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# Carbohydrate Exposure of Human Promyelocytic HL60 Cells and Histiocytic U937 Cells during Phagocytic Differentiation Assessed with Fluoresceinated Lectins

KARL-ERIK MAGNUSSON* and OLLE STENDAHL
*Department of Medical Microbiology, University of Linköping, S-581 85 Linköping, Sweden*

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**The display of carbohydrate structures was measured in promyelocytic HL60 cells and in histiocytic U937 cells induced to differentiate to phagocytic cells *in vitro* during three to seven days of cultivation in the presence of dimethylsulfoxide (DMSO). It was assessed by micro- or spectrofluorometric quantification of the binding of fluorescent lectins. Changes in the cell size and the association and uptake of IgG- or complement-opsonized yeast cells (*Saccharomyces cerevisiae*) were used as signs of phagocyte differentiation.**

**The binding of wheat germ agglutinin (WGA), concanavalin A (Con A), *Ricinus communis* agglutinin-I (RCA-I) and *Ulex europaeus* agglutinin-I (UEA-I) varied due to the presence of DMSO during cultivation, and without DMSO also on the number of days in culture and the type of cell.**

Cell-cell recognition employs specific reciprocal protein-carbohydrate interactions, as well as non-specific hydrophobic and ionic forces. The recognition is reciprocal in the sense that lectin-like substances and corresponding carbohydrate receptors may occur either on bacterial [1-3] or on mammalian [4, 5] cell membranes; either way, cell-cell contact is promoted [5, 6].

Different glycoconjugates have come into focus as receptor molecules for bacterial fimbriae (pili) which bind specifically to different short oligosaccharides [2]. The role of mammalian lectins is particularly well-studied in different liver cells which show affinity for molecules and particles displaying mannose, galactose or fucose residues [7-9]. Lectin-like activity has also been identified as important recognition markers of malignant transformation [5, 6, 11, 12].

**Abbreviations:** DMSO, dimethylsulfoxide; PMA, phorbol 12-myristate 13-acetate; KRG, Krebs-Ringer phosphate buffer with glucose; WGA, wheat germ agglutinin; Con A, concanavalin A; RCA-I, *Ricinus communis*, agglutinin-I; UEA-I, *Ulex europaeus* agglutinin-I.

*Author for correspondence.

During induction with DMSO, HL60 cells [13, 14] have been shown to develop polymorphonuclear leucocyte (PMNL) characteristics, e.g. phagocytic capacity and oxidative response [15, 16]. They are, however, deficient in some granular enzymes in comparison to mature PMNL [17]. When PMA (phorbol 12-myristate 13-acetate) is used instead of DMSO they are assumed to undergo macrophage maturation [18, 19]. Also the histiocytic cell line U937 [19-22] matures in the presence of DMSO and PMA, which is accompanied by increased adhesiveness and other membrane-mediated properties [21, 22]. Both HL60 and U937 cells show altered carbohydrate-specific affinity for glycolipid-containing liposomes, indicating varying lectin-like activities (unpublished results).

The present study was done to assess relative exposure of major surface carbohydrate structures during phagocyte induction with DMSO of HL60, U937 and clones (Cl 1 - Cl 3) derived from U937, thereby forming a background for the recognition and activation of these cells by fimbriated bacteria and other particles with lectin-like appendages. The binding of fluorescent lectins and microfluorometry was used to quantify the display of carbohydrates.

## Materials and Methods

### *Cell Lines*

The HL60 cells were a gift from Dr. Inge Olsson, University of Lund, Sweden (originally provided by Dr. R.C. Gallo, NCI, NIH, Bethesda, MD, USA [13, 14]. U937 cells [20, 22] and the U937-derived clones, Cl 1, Cl 2 and Cl 3 were obtained from Dr. Kenneth Nilsson, University of Uppsala, Sweden. They were maintained in suspension culture as described elsewhere [16]. Generally, the cells were subcultivated every seven days or when they had reached a density of $2 \times 10^6$ cells/ml. Then 6 ml of the cells were diluted with 20 ml of fresh medium. They were induced to differentiate with DMSO at a final concentration of 1.3%.

