Developmentally-related changes in surface membrane glycopeptides of murine haemopoietic cells

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We have carried out a comparative study of mature murine granulocytes with two immature haemopoietic cell lines (multipotential cells, FDCP-Mix, and granulocyte progenitor cells, FDCP-2) with respect to the structure and composition of their surface membrane glycopeptides. The glycopeptides were labelled biosynthetically by incubation of the cells for 1–3 days with [³H]glucosamine. Cell-associated glycopeptides were released by treatment with trypsin and the trypsin extract was exhaustively digested with Pronase to remove most residual peptide. Radiolabelled materials were fractionated by chromatography on lectin affinity columns connected in the series: lentil lectin (LCA), concanavalin A (Con A) and wheat germ agglutinin (WGA). Lectin-binding glycopeptides were eluted with appropriate competing sugars and further analysed by gel filtration, base/borohydride elimination and susceptibility to degradation by glycosidases including endo- β -galactosidase. Abundant quantities of *N*-linked polylactosamine-type glycopeptides, which bound only to the WGA columns, were identified on mature granulocytes but the molecules were highly-branched (i.e. resistant to endo- β -galactosidase). In contrast, there seemed to be very little branching in the polylactosamine chains from FDCP-2 cells, whilst corresponding carbohydrates from multipotential FDCP-Mix cells gave evidence for both linear and branched domains in the same, large complex glycans. *O*-Linked tetrasaccharides of general structure: NeuAc-Gal-(NeuAc)-GalNAc were found in clusters on WGA-binding glycopeptides from all cell types, these components being especially prominent on mature granulocytes. FDCP-2 cells were distinguished by the presence of monosialylated and non-sialylated counterparts of the foregoing tetrasaccharides. The relative amount of LCA-binding glycopeptides was low on FDCP-Mix cells by comparison with FDCP-2 cells and mature granulocytes. Our findings therefore demonstrate that notable differences in gross composition and molecular fine structure of surface membrane glycopeptides are detectable in haemopoietic cells at different stages of development. The relationship of these differences to the biological properties of cell surfaces remains to be established.

INTRODUCTION

The importance of cell surface glycoconjugates, and in particular polylactosamine-type carbohydrate, in cellular recognition and behaviour has been emphasized in several recent reviews (Fukuda, 1985; Feizi, 1985; Viitala & Jarnefelt, 1985). Polylactosamine-type glycans inhibit the binding of cultured cells to laminin substrates and may affect the metastasis of tumour cells and their recognition by the host defence mechanisms (Dennis *et al.*, 1984; Dennis & Laferte, 1985). Cell surface expression of carbohydrate antigens that are specified by branched and linear arrangements of polylactosamine sequences changes during embryonic development and differentiation (Fukuda *et al.*, 1981; Feizi, 1983). Furthermore, the full complexity of the carbohydrates which contain the polylactosamine sequences is gradually being elucidated. These molecules have been isolated from a range of normal (Fukuda, 1985; Spooncer *et al.*, 1984b) and malignant (Fukuda *et al.*, 1985; Mizoguchi *et al.*, 1986) haemopoietic cells. They are attached to protein via GlcNAc–asparagine *N*-glycosidic linkages and they may display complex branching patterns in the mannan core and in the outer chains.

In previous studies we have examined cell surface glycopeptides from two murine haemopoietic cell lines: growth factor (interleukin 3)-dependent, multipotential FDCP-Mix cells (Morris *et al.*, 1986), which are similar in many respects to normal bone marrow stem cells

