin activity, since such particles should present a way to mimick carbohydrate-ligand interactions at a natural membrane; (2) whether U 937 derived clones (Cl 1, Cl 2 and Cl 3) represented differentiated states with more stable properties.

Materials and Methods

Cell Lines

The HL 60 cells were a gift from Dr. Inge Olsson, University of Lund, Lund, Sweden (originally provided by Dr. R.C. Gallo, NCI, Bethesda, MD, [13]). U 937 cells [21-23] and the U 937-derived clones, Cl 1, Cl 2 and Cl 3, were given to us by Dr. Kenneth Nilsson, University of Uppsala, Uppsala, Sweden. They were maintained in suspension culture as described elsewhere [15]. Generally, the cells were subcultivated every 5-7 days or when they had reached a density of 2×10^6 per ml. Then 6 ml of the cells were diluted with 20 ml fresh medium.

Differentiation

(a) Induction. The cells were induced to differentiate either with dimethylsulfoxide (DMSO) for 1-7 days or phorbol myristate acetate (PMA) for 1-5 days at final concentrations of 1.3% and 10^{-7} M respectively.

(b) Cell size. The cells were counted with a Coulter Counter (Model ZF, Coulter Electronics Ltd, South Dunstable, Beds., England) equipped with a 100 μm aperture and a Channelyzer C-1000, Coulter Electronics, Highleah, FL, USA). The aperture current (I) was set at 64, the attenuation (A) at 1 and the threshold (T) varied between 0 and 100 to assess the size distribution. A base channel threshold (BCT) of 5 was used to discriminate between noise and cells.

(c) Phagocytic capacity. Phagocytosis was assayed with a modification of the fluorescence quenching method according to Hed [24, 25]. Fluorescein isothiocyanate (FITC)-labelled heat-treated yeast cells (*Saccharomyces cerevisiae*; 5×10^7 per ml) were opsonized with rabbit hyper-immune anti-yeast IgG [26] at 32 μg per ml or fresh normal serum (50%; complement-coating primarily with factor C3b), washed thrice in Krebs-Ringer phosphate buffer with glucose without Ca^{2+} and Mg^{2+} (KRG; pH 7.3, containing 120 mM NaCl, 4.9 mM KCl, 1.7 mM KH_2PO_4, 8.3 mM $Na_2HPO_4.2H_2O$ and 10 mM glucose) and then resuspended in KRG. HL 60 Cells (0.2 ml, 5×10^6 per ml) and 0.2 ml yeast cells were incubated in siliconized glass tubes at 37°C for 45 min, then a 50 μl aliquot (or one drop) was taken and mixed with an equal volume of PBS (phosphate buffered saline, pH 7.3, containing per 1000 ml: NaCl, 8 g; KCl, 0.2 g; Na_2HPO_4, 1.2 g; and KH_2PO_4, 0.2 g) or trypan blue solution (2 mg/ml in a 0.1 M citrate-phosphate buffer, pH 4.4, diluted 1:4 with 0.15 M NaCl) on a microscope slide. The fluorescence of adhering, but not of ingested yeast cells was quenched with trypan blue. Furthermore, the percentage of cells phoagyctizing at least one yeast cell was determined.

Assay of Lectin-like Activity

(a) Formation of liposomes. Liposomes were prepared according to the reverse phase evaporation method (REV;[27, 28]) in PBS and diluted to 10 μmol lipid per ml. They generally contained cholesterol (Chol)/egg phosphatidyl choline (lecithin; E-PC)/X-Chol [or lactosylceramide (LC), phosphatidylinositol (PI) or brain gangliosides (Gangl)], 8/8/2 on a molar basis. The liposomes were labelled with 0.025 μmol of fluorescent lipid *N*-4-nitrobenzo-2-oxa-1,3-diazole-phosphatidylethanolamine (NBD-PE), and in some instances with 1 μmol of phosphatidylglycerol (PG) to achieve a net negative surface charge. NBD-PE was obtained from Polar Lipids, Inc. (Birmingham, AL, USA), PG, PI and gangliosides from Sigma Chemical Co. (St. Louis, MO, USA), and X-Chol (X = mannose, galactose, *N*-acetylgalactosamine, fucose) was a kind gift of Merck Sharp & Dohme Research Laboratories, Rahaway, NJ, USA. The biochemical structure and properties of the cholesterol derivatives have been described in detail elsewhere [29, 30]. Before using the liposomes they were sonicated briefly for 20 s to disrupt larger aggregates.

(b) Characterization of the liposomes. The exposure of the carbohydrate moiety of the glycolipid was ascertained whenever possible by aggregation with the appropriate lectin, as assessed visually or under the microscope.

