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Lectin-binding components of normal granulocytes and leukaemic myeloid cells

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A panel of lectins was used to analyse glycoproteins of normal granulocytes and leukaemic myeloid cells. The glycoproteins of detergent-solubilized whole cells were separated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and their lectin-binding properties determined by incubation of the fixed gels with radioiodinated lectins. Normal granulocytes and leukaemic myeloid cells in different stages of maturation possess a cell-surface sialic acid-rich glycoprotein of apparent mol.wt. 115000 (GP115), that can be labelled by both the lactoperoxidase and periodate/NaB^3H_4 cell-surface labelling techniques. The sialoglycoprotein of leukaemic myeloblasts has a slightly lower apparent mol.wt., 112000 (GP112). After neuraminidase treatment before cell solubilization, both GP115 and GP112 bind the lectins from *Arachis hypogaea* (peanut) and *Helix pomatia* (snail) and have an increased apparent molecular weight of 125000. Two concanavalin A-binding glycoproteins of apparent mol.wts. 98000 and 90000 are present in leukaemic myeloblasts. Concanavalin A binding to these glycoproteins is decreased in more mature leukaemic cells and absent in granulocytes. As concanavalin A binding decreases in the maturer forms, there is a concomitant increase in the binding of *Ricinus communis* (castor bean) and *Maclura aurantiaca* (osage orange) lectins to these glycoproteins. Whole granulocytes, but not leukaemic myeloblasts, contain a major cell-surface concanavalin A binding glycoprotein of apparent mol.wt. 130000, which is labelled by the periodate/NaB^3H_4 technique. Concanavalin A binding to this glycoprotein increases as the morphology of leukaemic cells approaches that of mature granulocytes.

Very little is known regarding the structure and function of the cell-surface glycoproteins of human leukocytes. A precise understanding of the surface structure of the glycoproteins of normal leukocytes would allow comparison between normal and malignant-cell surfaces and might yield useful information about the nature and origin of malignant cells. Such information may be useful in defining the changes occurring during haemopoiesis and may also be an aid for the clarification and diagnosis of human haemopoietic malignancies.

Two techniques (periodate/NaB^3H_4 and galactose oxidase/NaB^3H_4) for labelling cell-surface carbohydrate have already been used to define differences between the surface-glycoprotein patterns of normal and malignant leukocytes after SDS/polyacrylamide-gel electrophoresis and subsequent autoradiography (Gahmberg & Andersson, 1978; Andersson & Gahmberg, 1978; Andersson *et al.*, 1979). Lectins provide an alternative approach to defining specific carbohydrate structures carried on glycoproteins of cells and cell surfaces. The sugar specificities of numerous lectins have now been defined in considerable detail, so that it is possible to select particular lectins in order to detect glycoproteins with particular carbohydrate moieties. A panel of radioiodinated lectins can be used to detect different glycoproteins after separation by SDS/polyacrylamide-gel electrophoresis (Tanner & Anstee, 1976; Rostas *et al.*, 1977). In the present study we have used this approach to analyse the major glycoproteins of normal human granulocytes and leukaemic myeloid cells. The results clearly

Abbreviations used: AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; HP, *Helix pomatia* (snail) lectin; MA, *Maclura aurantiaca* (syn, *pomifera*) (osage orange) lectin; PAS, periodic acid/Schiff; PNA, *Arachis hypogaea* (peanut) lectin; RCA, *Ricinus communis* (castor bean) lectin; SDS, sodium dodecyl sulphate; UM, unfractionated mononuclear cells.

demonstrate different lectin-binding patterns between normal granulocytes and different leukaemic myeloid cells, and these differences can be correlated with the morphological stage of maturation of the leukaemic cells.

Materials and methods

Normal leukocytes

Normal leukocytes were obtained within 4h of collection as buffy-coat preparations prepared from 500ml of citrated whole blood. Normal unfractionated mononuclear (UM) cells were prepared from buffy coat by the method of Boyum (1968) by using a solution of 9% (w/v) Ficoll 400 (Pharmacia Ltd., Hounslow, Middx., U.K.) and 34% (v/v) Triosil (Vestric Ltd., Kingswood Trading Estate, Bristol, U.K.) with a relative density (d) of 1.077. Normal granulocytes were prepared from buffy coat by the method of Aguado *et al.* (1980), with Ficoll/Triosil for solution A ($d = 1.077$) and 14.6% (w/v) Ficoll 400 with Urografin 150 (Schering Ltd., Burgess Hill, West Sussex, U.K.) for solution B ($d = 1.097$). Contaminating erythrocytes were lysed in NH_4Cl solution (0.15M-NH_4Cl/0.01M-$KHCO_3$/0.001M-K_2EDTA, pH 7.5) at 4°C for 15min.