(Spooncer *et al.*, 1984a; Spooncer *et al.*, 1986), and a myeloid precursor cell population (416B) which grows in the absence of added growth factors (Gallagher *et al.*, 1985). In the course of these studies we found that the lectin wheat germ agglutinin (WGA) interacted strongly with polylactosamine chains and that the binding strength of glycopeptides for this lectin, combined with gel filtration analysis of endo- β -galactosidase digestion products, yielded useful structural information on these complex carbohydrates. In the present work we have used these and other techniques to examine the surface glycopeptides of two additional groups of myeloid cells, these being: (1) mature granulocytes (NBMDG), and (2) a growth-factor (interleukin-3)-dependent cell line (FDCP-2) which is diploid, non-leukaemic and which represents a committed granulocyte progenitor (Dexter *et al.*, 1980b; Bazill *et al.*, 1983). The findings have been compared with the previously published data on the FDCP-Mix ‘stem cells’ (Morris *et al.*, 1986). Our results indicate that significant changes occur in the molecular size and branching patterns of polylactosamine chains during haemopoietic cell development.

MATERIALS AND METHODS

Cell cultures

FDCP-2 and FDCP-Mix cells were grown in culture in the presence of medium conditioned by the growth of

Abbreviations used: LCA, lentil lectin; Con A, concanavalin A; WGA, wheat germ agglutinin.

Fig. 1. Serial lectin affinity chromatography of cell-surface glycopeptides labelled with [^3H]glucosamine

Proteinase-digested glycopeptides were applied to lectin affinity columns coupled in series. The affinity column series was then washed with buffer. Columns were then separated and eluted with buffer, followed by different concentrations of specific sugar as described in the Materials and methods section. The LCA and Con A

RESULTS

Affinity chromatography

Proteinase-digested, trypsin-sensitive glycopeptides from the cell surfaces of each cell line which had been metabolically labelled with [^3H]glucosamine were applied to affinity columns of LCA, Con A and WGA connected in series. The columns were step-eluted with specific sugars as described in the legend to Fig. 1. In total, 75–80% of the glycopeptides in the original Pronase digest were bound by these lectin columns. The majority of the glycopeptides were in the LCA- and WGA-binding fractions. However, the relative quantity of LCA-binding glycopeptide is much smaller in FDCP-Mix cells than in FDCP-2 and mature granulocytes (NBMDG): 16% in total compared with about 30% in NBMDG and FDCP-2. In contrast, the relative proportion of WGA-binding material is highest in FDCP-Mix cells (74%). Regarding the individual WGA-affinity classes, FDCP-Mix cells contain the largest amounts of the high-affinity WGA-S and WGA-SS fractions, while granulocytes yielded the largest percentage of components showing weak (WGA-W) and intermediate (WGA-I) affinity for WGA (Fig. 1).

Characterization of WGA-binding glycopeptides

Reductive alkaline elimination. Each affinity class of WGA-binding glycopeptide was purified by dialysis and fractionated either untreated, or after treatment with mild alkali/borohydride, on a column of Bio-Gel P-6 (Fig. 2). Data are given only for the WGA-W glycopeptides. Untreated samples eluted exclusively in the void volume of the P-6 column. Following alkali treatment, significant proportions of material in WGA-W and WGA-I fractions from each cell type were retarded by the gel, the major retarded fraction having a K_{av} of 0.31. This peak was particularly prominent in granulocyte-derived glycopeptides (Fig. 2c). We have characterized this material as an *O*-linked tetrasaccharide of structure: NeuAc-Gal-(NeuAc)-GalNAc-ol (Gallagher *et al.*, 1985). Presumably these materials were clustered in the original glycopeptides so that proteinase was unable to degrade the molecules into components with single oligosaccharide chains. The large molecules (see Fig. 2) which continue to elute in the void volume of the column after alkali treatment are probably *N*-linked glycopeptides, based on additional experimental criteria described previously (Gallagher *et al.*, 1985).