### *Counting and Sizing of the Cells*

The cells were counted with a Coulter Counter (Model ZF, Coulter Electronics Ltd, Dunstable, UK) equipped with a 100 $\mu$m 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) at varying values depending on the type of cell. A base channel threshold (BCT) of 5 was used to discriminate between cells and noise.

### *Binding of Fluorescent Lectins*

Fluorescein (FITC)-conjugated lectins were obtained from E-Y Labs (San Mateo, CA, USA). The lectins used had the following characteristics (carbohydrate specificity in parenthesis): WGA, Wheat Germ Agglutinin from *Triticum vulgaris* [*N*-acetylglucosamine, (sialic acid)]; RCA-I, *Ricinus communis* ($\beta$-Gal); Con A, *Canavalia ensiformis* ($\alpha$-Man, $\alpha$-Glc); and UEA-I, *Ulex europaeus* ($\alpha$-L-Fuc).

Cells were washed twice in modified Krebs-Ringer phosphate buffer 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) by centrifugation (1250 rpm $\times$ 10 min in a Sorvall

**Table 1.** Relative capacity in each of the differentiated[a] cell lines to recognize and ingest complement (C3)-coated yeast cells in relation to IgG-sensitized yeast cells[b].

| Cell line | Attachment (%) | Ingestion (%) |
|---|---|---|
| HL60 | 80 | 93 |
| U937 | 72 | 52 |
| Cl 1 | 73 | 77 |
| Cl 2 | 82 | 91 |
| Cl 3 | 37 | 37 |

[a] Cultivation for 7 days with DMSO.
[b] 100% for each cell line.

SS-34 rotor) and then counted and resuspended to 2 × $10^7$ cells/ml in KRG. For microfluorometric determination of individual cell fluorescence, 50 μl of cells (2 × $10^7$/ml) was mixed with 250 μl lectin solution (20 μg/ml) and incubated for 30 min at 4°C. The reaction mixture was then diluted with 1.7 ml cold (4°C) KRG and 0.5 ml spun down on to microscope slides (5 min at 900 rpm, Shandon Cytospin 2, Shandon Southern Products, Astmoor, UK). The slides were washed and then stored at 4°C in KRG until analyzed.

*Microfluorometric Measurements*

Lectin-derived and background fluorescence of individual cells was assessed with a Zeiss Universal Microscope (Carl Zeiss, Oberkochen, W. Germany) equipped with a stabilized mercury lamp (HBO-100), epifluorescence condenser with a bandpass filter (485 nm), a fluorescence transmission filter (510 nm) and low-pass filter (590 nm), × 25 salt-water objective (Zeiss, Plan-Neofluor), microscope photometer (Type SF), power-supply with digital light meter (Type P), shutter-control unit (Type FL), and time-selector (for Prontor-Magnetic shutter). A 0.25 mm diameter aperture was used and focused in the cell membrane. It covered one cell (about 10 μm in diameter). Cells were selected randomly in white light, and fluorescence measured for 0.5 sec. The shutters were handled either manually or with the aid of a small computer (ABC-80, Luxor, Motala, Sweden) equipped with an interface for the shutter control unit, and a printer for data collection. To obtain a figure representing relative lectin binding, the photometer readings were first corrected for the fluorescence to protein ratio (F/P = 1.4, 3.8, 1.0 and 2.6 for Con A, WGA, RCA-I and UEA-I, respectively), which means that the microfluorometer values were divided by F/P for each lectin. Secondly, the latter figures were divided by a factor proportional to the molecular weight of the lectins, viz. 1.02, 0.36, 1.20 and 1.70 for Con A (102 000), WGA (36 000), RCA-I (120 000) and UEA-I (170 000), respectively.

*Phagocytosis*

Phagocytosis was assayed with a modification of the fluorescence quenching method as described by Hed and coworkers [23, 24]. FITC-labelled yeast cells (*Saccharomyces cerevisiae*; 5 × $10^7$/ml) were opsonized with rabbit hyper-immune anti-yeast IgG [25] at

**Table 2.** Relative distribution of lectin-binding sites on HL60 cells during culture (day 3,5, and 7) in the absence or presence of DMSO.