Leukaemic samples

All samples from leukaemic patients were used within 2h of collection. A 20ml portion of citrated peripheral-blood samples from patients with chronic myeloid leukaemia (CML) was treated in the way described above for the isolation of normal granulocytes. The cells harvested from the surface of solution A were termed 'immature myeloid' cells, and cells harvested from the interface of solution A and solution B were termed 'granulocyte' layer cells. 'Immature myeloid' cell fractions comprised 0–8% promyelocytes, 30–40% myelocytes, 30–40% metamyelocytes, 5–10% band forms, 1–3% granulocytes and 5–20% lymphocytes. Granulocyte fractions contained 60–80% mature granulocytes, 2% lymphocytes, the remaining cells being band forms with a smaller percentage of metamyelocytes. Citrated bone marrow from two untreated patients with acute myeloid leukaemia were processed by lysing the erythrocytes in NH_4Cl solution at 4°C followed by washing three times at a concentration of 5×10^6 cells/ml in phosphate-buffered saline, pH 7.4 (Dulbecco A; Oxoid, Basingstoke, Hants., U.K.) for 5min at 500g and 4°C.

Assessment of purity of cell preparation

Cells (5×10^6) were incubated in autologous plasma for 1h at 37°C. The cells were then pelleted, resuspended in 150μl of autologous plasma and spread on a clean glass slide. Slides were air-dried, fixed for 1min in methanol and stained with Leishman's stain (BDH, Poole, Dorset, U.K.) within 24h. Cells were identified morphologically and scored.

Neuraminidase treatment of leukocytes

Cells (10^8) suspended in 20ml of 0.9% unbuffered saline containing 10mM-$CaCl_2$, pH 5.6, and 500μl (0.5 unit) of *Vibrio cholerae* neuraminidase (Hoechst Pharmaceuticals Ltd., Brentford, Middx., U.K.) were incubated at 37°C for 30min with shaking, then washed three times in phosphate-buffered saline, pH 7.4, as described above.

Cell-surface labelling

Granulocytes and erythrocytes were surface-labelled with ^{125}I by the method of Moore *et al.* (1982), with only 110μl of glucose and 20μl of β-D-glucose oxidase (Type V; Sigma, Poole, Dorset, U.K.) for either 0.7ml of packed erythrocytes in 0.1ml of phosphate-buffered saline, pH 7.4, or 2×10^7 granulocytes in 0.5ml of phosphate-buffered saline, pH 7.4. Lactoperoxidase (Sigma) was used at a concentration of 2.5mg/litre (76 units/mg). Autoradiography was carried out by using No-Screen film (Kodak Ltd., Hemel Hempstead, Herts., U.K.). Granulocytes and immature myeloid cells were surface-labelled with ^3H by the method of Andersson & Gahmberg (1978). Fluorography was by the method of Chamberlain (1979). Briefly, after staining, the gel was washed twice in water then incubated for $\frac{1}{2}$h at room temperature in 1M-sodium salicylate in 20% (v/v) methanol, then dried down immediately. X-Omat AR film (Kodak Ltd.) was pre-fogged on top of the gel to give $\Delta A_{500} = 0.1$ with respect to un-fogged film, then left in the vapour phase of liquid N_2 for 3 weeks.

Solubilization of cells before SDS/polyacrylamide-gel electrophoresis

Cells were solubilized at 10^8/ml for 5min on ice in a solution of phosphate-buffered saline, pH 7.4, containing 1% Triton X-100 (BDH, Poole, Dorset, U.K.) and 2mM-phenylmethanesulphonyl fluoride (Sigma) by the method of Andersson & Gahmberg (1978). Samples were centrifuged (2000g for 10min at 4°C) and the supernatant diluted with an equal volume of sample buffer (Laemmli, 1970). A sample of undiluted supernatant was retained for protein assay by the method of Lowry *et al.* (1951), with bovine serum albumin in 0.1% SDS as the reference standard. Comparison of Triton-solubilized material prepared by centrifugation at 100000g at 4°C for 1h by SDS/polyacrylamide-gel electrophoresis with subsequent Coomassie Blue or PAS staining did not reveal any marked differences in the number of bands or their staining intensity.