In addition to sialylated tetrasaccharides two additional peaks were produced by alkali elimination of the

columns were step-eluted with 5 mM- and 0.5 M- α -methyl mannoside (MeM) to give weak (LCA-W and Con A-W) and strong (LCA-S and Con A-S) affinity preparations. Fractions (2 ml) were collected and 50 μl aliquots were taken for counting of radioactivity. The WGA-column was step-eluted with 0.01 M-, 0.1 M- and 0.5 M- and 1.0 M-GlcNAc to give weak (WGA-W), intermediate (WGA-I), strong (WGA-S) and very strong (WGA-SS) binding glycopeptides. Fractions (4 ml) were collected and 100 μl aliquots were counted. Fractions 1–7, 36–42 and 71–75 represent elution with buffer containing 1 M-NaCl. Arrows on the graph mark the beginning of each step elution. (a) Multipotential cells, FDCP-Mix; (b) granulocyte progenitor, FDCP-2; (c) mature granulocytes, NBMDG.

Fig. 2. Gel-filtration on Bio-Gel P-6 of alkali/borohydride-treated WGA-W-binding glycopeptidases

$[^3\text{H}]$ Glucosamine-labelled WGA-W-binding glycopeptides from Fig. 1 were dialysed against distilled water and treated with alkali/borohydride. They were then applied to a column (1.0 cm $\times$ 57 cm) of Bio-Gel P-6 (200–400 mesh) and eluted at about 3 ml/h with 0.25 M- NH_4HCO_3 . Elution volumes of reference mucin-type tetrasaccharide (Tetra), disaccharide (Disacc), and $[^{14}\text{C}]$ mannose are

Fig. 3. Relative proportions of *N*-linked glycopeptides of murine haemopoietic cells

The histograms have been compiled following subtraction of the *O*-linked carbohydrate content of the WGA-binding glycopeptides. 'W', 'I', 'S' and 'SS' refer to the four categories of WGA-binding glycopeptides as defined in the legend to Fig. 1.

FDCP-2 cell glycopeptides (Fig. 2*b*). The smallest peak corresponded in size to a reference disaccharide of structure Gal-GalNAc-ol, and a second slightly larger component was equivalent to a monosialylated form of this disaccharide. Therefore the *O*-linked chains of FDCP-2 contain a significant proportion of undersialylated structures compared with those from FDCP-Mix and NBMDG. Furthermore, the WGA-S and WGA-SS fractions from FDCP-2 contained *O*-linked carbohydrate similar in quantity and composition to that illustrated in Fig. 2(*b*) for the WGA-W fraction. However, in FDCP-mix cells and granulocytes the corresponding strong WGA-binding materials contained very little (< 15%) *O*-linked carbohydrate (results not shown).

Comparison of the *N*-linked carbohydrate content of each cell type. The amount of radiolabelled *N*-linked glycopeptides in the WGA-binding fractions is readily calculated following degradation of the *O*-linked glycopeptides by alkaline elimination (Fig. 2). LCA and Con A also bind *N*-linked glycans but they interact mainly with small, complex chains and, in the case of Con A, high-mannose oligosaccharides (Table 1). Fig. 3 gives a breakdown of the contribution of LCA-, Con A- and WGA-binding glycopeptides to the total *N*-linked material isolated by lectin affinity chromatography. Of the *N*-linked glycopeptides from FDCP-Mix cells, 60% bind only to WGA whereas the corresponding value for FDCP-2 and mature granulocytes is 30%. FDCP-Mix cells are the only cells which produce significant amounts of the highest-affinity WGA-SS fraction (see Fig. 1).

indicated. Alkali-stable material from the void volume peak (fractions 20–28 or 12–17) was taken for further analysis. Cell lines are: (a), FDCP-Mix; (b), FDCP-2; (c), NBMDG. Fraction sizes: (a) 0.5 ml; (b) and (c), 1 ml.

Structural comparison of *N*-linked glycopeptides from each cell line

The *N*-linked fractions of each WGA-binding class of glycopeptides were then analysed by gel filtration and glycosidase digestions.