(c) Cell affinity for liposomes. To assess carbohydrate affinity, 100 μl cells (2×10^6 per ml) were incubated with 10 μl liposomes (10 μmol per ml) for 20 min at 22°C, and then examined under the fluorescence microscope. The association of liposomes with the cells was described semi-quantitatively on a 5 point graduated scale, where - = no association and +/++++ = the range from weak to strong association.

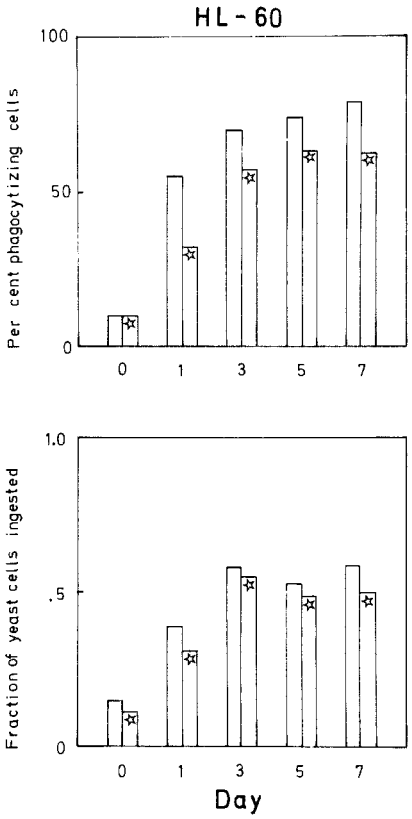

Figure 1. Effect of DMSO (1.3%) during cultivation on the ability of HL 60 cells to phagocytize yeast cells (one or more; upper panel) and ingest associated yeast cells (lower panel); open bars and bars with an asterisk (*) represent yeast cells sensitized with IgG and normal human serum (complement), respectively.

Results

Phagocytosis of IgG- and Complement-coated Yeast Cells

Figs. 1 and 2 describe the effect of DMSO on HL 60 and U 937 cells with respect to the percentage of phagocytozing cells and percentage of ingested yeast cells. The data show that the capacity to phagocytoze both IgG- and complement-coated yeast cells is gradually gained in the presence of DMSO. It is also obvious that DMSO induced increased phagocytic capacity of C3b-coated yeast much more slowly in U 937 cells than in HL 60 cells. Table 1 summarizes the phagocytosis by the clones Cl 1 - Cl 3 on the seventh day after subcultivation. Cl 3 is apparently much less efficacious than Cl 1 and Cl 2. With the addition of DMSO to the culture, HL 60 cells become smaller, whereas Cl 1 and Cl 2 maintain approximately the same size, and U 937 and Cl 3 increase in volume (Fig. 3). The effect of PMA induction was not measured in this respect.

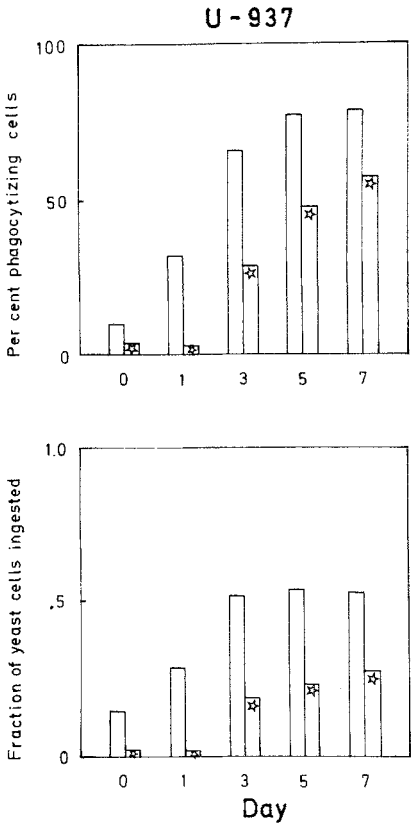

Figure 2. Effect of DMSO (1.3%) during cultivation on the ability of U 937 cells to phagocytize yeast cells (one or more; upper panel) and ingest associated yeast cells (lower panel); open bars and bars with an asterisk (*) represent yeast cells sensitized with IgG and normal human serum (complement), respectively.

Binding of Glycolipid-containing Liposomes

The interaction between fluorescent liposomes and cells was studied with the epifluorescence microscope and evaluated semi-quantitatively from - to ++++ (Table 2). Since liposomes also adhered to the background, and since the differentiated cells differed in size, no quantitative microfluorometric assay was used, although our microscope was equipped with such capability. The results at first glance appear rather confusing. There are, however, some general patterns. Starting with HL 60 cells, it is evident that DMSO cultivation increased conspicuously the recognition of mannosyl and ganglioside residues, and perhaps also of galactosyl and lactosyl ligands. With PMA the carbohydrate affinity profile was similar, but clearly increased for fucose and inositol epitopes.