SDS/polyacrylamide-gel electrophoresis and autoradiography

Slab-gel electrophoresis was carried out by using the discontinuous buffer system of Laemmli (1970), with acrylamide concentrations of 8% (w/v) for the separating gel and 3% for the stacking gel. A 350 μg portion of leucocyte protein was applied to each gel slot. Erythrocyte ghosts were prepared by the method of Dodge *et al.* (1963) and solubilized with an equal volume of sample buffer (Laemmli, 1970). A 150 μg portion of erythrocyte ghost protein was applied to each gel slot. Electrophoresis was at 30 V per slab (12 cm × 15 cm × 1 mm) until the tracking dye had almost reached the end of the gel (16–24 h). The gels were sliced in two (a test slab and a control slab), then fixed overnight in propanol/acetic acid/water (25:7:68, by vol.). They were then washed in distilled water, equilibrated with 1 M-potassium phosphate buffer, pH 7.5, followed by 0.2 M-potassium phosphate buffer, pH 7.5, and incubated with radioiodinated lectin by the method of Rostas *et al.* (1977). Briefly, 0.9 mg of radioiodinated lectin in 150 ml of 0.2 M-potassium phosphate buffer, pH 7.5, was added to the test gel, and 0.1 mg of radioiodinated lectin in 50 ml of the same buffer containing 0.3 M inhibiting sugar was added to the control gel and incubated for 3 h with shaking. Both gels were washed in buffer until no radioactivity could be detected in the washing buffer. After thorough washing, gels were washed three times with distilled water and then stained with Coomassie Brilliant Blue R or PAS stain before being dried down on to filter paper and mounted on cardboard. Autoradiographs of the gels were developed after sufficient time (3 days–8 weeks) had elapsed for optimum detection of bands. No-Screen Film NS2T, D-19 developer powder and Unifix were from Kodak Ltd. Assignment of apparent molecular weights to bands was made by comparison with those of erythrocyte membrane proteins separated under the same conditions and stained with Coomassie Blue (as defined by Steck, 1974).

Source and preparation of lectins

Helix pomatia (HP) and concanavalin A lectins were from Pharmacia. *Arachis hypogaea* (PNA) lectin, obtained from peanuts from a local food store, was prepared by the method of Anstee *et al.* (1977). *Maclura aurantiaca* seeds, the source of MA, were obtained from Schumacher Co., Sandwich, MA, U.S.A., and *Ricinus communis* seeds, the source of RCA, were obtained from Thompson and Morgan, Ipswich, Suffolk, U.K. Both lectins were prepared by the method of Tanner & Anstee (1976).

Radioiodination of lectins

Lectin (1 mg in 0.2 M-phosphate buffer, pH 7.5, containing 0.3 M of the lectin's respective inhibiting sugar) was labelled with 1 mCi of ^{125}I by the chloramine-T method (Klinman & Taylor, 1969). The labelled lectin was dialysed against saline (0.9% NaCl) until there was very little detectable radioactivity in the saline (usually after 5 days) as detected by a scintillation meter, type 540 (^{125}I probe 544) (Mini Instruments Ltd., Burnham-on-Crouch, Essex, U.K.). Lectin activity was assayed by haemagglutination using normal human erythrocytes (MA, RCA), group A_1 human erythrocytes (HP), trypsin-treated human erythrocytes (concanavalin A) or neuraminidase-treated human erythrocytes (PNA). The methods of trypsin treatment and neuraminidase treatment were as described by Tanner *et al.* (1980) and Anstee *et al.* (1979).

Results

The glycoprotein components of Triton-solubilized whole cells were separated by SDS/polyacrylamide-gel electrophoresis and detected with the PAS stain. The major PAS-staining bands of normal granulocytes and the granulocyte fraction from the peripheral blood of CML patients have apparent mol.wts. 130000 (GP130) and 98000 (GP98); less intensely stained bands of apparent mol.wts. 115000 (GP115), 110000 (GP110) and 90000 (GP90) are also present (Figs. 1*a* and 1*e*). Neuraminidase treatment of mature granulocytes resulted in the loss of PAS staining in regions of GP115 and GP110 (Fig. 1*b*). Immature myeloid cells prepared from the peripheral blood of patients with CML (see the Materials and methods section) contained the same PAS-staining bands, but the relative staining intensities were different from that of mature granulocytes (Fig. 1*c*). In particular, there was a marked reduction in the PAS-staining intensities of GP130 and GP98 and a marked increase in the PAS-staining intensity of GP115 and GP110. Neuraminidase treatment of immature myeloid cells resulted in the loss of PAS staining of GP115 and GP110 (Fig. 1*d*). Myeloblasts from a patient with acute myeloid leukaemia show only one major PAS-staining band (Fig. 1*f*) of apparent mol.wt. 112000 (GP112) and a fainter band of apparent mol.wt. 107000 (GP107). GP115 is the major labelled component after lactoperoxidase-catalysed radioiodination of intact granulocytes (Fig. 2*a*). Neuraminidase treatment of lactoperoxidase radioiodinated granulocytes yields a single major band of apparent mol.wt. 125000 (GP125), whereas GP115 is absent (Fig. 2*b*). After periodate/NaB^3H_4 labelling, GP115 is the major labelled component (Fig. 2*d*). GP115 is more intensely labelled in leukaemic immature myeloid cells (Fig. 2*c*). After prolonged autoradiography GP130 is labelled in normal granulocytes but not in leukaemic immature myeloid