| Lectin | DMSO | Day 3 | | Day 5 | | Day 7 | |
|---|---|---|---|---|---|---|---|
| | | RLB[a,b] | % | RLB | % | RLB | % |
| Con A | — | 49 ± 5 | 8.0 | 3 ± 1 | 0.5 | 9 ± 2 | 1.7 |
| | + | 44 ± 7 | 5.3 | 12 ± 6 | 1.4 | 18 ± 10 | 1.7 |
| WGA | — | 431 ± 11 | 70.3 | 507 ± 28 | 86.1 | 411 ± 53 | 81.4 |
| | + | 543 ± 85 | 66.3 | 664 ± 172 | 77.6 | 716 ± 120 | 67.5 |
| RCA-I | — | 132 ± 15 | 21.5 | 78 ± 13 | 13.2 | 83.0 ± 12.6 | 16.4 |
| | + | 232 ± 26 | 28.3 | 179 ± 40 | 20.9 | 327 ± 47.0 | 30.8 |
| UEA-I | — | 0.9 ± 0.4 | 0.14 | 0.8 ± 0.3 | 0.14 | 2.3 ± 0.9 | 0.46 |
| | + | — | 0 | — | 0 | — | 0 |

[a] RLB = Relative lectin binding, corrected for fluorescence to protein ratio (F/P) and molecular weight of each lectin (See also Materials and Methods)

[b] ± S.E.M., Standard Error of the Mean, n = 15

32 μg/ml, or fresh normal serum (50%; complement-coating primarily with C3b), washed thrice in KRG without $Ca^{2+}$ and $Mg^{2+}$ and then resuspended in KRG. HL60 Cells (0.2 ml, 5 × $10^6$/ml) and 0.2 ml yeast cells were incubated in siliconized glass tubes at 37°C for 45 min, when 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; $Na_2HPO_4.2H_2O$, 1.2 g and $KH_2PO_4$ 0,2 g) or trypan blue solution (2 mg/ml in 0.1 M sodium 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 phagocytizing at least one yeast cell was determined.

## Results

### *Signs of Phagocytic Differentiation*

The ability to recognize and ingest IgG- or complement(C3)-sensitized yeast cells was used as a specific measure of phagocyte maturation. In all cell lines there was an increase in the capacity to phagocytize, as evidenced by the number of cells with at least one adherent yeast cell and by the fraction of internalized yeast cells, i.e. yeast cells remaining fluorescent after addition of crystal violet. However, the different cell lines behaved differently both qualitatively and quantitatively. As a general phenomenon, IgG-coated particles were recognized and ingested more easily than C3-coated particles (Table 1). Furthermore, the C3-mediated phagocytosis developed more slowly than the IgG-mediated. With HL60 cells, after one day with DMSO, the number of phagocytozing cells and the fraction of ingested yeast cells was 50 and 69%, and 56 and 66%, respectively, of the maximum capacities obtained for C3- and IgG-coated particles. For U937 cells the difference in the development of C3- and IgG-receptor-mediated processes was even more pronounced; 5 and 41% in attachment, and 7 and 54% in ingestion after one day with DMSO, respectively. The clones were studied only after seven days

**Table 3.** Relative distribution of lectin-binding sites on U937 cells during culture (day 3, 5 and 7) in the absence or presence of DMSO.

| Lectin | DMSO | Day 3 | | Day 5 | | Day 7 | |
|---|---|---|---|---|---|---|---|
| | | RLB[a,b] | % | RLB | % | RLB | % |
| Con A | — | 35 ± 3 | 4.2 | 51 ± 4 | 4.1 | 32 ± 3 | 4.9 |
| | + | 23 ± 3 | 4.1 | 39 ± 3 | 4.1 | 38 ± 4 | 4.9 |
| WGA | — | 655 ± 101 | 77.9 | 922 ± 95 | 74.6 | 261 ± 59 | 40.0 |
| | + | 463 ± 52 | 81.7 | 646 ± 67 | 69.0 | 278 ± 29 | 35.8 |
| RCA-I | — | 149 ± 20 | 17.8 | 261 ± 16 | 21.2 | 359 ± 41 | 55.1 |
| | + | 80 ± 12 | 14.1 | 252 ± 17.7 | 26.9 | 459 ± 50 | 59.2 |
| UEA-I | — | 1.1 ± 0.3 | 0.13 | 1.0 ± 0.1 | 0.08 | 0.4 ± 0.3 | 0.06 |
| | + | 0.20 ± 0.3 | 0.04 | — | 0 | — | 0 |

[a,b] See footnotes to Table 2.