Gel filtration. The molecular size distributions of the *N*-linked WGA-binding glycopeptides was analysed by chromatography on Sephadex G-100 (Fig. 4). The plots show data for FDCP-Mix and FDCP-2. Granulocyte-derived glycopeptides were very similar in molecular size to those from FDCP-Mix. The majority of the glycopeptides were larger than α_1 -acid glycoprotein-derived oligosaccharides (which contain some complex tri- and tetra-antennate *N*-glycans), so the WGA-binding components are relatively large. In the case of FDCP-Mix- and granulocyte-derived materials there was a direct correlation between WGA-binding strength and molecular size, with the WGA-SS fraction emerging just after the excluded volume on G-100 (K_{av} 0.16; Fig. 4d), whereas the WGA-W glycopeptides gave a K_{av} value of 0.53 (Fig. 4a). In general the glycopeptides from FDCP-Mix and granulocytes were reasonably homogeneous on gel filtration. WGA-W and WGA-I preparations from FDCP-2 cells were slightly smaller than counterparts from FDCP-Mix (Figs. 4a and 4b). However, the WGA-S and WGA-SS fractions from FDCP-2 cells yielded two distinct populations of glycopeptides (Figs. 4c and 4d). In each case the larger species were of comparable size to the large glycopeptides from the FDCP-Mix cells but the smaller components were of a similar size to the WGA-W binding glycopeptides.

Neuraminidase treatment. All the WGA-binding, *N*-linked glycopeptide preparations from each cell type contained moderate quantities of neuraminidase-sensitive ${}^3\text{H}$ radioactivity, which was presumably in the form of sialic acid. Broadly similar quantities of ${}^3\text{H}$ label were released, mainly in the range of 11–17% of the total ${}^3\text{H}$ content. The one exception was the WGA-W fraction from FDCP-Mix cells: 33% of the ${}^3\text{H}$ content of these glycopeptides was released by neuraminidase treatment.

Endo- β -galactosidase treatment

Intact *N*-linked WGA-binding glycans were treated with endo- β -galactosidase at an enzyme concentration of 0.125 units/ml and fractionated on Sephadex G-50 (Fig.

Fig. 4. Molecular size of *N*-linked WGA-binding glycopeptides on Sephadex G-100

Alkali-stable glycopeptides from Fig. 2 were applied to a column (0.65 cm $\times$ 48 cm) of Sephadex G-100 (superfine grade) and eluted at about 2 ml/h with 0.25 M-NH₄HCO₃. (a), WGA-W; (b), WGA-I; (c), WGA-S; (d) WGA-SS. V_0 , void volume (Dextran Blue); V_t , total included volume (sodium dichromate). The elution positions of α_1 -acid glycopeptides (α_1) and LCA-W glycopeptides are indicated. ●, FDCP-Mix; ○, FDCP-2. *N*-linked glycopeptides from granulocytes were similar in molecular size to the glycopeptides from FDCP-Mix cells. Fraction size; 0.41 ml.

Fig. 5. Sephadex G-50 chromatography of untreated and endo- β -galactosidase-treated glycopeptides

[${}^3\text{H}$]Glucosamine-labelled *N*-linked WGA-binding glycopeptides were run either untreated or endo- β -galactosidase-treated on a column (59 cm $\times$ 0.9 cm) of Sephadex G-50 (superfine grade) and eluted at 3 ml/h with 0.25 M NH_4HCO_3 . Each profile represents a separate column run. (—), untreated samples; (---), endo- β -galactosidase-treated samples. Reference molecules indicated with an arrow are the trimannan core prepared from LCA-W-binding glycopeptides and a disaccharide of structure Gal-GalNAc-ol (see the Materials and methods section). Cell lines are: (a), FDCP-Mix; (b), FDCP-2; (c) NBMDG. WGA-affinity fractions are (left to right): WGA-W, WGA-I, WGA-S and WGA-SS. There was insufficient material in the WGA-SS fraction from granulocytes (see Fig. 1c) for satisfactory analysis. Fraction size: 0.47 ml.