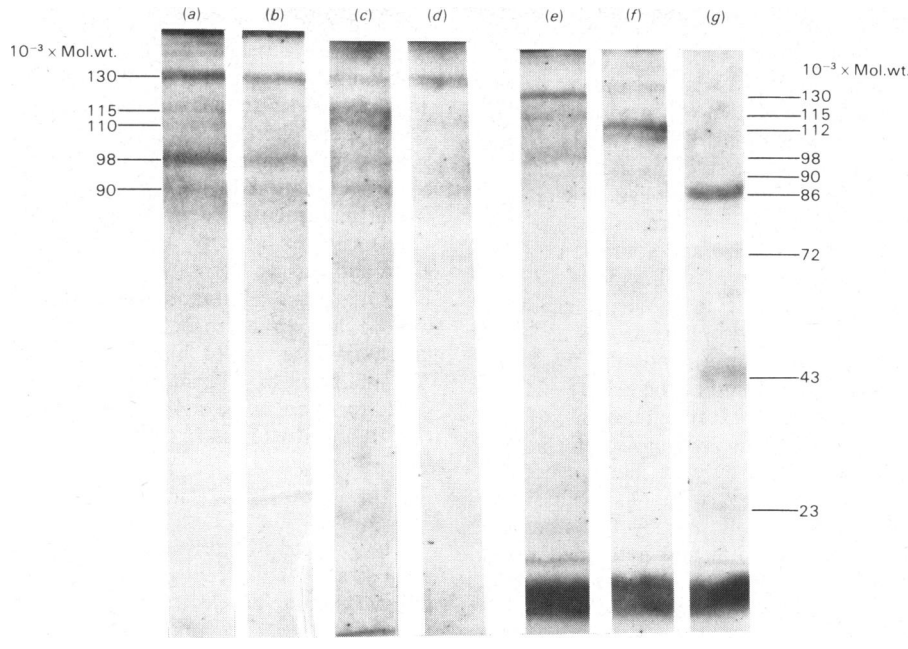

Fig. 1. *PAS-staining bands of electrophoretically separated components of normal granulocytes and leukaemic myeloid cells*

Sample preparation and conditions of electrophoresis were as described in the Materials and methods section. (*a*) CML granulocytes; (*b*) CML granulocytes (neuraminidase-treated); (*c*) CML immature myeloid cells (patient M.K.W.); (*d*) CML immature myeloid cells (neuraminidase-treated); patient M.K.W.) taken on the same day as sample (*c*); (*e*) normal granulocytes; (*f*) AML myeloblasts (patient D.C.); (*g*) normal erythrocyte ghosts. The illustration is compiled from the results of three separate gel-electrophoresis experiments. Samples (*a*) and (*b*) are from one experiment, (*c*) and (*d*) from another experiment, and (*e*), (*f*) and (*g*) from a third experiment.

cells, whereas GP90 is labelled in both cell preparations (Figs. 2*c* and 2*d*). Other lower-molecular-weight components common to both cell types are also labelled.

The binding of five radioiodinated lectins (concanavalin A, RCA, MA, HP and PNA) to the electrophoretically separated components of normal granulocytes and leukaemic myeloid cells was determined.

Binding of radioiodinated concanavalin A lectin

In normal mature granulocytes, concanavalin A lectin bound predominantly to GP130 and to a lesser extent to a rather diffuse area of apparent mol.wt. 68000 (GP68) (Fig. 3*a*). An identical concanavalin A binding pattern to that of normal granulocytes was observed for leukaemic granulocytes (Fig. 3*b*). The leukaemic immature myeloid cell fraction gave the concanavalin A-binding pattern shown in Fig. 3(*c*). A marked decrease in concanavalin A binding to GP130 relative to normal and leukaemic granulocytes was apparent, but bands denoted GP98 and GP90 were evident. Concanavalin A binding to GP90 was observed in all 13 patient samples analysed, whereas binding to GP98 was variable. Concanavalin A binding to leukaemic myeloblasts (two patients) demonstrated labelling of GP98 and intense labelling of GP90 (Fig. 3*d*), whereas GP130 was not apparent (Fig. 3*d*). In no case was the electrophoretic mobility of concanavalin A-binding components affected by prior neuraminidase treatment of the intact cells or by omitting 2-mercaptoethanol from the sample buffer used for cell solubilization (results not shown).

An attempt was made to correlate more clearly the presence of GP130 with the morphology of the myeloid cells from individual patients. Sample 1 (Fig. 3*f*), taken from a CML patient (M.K.W.) before treatment, was a buffy-coat preparation isolated from peripheral blood with a Haemonetics cell separator. The cell population comprised 10% myeloblasts, 55% promyelocytes, 20% myelocytes and 10% metamyelocytes, the remaining 5% of the cells being band forms, mature granulocytes, lymphocytes and normoblasts. Sample 2 (Fig. 3*g*) comprised immature myeloid cells (see the Materials and methods section) from the peripheral blood of the same patient 3 weeks later [during which time