**Table 4.** Relative distribution of lectin-binding sites on Cl 1 cells during culture (day 3, 5 and 7) in the absence or presence of DMSO.

| Lectin | DMSO | Day 3 | | Day 5 | | Day 7 | |
|---|---|---|---|---|---|---|---|
| | | RLB[a,b] | % | RLB | % | RLB | % |
| Con A | — | 52 ± 7 | 6.0 | 64 ± 6 | 5.9 | 48 ± 3 | 5.4 |
| | + | 70 ± 5 | 6.7 | 54 ± 7 | 4.6 | 47 ± 4 | — |
| WGA | — | 619 ± 111 | 70.3 | 689 ± 96 | 63.6 | 612 ± 74 | 68.8 |
| | + | 582 ± 85 | 55.9 | 814 ± 81 | 69.4 | 744 ± 109 | — |
| RCA-I | — | 209 ± 29 | 23.7 | 329 ± 37 | 30.4 | 228 ± 37 | 25.6 |
| | + | 390 ± 85 | 37.4 | 304 ± 42 | 25.9 | N.D.[b] | — |
| UEA-I | — | 0.5 ± 0.4 | 0.06 | 1.3 ± 0.4 | 0.12 | 0.7 ± 0.2 | 0.08 |
| | + | — | 0 | 1.7 ± 0.7 | 0.14 | — | — |

[a,b] See footnotes to Table 2.

**Table 5.** Relative distribution of lectin-binding sites on Cl 2 cells during culture (day 3, 5 and 7) in the absence or presence of DMSO.

| Lectin | DMSO | Day 3 | | Day 5 | | Day 7 | |
|---|---|---|---|---|---|---|---|
| | | RLB[a,b] | % | RLB | % | RLB | % |
| Con A | — | 51 ± 10 | 5.3 | 34 ± 3 | 3.7 | 30 ± 3 | 4.8 |
| | + | 52 ± 7 | 4.3 | 37 ± 3 | 3.1 | 18 ± 8 | 2.9 |
| WGA | — | 560 ± 98 | 56.8 | 592 ± 50 | 64.3 | 400 ± 86 | 63.2 |
| | + | 633 ± 126 | 52.9 | 852 ± 104 | 73.3 | 434 ± 70 | 69.8 |
| RCA-I | — | 375 ± 73 | 38.0 | 291 ± 48 | 31.6 | 199 ± 91 | 31.4 |
| | + | 510 ± 133 | 42.6 | 270 ± 65 | 23.2 | 167 ± 41 | 26.9 |
| UEA-I | — | — | 0 | 3.2 ± 1.0 | 0.35 | 3.9 ± 0.9 | 0.62 |
| | + | 1.7 ± 0.5 | 0.14 | 3.7 ± 1.8 | 0.32 | 2.5 ± 1.0 | 0.40 |

[a,b] See footnotes to Table 2.

**Table 6.** Relative distribution of lectin-binding sites on Cl 3 cells during culture (day 3, 5 and 7) in the absence or presence of DMSO.

| Lectin | DMSO | Day 3 | | Day 5 | | Day 7 | |
|---|---|---|---|---|---|---|---|
| | | RLB[a,b] | % | RLB | % | RLB | % |
| Con A | — | 195 ± 13 | 10.8 | 25 ± 3 | 3.9 | 26 ± 2 | 5.4 |
| | + | 69 ± 5 | 4.9 | 52 ± 5 | 7.4 | 46 ± 5 | 8.7 |
| WGA | — | 1552 ± 123 | 86.0 | 521 ± 68 | 81.3 | 358 ± 65 | 73.8 |
| | + | 974 ± 81 | 68.6 | 483 ± 85 | 68.7 | 258 ± 56 | 48.6 |
| RCA-I | — | 58 ± 5 | 3.2 | 92 ± 13 | 14.4 | 102 ± 17 | 21.0 |
| | + | 373 ± 44 | 26.3 | 164 ± 14 | 23.4 | 225 ± 39 | 42.4 |
| UEA-I | — | — | 0 | 1.9 ± 0.5 | 0.30 | 2.4 ± 0.6 | 0.49 |
| | + | 2.6 ± 0.9 | 0.18 | 4.0 ± 1.1 | 0.57 | 1.9 ± 0.4 | 0.36 |