5). At this concentration the enzyme selectively degrades repeat *N*-acetyl-lactosamine sequences where galactose residues are unbranched:

(Fukuda, 1981). Galactose units in branched sequences of structure:

are generally resistant to the enzyme. Substitution of the lactosamine sequences with fucose residues may also inhibit the enzyme, although this is unlikely to be a major factor in this study since fucosylation of these sequences also inhibits the binding to WGA (Carlsson *et al.*, 1976). The situation is complex, however, because it has recently been shown that fucose residues may strengthen the binding of some poly-lactosamine chains to WGA (Ivatt *et al.*, 1986). The arrangement of the fucose residues in these molecules has not been determined.

The pattern of breakdown of poly-lactosamine chains caused by endo- β -galactosidase will reflect the numbers and arrangement of branched galactose residues. Molecular size profiles of the degradation products therefore provide a fingerprint of the molecular structure of the intact polymer. Separations on Sephadex G-50 of the enzyme-treated and untreated WGA-binding glycopeptides are shown in Fig. 5. Susceptibility to the enzyme is shown by an overall reduction in molecular size relative to the untreated materials.

The smallest degradation products were disaccharides (peak I) of structure GlcNAc-Gal (these originate from linear regions of the glycan chain) and the peak II fraction which we believe to be composed of sialylated tetrasaccharides of structure NeuAc-Gal-GlcNAc-Gal-. This assumption is based on the findings that: (a) the molecular size of the peak is reduced following neuraminidase treatment (see Morris *et al.*, 1986) and (b) the specificity of endo- β -galactosidase is such that the smallest sialylated fragment that the enzyme can produce is a four-sugar sequence as described. A terminal sugar sequence of NeuAc-Gal in all the poly-lactosamine structures studied here is indicated by the finding that these components bind quantitatively to a ricin affinity column following neuraminidase treatment (results not shown).

The proportions of radioactivity in peaks I (disaccharide) and II (tetrasaccharide) give an indication of the amounts of internal and external linear sequences. In general the amount of radioactivity in peak II relative to that in peak I decreases with increasing affinity of the glycans to WGA. This finding is consistent with the observed general correlation between molecular size and WGA affinity (Figs. 4 and 5) with a higher content of internal, linear *N*-acetyl-lactosamine units presumably contributing to these molecular size increases.

Conspicuous differences were observed in the endo- β -galactosidase degradation profiles of the poly-lactosamine chains from the three cell populations studied in this paper. Limited breakdown only was observed in the WGA-binding fractions from mature granulocytes (Fig. 5c). The yields of di- and tetra-saccharides were low in all fractions and the majority of the glycopeptides after

endo- β -galactosidase treatment eluted as a major peak only slightly more retarded than the untreated preparations. These findings are indicative of a predominance of highly-branched sequences in the poly-lactosamine chains from granulocytes.

In contrast with the data obtained with granulocytes, the WGA-binding poly-lactosamine chains from FDCP-2 cells were more extensively degraded by endo- β -galactosidase (Fig. 5b), with relatively high yields obtained in both the di- and tetra-saccharide peaks (peaks I and II). The only other major fraction in all FDCP-2 derived glycans was a component of intermediate size, K_{av} 0.44. This peak may represent a common 'core' oligosaccharide attached directly to polypeptide which occurs in all these WGA-binding glycopeptides. Little branching in the outer chains of these molecules is evident as the only low- M_r products (peaks I and II) originate from linear domains of the *N*-acetyl-lactosamine repeat units.