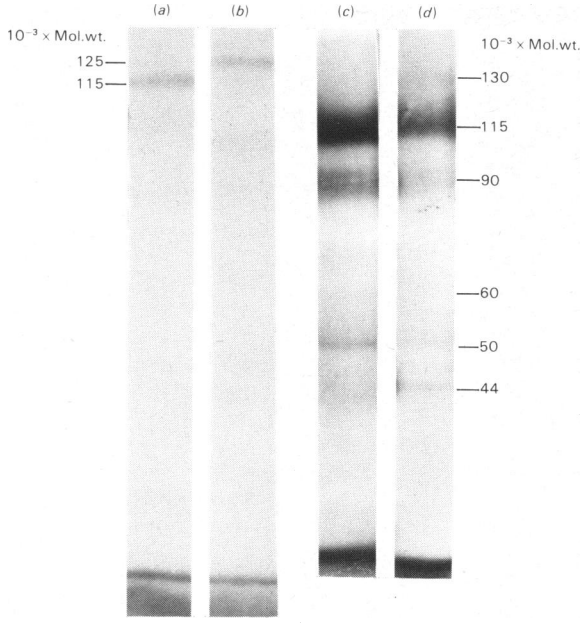

Fig. 2. *Lactoperoxidase- and periodate/NaB^3H_4-labelled surface glycoproteins of electrophoretically separated normal granulocytes and leukaemic immature myeloid cells*

Neuraminidase treatment was carried out on labelled cells. (*a*) Normal granulocytes surface-labelled by lactoperoxidase (5 days exposure); (*b*) normal granulocytes surface-labelled by lactoperoxidase followed by neuraminidase treatment (5 days exposure); (*c*) leukaemic immature myeloid cells (patient W.W.), surface-labelled by periodate/NaB^3H_4 (3 weeks exposure); (*d*) normal granulocytes surface-labelled by periodate/NaB^3H_4 (3 weeks exposure). Samples (*a*) and (*b*) were from the same experiment, and samples (*c*) and (*d*) were from another experiment.

the patient had been treated with Busulphan (4 mg/day) and Allopurinol (300 mg/day)] and consisted of 8% promyelocytes, 35% myelocytes, 38% metamyelocytes, 16% band forms, 16% lymphocytes and 3% granulocytes. Sample 1 (Fig. 3*f*) showed little concanavalin A binding to GP130, but prominent binding to GP90. Sample 2 showed binding to GP90 and GP130 (Fig. 3*g*). Significant binding to GP98 was not evident in this patient. The increased binding to GP130 in sample 2 correlates with the presence of a higher percentage of the more mature myeloid cells in this sample. Sequential samples from four other patients (W.W., H.J.L., R.B. and J.G.) gave similar results.

Binding of radioiodinated MA and RCA lectins

MA and RCA lectins gave similar binding patterns (Table 1; Fig. 4). Normal granulocytes bound lectin to bands GP130, GP115, GP110, GP98 and GP90 and to several other bands. GP125 was clearly labelled in neuraminidase-treated granulocytes. Leukaemic granulocytes and immature myeloid cells gave similar MA- and RCA-binding patterns to that obtained with normal granulocytes (Table 1). The binding of MA and RCA lectins to leukaemic myeloblasts was different from that obtained with mature granulocytes and immature myeloid cells. Only one major lectin-binding component was present (GP112), plus a minor band (GP107) (Fig. 4*f*). GP112 and GP107 were absent in neuraminidase-treated myeloblasts; however, new bands with apparent mol.wts. 125000 and 120000 were evident (Fig. 4*g*).

Binding of radioiodinated HP and PNA lectins

HP and PNA did not show significant binding to normal granulocytes, granulocytes or immature myeloid cells from the peripheral blood of patients with CML or to bone-marrow-derived myeloblasts from two patients with AML. However, prior treatment of the cells with neuraminidase resulted in binding of both HP and PNA to a single major band of apparent mol.wt. 125000 in all cell samples (Table 1).

Discussion

There are now many lectins with well-characterized sugar specificities, and so it is possible to select a panel of lectins with different sugar specificities to use as tools for the detection and characterization of glycoproteins in cells and at cell surfaces. In the present paper we have selected five lectins to look at the major glycoprotein components of normal granulocytes and leukaemic myeloid cells. The sugar specificities of these lectins are illustrated in Table 2. Lectin binding and PAS staining was performed on Triton-solubilized whole cells. The comparisons of different samples were made by addition of identical amounts of solubilized protein to each gel slot. In order to assess whether or not the major glycoprotein components detected using these techniques are accessible at the outer surface of intact cells, surface labelling was carried out by using lactoperoxidase-catalysed radioiodination (Moore *et al.*, 1982) and periodate/NaB^3H_4 (Andersson & Gahmberg, 1978). Lactoperoxidase-catalysed radioiodination of intact granulocytes yielded a single band of apparent mol.wt. 115000 (GP115). Neuraminidase treatment of radioiodinated intact granulocytes resulted in the appearance of a band of apparent mol.wt. 125000 (GP125) and the disappearance of GP115. Since this method labels the polypeptide moiety, this result suggests that GP125 is the desialylated form of GP115. GP115 therefore clearly corresponds to the sialo-