For U 937 there was very striking binding of mannose containing liposomes already in the uninduced state, and with DMSO the affinities for galactosyl, fucosyl, inositol and

Table 1. Phagocytosis of IgG- and complement-coated yeast cells by the U937-derived clones Cl 1, Cl 2 and Cl 2 and Cl 3 grown for seven days without and with 1.3% DMSO.

Phagocytosis characteristics	Cell line											
	Cl 1				Cl 2				Cl 3			
Yeast sensitization	IgG		Complement		IgG		Complement		IgG		Complement	
DMSO cultivation	−	+	−	+	−	+	−	+	−	+	−	+
Fraction of associated yeast cells ingested	0.28	0.55 ± 0.14[a]	0.29	0.40 ± 0.14[a]	0	0.39 ± 0.06[a]	0.01	0.32 ± 0.16[a]	0	0.41 ± 0.10[a]	0.01	0.15 ± 0.04[a]
Per cent cells phagocytizing (one or more yeast cells)	22	44 ± 6[b]	20	34 ± 4[b]	0	32 ± 1[b]	2	29 ± 12[b]	0	30 ± 8[b]	2	11 ± 5[b]

[a] mean ± S.D.; five experiments.
[b] mean ± S.D., three experiments.

Table 2. Binding[a] of glycolipid-containing liposomes to cells grown in the absence or presence of either DMSO (1.3%) for seven days or PMA (10^{-7} M) for five days.

	Carbohydrate ligand							
Cell line	None	Man	Gal	GalGlc-(Lac)	GalNAc	Fuc	Inositol	Ganglioside
HL 60	−	−	+	−	+ +	−	+ +	+
HL 60 + DMSO	−	+ + +	+ +	+ / + +	+ +	+	+ +	+ + +
HL 60 + PMA	+	+ +	+ +	+ +	+ + +	+ +	+ + +	+ +
U 937	−	+ + +	+	+	+	−	+	+
U 937 + DMSO	+	+ + + +	+ + + +	+	+ +	+ + / + + +	+ + + +	+ + + / + + + +
U 937 + PMA	−	+	+ +	+ + / + + +	+ +	+ +	+ +	+ +
Cl 1	− / +	+	+ / + + + +	− & + +	− & + + + +	+	+ + + +	+ + / + + + +
Cl 1 + DMSO	− / +	+	+	+	− & + + +	+ +	+ + +	+ + +
Cl 1 + PMA	+	+ + +	+	+ +	+ +	+	+ / + + +	+ +
Cl 2	− / +	− / + + + +	+ / + +	+ / + +	+ + + +	+ +	+	+ / + +
Cl 2 + DMSO	− / +	+ +	+ / + +	+	+ +	+ +	+ +	+
Cl 2 + PMA	+	+ + / + + + +	+ + + +	+ + + +	+ + + +	+ +	+ +	+ + + +
Cl 3	+	− & + +	−	+ + / + + +	+ + + / + + + +	+ / + +	+ + / + + +	+
Cl 3 + DMSO	−	+ +	+ / + +	+ +	+ + / + + + +	+ +	+ / + +	+ + / + + +
Cl 3 + PMA	+	+ + / + + +	+	+	+ +	+	+ / + +	+ + +

[a]Arbitrary units: − = no bindning, + / + + + + = weak to strong binding, & = two distinct populations.

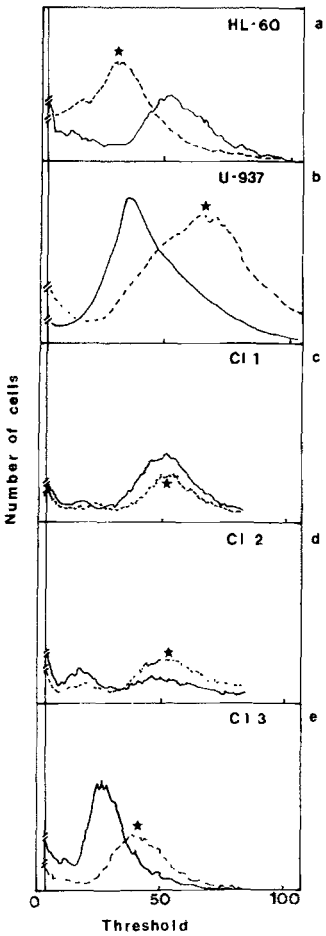

Figure 3. Size (volume; arbitrary units) distribution of (a) HL 60, (b) U 937, (c) Cl 1, (d) Cl 2 and (e) Cl 3 cells after seven-day cultivation without or with (*) 1.3% DMSO.

ganglioside residues were clearly augmented. With PMA the effects were smaller, except for a down-regulation of the binding of mannosyl, and an up-regulation of the binding of lactosyl vesicles.