The WGA-W fraction from FDCP-Mix gave an enzyme-degradation profile that was similar to that of WGA-W from FDCP-2 cells (cf. Fig. 5a, WGA-W, and Fig. 5b, WGA-W). The contrast between these two WGA-W profiles and that for the WGA-W preparation from granulocytes (Fig. 5c, WGA-W) is particularly striking. The remaining FDCP-Mix-cell glycopeptides gave the most complex and heterogeneous degradation profiles observed in this study (Figs. 5a, WGA-I, WGA-S and WGA-SS). The plots show that endo- β -galactosidase releases di- and tetrasaccharides together with a range of intermediate and larger sized fragments, covering most of the fractionating range of the column. These findings are indicative of mixed arrangements of linear and branched domains in the poly-lactosamine-type glycopeptides and they clearly differentiate FDCP-Mix poly-lactosamine chains from those produced by granulocytes (branched chains) and FDCP-2 cells (linear chains).

DISCUSSION

The methods used in this study have led to the identification of several variations in carbohydrate structure and composition of murine haemopoietic cells at different stages of development. Because the FDCP-Mix and the FDCP-2 cells are both interleukin-3-dependent continuously growing cells, the variations observed are likely to be due to their developmental potential rather than their dependency on, or their response to, interleukin-3. Similarly, the labelling studies with mature granulocytes were also performed in the presence of interleukin-3, thus ruling out the possibility that the changes observed are simply related to the presence or absence of growth factor. Indeed, the differences found between these different cells probably reflect fundamental changes in their progressive development from multipotent cells (FDCP-Mix) through committed progenitor cells (FDCP-2) to mature, functional granulocytes.

The saccharide-binding specificities of the lectins used in this study are given in Table 1. All cell types produced some glycopeptides that bound to each lectin but the highest-affinity WGA-binding structures (large poly-lactosamine-type molecules) were virtually absent from the granulocyte extracts (Fig. 1). A low expression of fucosylated complex *N*-glycans (LCA-binding materials)

Table 1. Carbohydrate-binding properties of lectins used in this study

Further details and references on the carbohydrate-binding properties of these and other lectins are reviewed in Gallagher (1984).

Lectin	Structure recognized
LCA	<div>NeuAc-Gal-GlcNAc-Man</div> <div>NeuAc-Gal-GlcNAc-Man</div> <div>Man-GlcNAc-GlcNAc-Asn</div> <div>Fuc</div>
Con A	<div>(a) <div>NeuAc-Gal-GlcNAc-Man</div><div>NeuAc-Gal-GlcNAc-Man</div><div>Man-GlcNAc-GlcNAc-Asn</div><div>Fuc ±</div></div> <div>(b) <div>Man-Man</div><div>Man-Man-Man</div><div>Man-GlcNAc-GlcNAc-Asn</div><div>Man-Man-Man</div></div>
WGA	<div>(a) Gal-GlcNAc-Gal-GlcNAc-Gal-GlcNAc</div> <div>(b) <div>NeuAc-Gal-GalNAc-</div><div>NeuAc</div></div> <div>(c) <div>R₁-Man</div><div>GlcNAc-Man-GlcNAc-GlcNAc-Asn</div><div>R₂-Man</div></div>

was a consistent and notable feature of the multipotential stem cells (FDCP-Mix cells), distinguishing them from FDCP-2 cells and granulocytes (Fig. 1).

The highest yields of glycopeptides were obtained from the WGA-affinity column (Fig. 1). Analyses of these materials gave the most interesting data in this study. WGA-binding *N*-linked glycopeptides were larger than the LCA- or Con A-binding components eluting from a Sephadex G-100 column, with *K_{av}* values ranging from 0.53 for WGA-W to 0.16 for WGA-SS (Figs. 4*a* and 4*d*). *O*-linked oligosaccharides also bound to the WGA-column, occurring as clusters of small carbohydrate chains attached to a common polypeptide backbone. In FDCP-Mix cells and granulocytes these molecules were predominantly disialylated tetrasaccharides (Table 1, structure *b*) and they were enriched in mature granulocytes (Fig. 2). FDCP-2 cells produced a heterogeneous population of small *O*-linked saccharides that included significant quantities of disaccharides of probable structure Gal-GalNAc, which were hardly detectable in the other two cell types (Fig. 2).