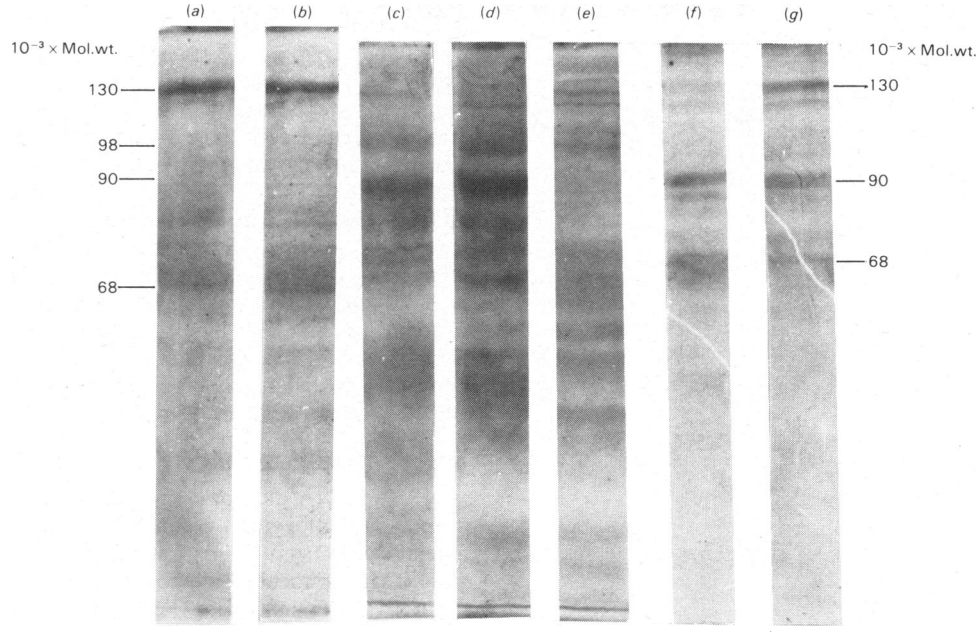

Fig. 3. *Binding of radioiodinated concanavalin A to electrophoretically separated components of normal granulocytes and leukaemic myeloid cells*

(*a*) Normal granulocytes; (*b*) granulocyte fraction from CML patient M.K.W.; (*c*) immature myeloid cells from CML patient T.B.; (*d*) bone marrow from patient D.C.; (*e*) normal unfractionated mononuclear cells; (*f*) immature myeloid cells from patient M.K.W. before treatment; (*g*) immature myeloid cells from patient M.K.W. after 3 weeks of treatment. The illustration is compiled from the results of three separate gel-electrophoresis experiments. Samples (*a*) and (*b*) are from one experiment, samples (*c*), (*d*) and (*e*) are from another experiment, and samples (*f*) and (*g*) are from a third experiment. Concanavalin A was radioiodinated as described in the Materials and methods section. The labelled lectin had an agglutination titre against Pronase-treated erythrocytes of 1:8000. About 1000 counts/s of labelled lectin were bound to the gel. Autoradiography was for 3 days.

glycoprotein of normal granulocytes described by Andersson & Gahmberg (1978) as having apparent mol.wt. 105000 when labelled with periodate and 130000 when labelled with galactose oxidase after neuraminidase treatment. MA and RCA lectins bound to GP115 in mature granulocytes, whereas binding of PNA and HPA lectins to GP125 was apparent in neuraminidase-treated granulocytes. An identical pattern of lectin binding was found in all immature myeloid cells examined. In leukaemic myeloblasts of a single patient, the major MA- and RCA-binding component had a slightly lower apparent molecular weight than in the other cells examined (112000 in comparison with 115000). This difference may simply reflect incomplete sialylation of GP112, since after neuraminidase treatment of both cell types the major MA- and RCA-binding component has apparent mol.wt. 125000. Our results also show that the PAS-staining bands GP110 of granulocytes and GP107 of myeloblasts bind RCA and MA and suggest a shift in their apparent molecular weights after neuraminidase treatment to 120000. It is therefore possible that a second minor sialoglycoprotein occurs in normal granulocytes and leukaemic myeloid cells. The presence of this band is most noticeable in neuraminidase-treated myeloblasts (Fig. 4*g*).