The U 937-derived clones were included in the study since they are assumed to represent distinct, stable subclones of U 937. Indeed, with respect to lectin-like activity they largely mimicked DMSO-induced U 937, although mannose affinity was not seen in Cl 1 (and Cl 3), but in Cl 2. The effects of DMSO and PMA in these cells are either negligible or contradictory, with one exception; PMA-cultured Cl 2 cells displayed a complete ensemble of carbohydrate affinities. It therefore seems as if a range of carbohydrate-binding membrane structures can be induced in HL 60 and U 937 cells, similar to what has been found in mature macrophages and non-parenchymous liver cells. These properties appear to be permanently present in the clones.

Discussion

The present investigation shows that DMSO-induced HL 60 and U 937 cells gain the ability to bind and ingest both IgG- and complement-coated yeast particles (Figs. 1 and 2), thus indicating phagocytic maturation. However, the rates are different in the two cell lines. This is particularly evident in relation to complement-opsonized yeast; HL 60 cells developed maximum capacity already after three days, whereas this occurred in U 937 only after five to seven days. Furthermore, HL 60 cells showed an almost equal ability to phagocytize IgG- and complement-coated yeast, while U 937 cells both bound and internalized complement-coated yeast less efficiently than IgG-coated yeast. The latter discrepancy was also obtained for Cl 3 cells (Table 1), whereas Cl 1 and Cl 2 cells recognized IgG- and complement-coated yeast to a similar extent, i.e. Fc- and C3-mediated interaction developed in parallel.

The interaction with liposomes that expose different carbohydrate portions differed between the cell lines, and in each cell line depending on the absence or presence of DMSO or PMA for seven or five days, respectively, when they were assumed to have reached maximum maturation (Figs. 1 and 2; Table 2). The results in Table 2 at first glance appear rather confusing, since the cell populations were apparently heterogeneous e.g. Cl 1 with respect to the binding of galactosyl, lactosyl, *N*-acetylgalactosaminyl and ganglioside liposomes, and responded differently to the inducing agents.

As a general observation, DMSO-induction of differentiation had less effect than PMA on the non-specific association of uncharged PC-Chol liposomes. For Cl 1 and Cl 3 there was virtually no effect of DMSO or PMA, whereas Cl 2 responded to PMA only. As a response to DMSO, which was supposed to promote the development to granulocyte-like cells from HL 60 and macrophage-like cells from U 937, HL 60 responded with greater affinity for mannose- and ganglioside-containing vesicles, and U 937 also for galactosyl, fucosyl and inositol liposomes; mannose-binding was present already in the uninduced state of U 937. In the presence of PMA, HL 60 showed a generally increased binding of all types of liposomes, but whether this was a specific effect or not, is unclear, since the binding of control vesicles (PC-Chol) was also augmented. It is interesting that the association with mannosyl-liposomes was decreased. In general the U 937 clones displayed more stable properties with respect to the effects of DMSO or PMA, and a more complete ensemble of carbohydrate affinities.

We have previously observed that DMSO- or PMA-induced differentiation of HL 60 and U 937 cells results in decreased lateral diffusion of glycoconjugates labelled with wheat germ agglutinin [31] with about a factor of 2. This effect could be interpreted as a consequence of increased membrane viscosity or increased contact between the labelled glycoconjugates and the cytoskeleton [31], but the present investigation raises the question whether it could also be due to interaction between surface lectins and glycoconjugates present within the membrane of the same cell.

In conclusion, during DMSO- or PMA induced maturation *in vitro*, HL 60 and U 937 cells change their surface properties both with respect to display of carbohydrate structures [19-23, 33] and recognition of carbohydrates on other particles (this study). Interestingly, these properties develop in parallel with Fc and C3 receptor-mediated immune recognition and phagocytosis. We think that the present results encourage an effort to demineate the molecular background of the lectin-like activity, for instance by separa-

tion of membrane components and identifying lectin activity by reaction with glycolipid-containing liposomes, or fluoresceinated neoglycoproteins [34]. We also suggest that *in vitro* induced maturation of HL 60 or U 937 cells offers a useful way to assess carbohydrate-ligand interactions in general.

Acknowledgements

We thank Kristina Orselius and Kerstin Hagersten for excellent technical assistance. The U 937 cells and the U 937-derived clones Cl 1, Cl 2 and Cl 3 were a kind gift of Dr. Kenneth Nilsson, Dept. Pathology, University of Uppsala, Uppsala. Sweden. The HL 60 cells were obtained from Dr. Inge Olsson, Dept. Haematology, University of Lund, Lund, Sweden, originally provided by Dr. R.C. Gallo, NCI, Bethesda, MD, USA. The research was supported by the Swedish Medical Research Council (Project Nos. 5968 and 6251, Erna and Victor Hasselblad Foundation, King Gustaf Vth 80-year Fund, Magn. Bergvalls Stiftelse and the Medical Research Council of the Swedish Life Insurance Companies.

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