[a,b] See footnotes to Table 2.

with DMSO. The modal volume (= threshold (T) value of the cell peak on a 0-100 graduated scale) differed between the cell lines. The response to DMSO induction was also distinct. In HL60 the average size decreased from 52 to 31, whereas it increased from 36 to 67 in U937. Cl 1 and Cl 2 were unaffected by DMSO, remaining at 52 units, whereas Cl 3 increased from 26 to 41. U937 showed a very broad and skewed distribution, possibly indicating a heterogenous cell population.

*Binding of Lectins*

Tables 2-6 describe the binding (arbitrary) of WGA, RCA-I, Con A and UEA-I to HL60, U937, Cl 1, Cl 2 and Cl 3 cells, as well as the relative amount (%) of the lectins on the cell surface. It is evident that the lectin binding, and thus the carbohydrate exposure, was affected by the number of days in culture, and the presence of DMSO. The major events occurred in glycoconjugates labelled with WGA and RCA-I, both with respect to the response to DMSO or age after subcultivation. However, Con A binding sites were also affected, for instance in Cl 3 cells, which also showed greater binding of UEA-I.

## Discussion

The present investigation shows that DMSO-induced HL60 and U937 cells gained the ability to bind and ingest both IgG- and complement-coated yeast particles, but the rates were different in the two cell lines. This was particularly evident in relation to complement-opsonized yeast. Furthermore, HL60 cells and almost equal ability to phagocytoze IgG- and complement-coated yeast, while U937 cells both bound and internalized complement-coated yeast much less efficiently than IgG-coated yeast (Table 1).

The size-distribution of U937 cells was observed to be broader than for the other cell lines (data not shown), possibly indicating a heterogenous population of cells. Also with respect to phagocytosis of C3- and IgG-coated particles, U937 showed an intermediate behavior of the clones.

The binding of lectins per cell surface area was conspicuously influenced by the number of days in culture (Tables 2-6). *N*-Acetylglucosamine (sialic acid) and galactose, recognized by WGA and RCA-I, were probably exposed to a greater extent than mannose or fucose, as evidenced by Con A- and UEA-I association, respectively. In the DMSO-induced HL60 cells the surface density of ligands for RCA-I, WGA and Con A was apparently increased in comparison to uninduced cells (Table 2), but the total number of glycoconjugates was barely affected due to the decrease in cell size with DMSO. These data confirm findings by Jenis *et al.* [18] using fluorescent Con A and DMSO-induced HL60. In addition, Tables 2-6 also show that the proportions of binding sites per surface area for the different lectins varied during the three to seven days of cultivation, whether the cell lines had been grown in the presence of DMSO or not.

After seven days in culture, different patterns are seen. Uninduced HL60 and Cl 3 cells, and to a lesser extent Cl 1 and Cl 2 cells have a predominance of *N*-acetylglucosamine (sialic acid). By contrast, U937 cells expose a higher proportion of galactose. U937 cells and the clones also display relatively more mannose than HL60. However, DMSO induction of HL60 and Cl 1 increlased the proportion of galactose-residues, as recognized by RCA-I. Incidentally, Pincus [26], recently showed that guinea-pig eosinophils were agglutinated with 10-fold less WGA than Con A, thus indicating about a 10-fold greater exposure of *N*-acetylglucosamine than of mannose residues. About the same proportions of available glycoconjugates are demonstrated in Tables 2-6. Mizoguchi *et al.* [27] recently demonstrated that the quantity of neutral asparagine-linked sugar chains was increased duri ng PMA-induced monocytoid differentiation in HL60, which was attributed to the appearance of high mannose-type oligosaccharides. They also found that there was a decrease in high molecular weight oligosaccharides in the acidic fraction. Complex structural differences seen in the surface carbohydrates on glycolipids [28] or glycoproteins [29] thus appear to be accompanied by alterations of the lectin binding both on an absolute and a relative basis. The results might reflect DMSO-induced alterations of glycosylation of glycoconjugates particularly in HL60 and Cl 3 cells [30]. Such changes might, according to Hakomori [11], modulate different endogenous cellular functions and cell social events, including lectin interactions and the recognition and response to environmental soluble factors, bacteria and other immunologically active cells [5, 31].