GP130 was not labelled on intact granulocytes when lactoperoxidase-catalysed radioiodination was used, but could be labelled by the periodate/NaB^3H_4 method, indicating that it is a surface glycoprotein on these cells. GP130 binds the lectins from concanavalin A, MA and RCA in normal granulocytes. Concanavalin A binding to GP130 was absent in leukaemic myeloblasts, and the amount of concanavalin A binding to GP130 increased with the increasing maturity of myeloid cells in the samples tested. Thus Triton-solubilized material from immature-myeloid-cell samples rich in promyelocytes and myelocytes showed very poor concanavalin A binding to GP130, whereas those depleted of such cells and enriched with more mature cells showed an increase in concanavalin A binding. These differences in concanavalin A binding in different cell

Table 1. *Comparison of the PAS-staining and lectin-binding properties of the major glycoproteins of leukaemic myeloid cells and normal granulocytes*
Leukaemic myeloblasts were isolated from the bone marrow of a single patient with acute myeloid leukaemia. Immature myeloid cells (see the Materials and methods section) were derived from the peripheral blood of several different patients with chronic myeloid leukaemia. Mature granulocytes were derived from the peripheral blood of normal blood donors. Mature granulocytes from the peripheral blood of patient with chronic myeloid leukaemia gave PAS staining and lectin-binding patterns indistinguishable from mature granulocytes of normal blood donors. Intensity of PAS staining and lectin binding is indicated by –, (+), +, ++, +++, where – represents no staining or binding, and +++ represents maximal staining or binding. Abbreviation used: ND, no data.

	Staining intensity with PAS stain			Staining intensity ... Concanavalin A			*Ricinus communis*			*Maclura aurantiaca*			*Helix pomatia**/ *Arachis hypogaea**		
Glyco-proteins	Leukaemic myelo-blasts	Immature myeloid cells	Mature granul-ocytes	Leukaemic myelo-blasts	Immature myeloid cells	Mature granul-ocytes	Leukaemic myelo-blasts	Immature myeloid cells	Mature granul-ocytes	Leukaemic myelo-blasts	Immature myeloid cells	Mature granul-ocytes	Leukaemic myelo-blasts	Immature myeloid cells	Mature granul-ocytes
GP130	–	(+)	+++	–	+	+++	–	(+)	+	–	+	+++	–	–	–
GP125†	(+)	(+)/–	–	–	–	–	+++	+++	ND	+++	+++	+++	(+)	(+)	(+)
GP115	–	++	+	–	–	–	–	++	++	–	+	+	–	–	–
GP112	++	–	–	–	–	–	++	–	–	++	–	–	–	–	–
GP98	–	+	+++	+	++/+	–	–	++	+++	–	+	+++	–	–	–
GP90	–	+	++	+++	++	–	–	++	++	–	+	+	–	–	–

* Results for *Helix pomatia* and *Arachis hypogaea* lectins were obtained with neuraminidase-treated cells.
† GP125 is only present on neuraminidase-treated cells.

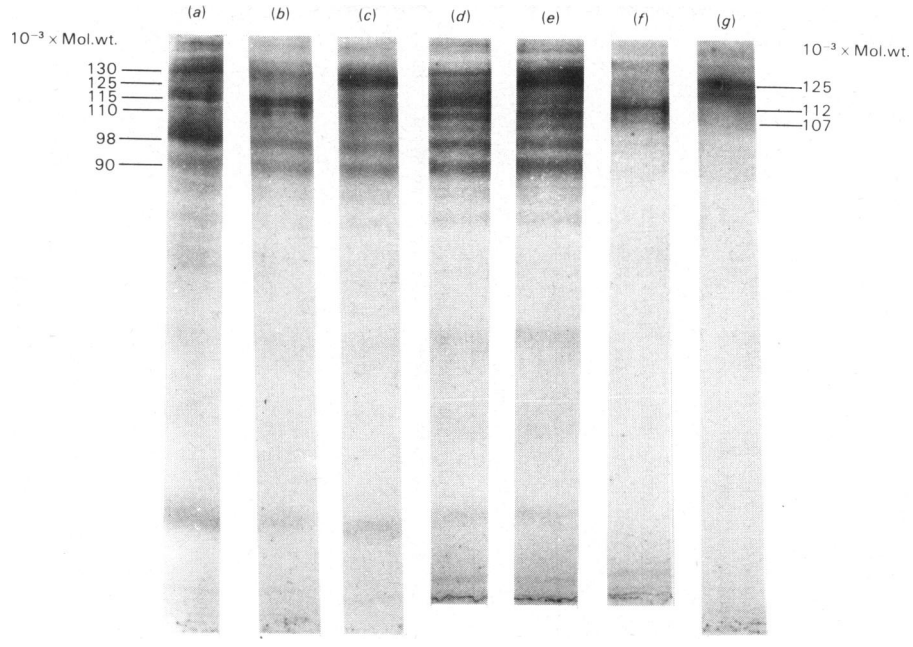

Fig. 4. *Binding of radioiodinated RCA to electrophoretically separated components of normal granulocytes and leukaemic myeloid cells*

(*a*) Normal granulocytes; (*b*) immature myeloid cells from patient M.K.W., sample as in Fig. 3(*g*); (*c*) neuraminidase-treated immature myeloid cells from patient M.K.W., sample as in (*b*) above; (*d*) immature myeloid cells from patient M.J.; (*e*) neuraminidase-treated immature myeloid cells from patient M.J.; (*f*) bone-marrow cells from a patient with AML (D.C.); (*g*) neuraminidase-treated bone-marrow cells from a patient with AML (D.C.). Samples (*a*), (*b*), (*c*) and (*g*) were from one experiment, and samples (*d*), (*e*) and (*f*) were from another experiment. RCA was radioiodinated as described in the Materials and methods section. The labelled lectin had an agglutination titre of 1:512 against human erythrocytes. About 20 counts/s of labelled lectin were bound to the gel. Autoradiography was for 8 weeks.