The *N*-linked glycopeptides in the WGA-binding fractions from each cell type were sialylated, polylactosamine type sequences of variable size and they displayed distinct, cell-type-related molecular structures as demonstrated by analysis of the degradation products of

endo- β -galactosidase treatment (Fig. 5). Rather small, enzyme-sensitive linear sequences predominated in FDCP-2 (Fig. 5*b*) cells, whilst the differentiated granulocytes synthesized large oligo- and poly-saccharides that appeared to be highly branched as demonstrated by their relative resistance to breakdown by endo- β -galactosidase (Fig. 5*c*). Fukuda and colleagues (Fukuda *et al.*, 1981) have shown that branching increased in polylactosamine structures of human myeloid cells during maturation. In the present study we had the advantage of being able to study a murine haemopoietic stem cell line, for which there is no available human equivalent. These multipotential stem cells (FDCP-Mix) produced very complex populations of polylactosamines (Fig. 5*a*) that contained both linear and branched domains. If the cells used in this study do indeed accurately reflect normal haemopoietic cell development *in vivo*, then structural changes in polylactosamine chains during maturation along the myeloid-to-granulocyte axis progress from large molecules with intermediate branching in multipotential cells (FDCP-Mix), to linear carbohydrate chains in granulocyte progenitors (FDCP-2) and finally to highly branched constituents in mature granulocytes (see Fig. 6).

A major similarity between FDCP-Mix and FDCP-2 cells was the structure of their WGA-W polylactosamine

Multipotential stem cells (FDCP-Mix)

Granulocyte progenitor cells (FDCP-2)

Mature granulocytes

Fig. 6. Structural changes in polylectosamine-type oligosaccharides during murine haemopoietic cell development

sequences (Figs. 5a, WGA-W, and 5b, WGA-W). Endo- β -galactosidase digestion of these molecules gave very little evidence for branching, the main low- M_r breakdown products being disaccharides and sialic acid-containing tetrasaccharides derived from linear regions. The third breakdown product was a larger relatively homogeneous component (K_{av} on G-50 of 0.44) which we have assumed to be the mannosyl-core region of the glycopeptide. This putative 'core' structure was larger than the intact, biantennate LCA-W material. Polylectosamine sequences of myeloid cells are often attached to tri- and tetra-antennary glycans (Spooncer *et al.*, 1984b; Fukuda *et al.*, 1984, 1985) and it seems most probable that the core structure in WGA-W (and most likely in the other WGA-binding fractions as well) is also a multi-branched domain. The WGA-affinity column was very useful for separating this group of rather small, unbranched molecules from the remainder of the polylectosamine chains. It was significant however that the WGA-W fraction from mature granulocytes was only slightly degraded by endo- β -galactosidase (Fig. 5c, WGA-W). Branched structures are thus evident throughout the size range of polylectosamine sequences produced by granulocytes (Fig. 5c).

Although they have been implicated in cell-to-cell and cell-to-substrate interactions (see the Introduction), the biological functions of polylectosamine chains remain unknown. Their relative abundance, large size, structural complexity and modulation during cell development argue strongly for important roles in recognition processes in the pericellular region, since interactions between immature cells and their microenvironment are essential for normal haemopoietic cell differentiation (Spooncer *et al.*, 1984a; Wyke *et al.*, 1986). Future studies should aim to identify the cell surface glycoproteins that contain polylectosamine sequences to see if individual glycoprotein species bear a more restricted

range of carbohydrate chains than can be found in trypsin extracts of the entire cell surface, and might, for example, evaluate polylectosamine chains for their ability to influence the binding and maturation of the stem cells (FDCP-Mix), and freshly isolated bone marrow stem cells, on inductive stromal cell layers *in vitro* (Dexter *et al.*, 1980a, 1985; Dexter & Testa, 1980). These studies should throw some light on the regulation of structural diversity and function of these complex carbohydrates.

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