In conclusion, the results show that cell differentiation and number of days in culture strongly influence the exposure of carbohydrate structures. They form a basis for further studies of, for instance, the recognition and stimulation of cellular oxidative metabolism by lectins or bacteria with lectin-like appendages (fimbriae).

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## References


1 Hart DA (1980) J Clin Nutr 33:2416-25.
2 Duguid JP, Old DC (1980) in Bacterial Adherence, Receptors and Recognition, Ser B, Vol 6, ed. Beachey EH, Chapman and Hall, London, p 185-217.
3 Eshdat Y, Sharon N (1984) Biol Cell 51:259-66.
4 Hughes RC, Pena SDJ (1981) in Carbohydrate Metabolism and Its Disorders, eds. Randle PJ, Steiner DF, Whelan, WJ, Academic Press, London, p 363-423.
5 Sharon N (1984) Biol Cell 51:239-46.
6 Monsigny M, Kieda C, Roche A-C (1983) Biol Cell 47:95-110.
7 Ashwell G, Harford J (1982) Annu Rev Biochem 51:531-54.
8 Wolkoff AW, Klausner RD, Ashwell G, Harford J (1984) J Cell Biol 98:375-81.
9 Wall DA, Hubbard AL (1985) J Cell Biol 101:2104-12.
10 Izhar M, Nuchamowitz Y, Mirelman D (1982) Infect Immun 35:1110-18.
11 Hakomori S (1985) Cancer Res 45:2405-14.
12 Hammarström S (1985) in Tumor Marker Antigens, ed. Holmgren J, Studentlitteratur, Lund, p 34-51.
13 Gallagher R, Collins S, Trujillo J, McCredie K, Aheaon M, Tsai S, Metzger R, Anlakh G, Ruschetti F, Gallo R (1979) Blood 54:713-32.
14 Collins SJ, Ruscetti FW, Gallagher RE, Gallo RC (1979) J Exp Med 149:969-74.
15 Stendahl O, Dahlgren C, Hed J (1982) in Biochemistry and Functions of Phagocytes, eds. Rossi F, Patriarca P, Plenum Press, New York, Adv Exp Med Biol 144:531-37.
16 Stendahl O, Dahlgren C, Hed J (1982) J Cell Physiol 112:217-21.
17 Stendahl O, Andersson T, Dahlgren C, Magnusson K-E (1986) Biochim Biophys Acta 881:430-36.
18 Jenis DM, Stepanowski AL, Blair OC, Burger DE, Sartorelli AC (1984) J Cell Physiol 121:501-7.
19 Harris P, Ralph P (1985) J Leucocyte Biol 37:407-22.
20 Sundström C, Nilsson K (1976) Int J Cancer 17:565-77.
21 Nilsson K, Forsbeck K, Gidlund M, Sundström C, Totterman T, Sällström J, Venge P (1981) in Haematology and Blood Transfusion, Vol. 26, Modern Trends in Human Leukemia IV, eds. Neth R, Gallo R, Graf L, Manweiler C, Winkler K, Berlin, Springer Verlag, p 215-21.
22 Nilsson K, Kimura A, Klareskog L, Andersson LC, Gahmberg CG, Wigzell H (1981) Leuk Res 5:185-93.
23 Hed J (1977) FEMS Microbiol Lett 1:357-60.
24 Sahlin S, Hed J, Rundqvist I (1983) J Immunol Meth 60:115-24.
25 Stendahl O, Tagesson C, Magnusson K-E, Edebo L (1977) Immunology 32:11-18.
26 Pincus SH (1986) Proc Soc Exp Biol Med 182:344-49.
27 Mizoguchi A, Takasaki S, Maeda S, Kobata A (1984) J Biol Chem 259:11949-57.
28 Klock JC, Macher BA, Lee WMF (1981) Blood Cells 7:247-55.
29 Thorpe SJ, Feizi T (1984) Biosci Rep 4:673-85.
30 Fukuda M, Koeffler HP, Minowada J (1981) Proc Natl Acad Sci USA 78:6299-303.
31 Brandley BK, Schnaar RL (1986) J Leucocyte Biol 40:97-111.