Table 2. *Oligosaccharide structures preferentially bound by the lectins used in the present study*

Lectin	Oligosaccharide	Reference
Concanavalin A	GlcNAcβ(1–2)Manα(1–3) Manβ(1–4)GlcNAc GlcNAcβ(1–2)Manα(1–6)	Baenziger & Fiete (1979*a*) Debray *et al.* (1981)
Ricinus communis lectins I and II	Galβ(1–4)GlcNAcβ(1–2)Manα(1–3) Manβ(1–4)GlcNAc Galβ(1–4)GlcNAcβ(1–2)Manα(1–6)	Baenziger & Fiete (1979*b*) Debray *et al.* (1981)
Ricinus communis lectin II	Galβ(1–3)GalNAc	Baenziger & Fiete (1979*b*)
Maclura aurantiaca	Galβ(1–3)GalNAc	Sarkar *et al.* (1981)
Helix pomatia	GalNAc-α-	Hammarström & Kabat (1971)
Arachis hypogaea	Galβ(1–3)GalNAc	Lotan *et al.* (1975) Terao *et al.* (1975) Pereira *et al.* (1976)

types suggest the possibility that GP130 is synthesized at a late stage in granulocyte maturation and may therefore have an important functional role in the mature granulocyte. Indeed, SDS/polyacrylamide-gel electrophoretic studies on cells from a boy with recurrent infections due to pyogenic organisms,

who was shown to have defective phagocytosis of opsonized particles, revealed the absence of the high-molecular-weight major PAS-staining glycoprotein which may correspond to the concanavalin A-binding protein described here (Arnaout *et al.*, 1982). A similar concanavalin A-binding protein (P180) has been reported to appear during differentiation of the mouse myeloid-leukaemia cell line M1 into macrophages and granulocytes (Sugiyama *et al.*, 1979). We cannot, however, rule out the possibility that absence of concanavalin A binding reflects different glycosylation, rather than a total absence, of GP130.

GP98 and GP90 were not labelled by lactoperoxidase-catalysed radioiodination; however, GP90 was clearly labelled on normal granulocytes and immature myeloid cells by using the periodate/NaB^3H_4 method. Glycoproteins GP98 and GP90 are prominently labelled by RCA and MA lectins in mature granulocytes and in leukaemic immature myeloid cells. However, myeloblasts from a single AML patient showed little, if any, binding of RCA or MA to GP98 and GP90. In contrast, concanavalin A lectin, although detecting bands of GP98 and GP90 in AML cells and to a lesser extent in more differentiated samples, did not bind GP98 and GP90 in mature granulocytes. From a consideration of the known specificity of concanavalin A and RCA lectins (Table 2), it is possible that the decrease in concanavalin A binding and concomitant increase in RCA binding reflects changes in the glycosylation of these glycoproteins occurring during maturation of the cells from myeloblasts to more mature forms. Concanavalin A binding would be reduced as galactose residues are added to the terminal *N*-acetylglucosamine residue of one or more *N*-glycosidically linked oligosaccharides on each glycoprotein, and, as the galactose residues are added, so RCA activity would be increased. Such a possibility defines an important consideration when using lectins or other anti-carbohydrate probes to monitor cellular differentiation, since the failure to detect a glycoprotein band may indicate altered glycosylation rather than total absence of the glycoprotein. A similar correlation of maturation with glycosylation has been noted by Reisner *et al.* (1979), who reported that PNA lectin will define two populations of human thymocytes. Reisner *et al.* (1979) also looked at the binding of PNA lectin to various types of leukaemic cells and noted that, in most cases of acute lymphoblastic leukaemia, stem-cell leukaemia and myeloid leukaemia, the blasts bound fluorescent PNA lectin directly, whereas in only 3 of 32 patients with chronic lymphocytic leukaemia was direct binding of the lectin observed. These authors suggest that the addition of sialic acid to PNA receptors correlates with cell maturation.

Remarkably little is known regarding the structure and function of the major surface glycoproteins of granulocytes and granulocyte precursors. The use of lectins described here has allowed a much more selective identification of the major glycoproteins than can be obtained with the periodate/NaB^3H_4 and lactoperoxidase-labelling techniques.

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