Proteoglycans in Cellular Recognition and Secretory Functions in the Haemopoietic System

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Introduction

Proteoglycans (PGs) are synthesised by all nucleated haemopoietic cells and are also present in the α-granules of platelets [65]. Their sulphated polysaccharide chains, the glycosaminoglycans (GAGs), have been recognised for many years as prominent components of secretory granules of mast cells and basophils, and the unusually high degree of sulphation of these GAGs is responsible for the pronounced metachromasia of the granular structures [31, 133]. Earlier studies also detected GAGs in lysosomes or prelysosomes of neutrophils [94]. The core protein of the major PG of secretory granules has been cloned and sequenced and appears to be quite widely distributed in the haemopoietic system. Recent immunological studies have also identified three distinct proteoglycan species in lymphocytes, and two of these, syndecan and the Hermes antigen, are expressed on the cell surface whilst the other, the invariant chain (Ii), is found inside the cells transiently in association with the class II antigens. In this review, we describe the structures and functions of the PGs in haemopoietic cells with particular emphasis on cells of the lymphoid lineage.

The Glycosaminoglycans (GAGs)

GAGs are linear polysaccharides largely composed of repeating disaccharide units [39, 73, 100]. The major GAG produced by most haemopoietic cells is chondroitin sulphate, the notable exception being the connective tissue mast cell which synthesises mainly heparin [65]. Dermatan sulphate and heparan sulphate have a more restricted distribution than chondroitin sulphate, dermatan sulphate being clearly identified only in activated mucosal mast cells [68, 133] while heparan sulphate is found mainly in cells of the lymphoid lineage where expression may be developmentally regulated [114]. The foregoing GAGs are all synthesised directly on core proteins by sequential addition of monosaccharide units. Serine residues within specific peptide sequences or "sequons" are sites of protein glycanation [34].

Several types of sequon have been identified, but a common feature is that serine is always adjacent to glycine [18], and the Ser-Gly dipeptide appears to be the minimum requirement for recognition of the protein by a xylosyl transferase which transfers xylose to serine, priming the protein for the synthesis of a protein-linkage sequence upon which the disaccharide repeating units of the GAG are assembled (Fig. 1).

Chondroitin and Dermatan Sulphate

In chondroitin sulphate the disaccharide repeat is *N*-acetylogalactosamine (GalNAc) and glucuronic acid (GlcA):

GalNAc β 1,4 G1cA
|
S

This is ester sulphated (denoted S) at C4 (type A) or C6 (type C) of GalNAc.

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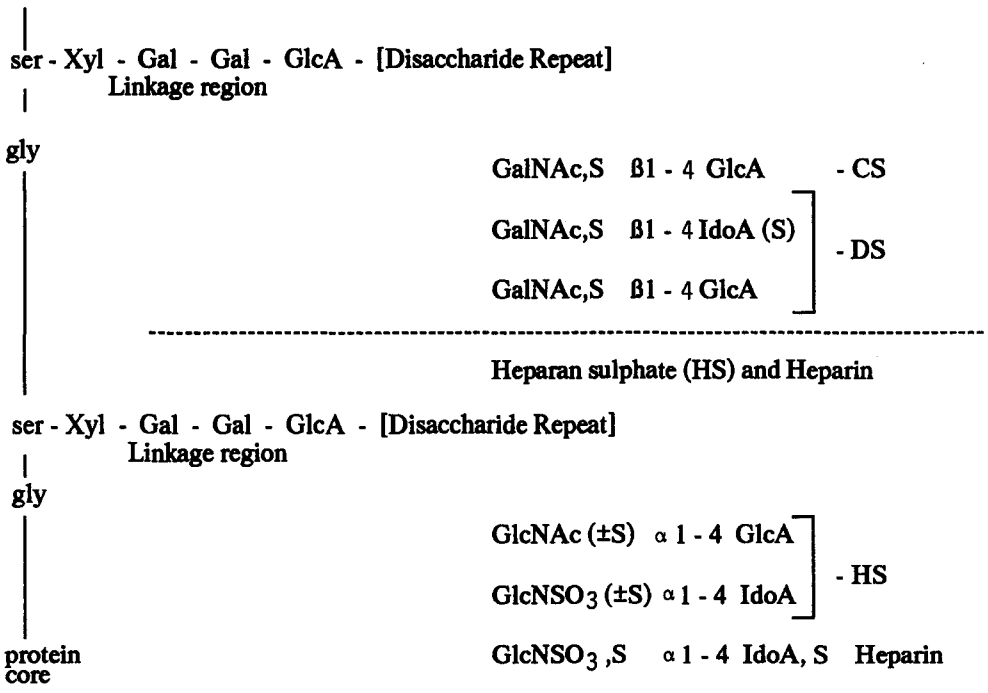

Fig. 1. Glycosaminoglycans in covalent linkage to protein. Sulphated glycosaminoglycans (*GAGs*) are commonly linked to protein by a tetrasaccharide linkage region in which a terminal xylose forms on O-glycosidic linkage to serine in a Ser-Gly sequence. The GAG chains contain from 50 to 200 disaccharide units. The chondroitin sulphate disaccharide repeat is N-acetylgalactosamine (*GalNAc*) and glucuronic acid (*GlcA*) with sulphation (indicated by *S*) at C4 or C6 of GalNac. Dermatan sulphate contains a variable proportion of disaccharides in which GlcA has been converted to iduronic acid (*IdoA*), and the latter may be sulphated at C2. In heparan sulphate (*HS*) and heparin a glucosamine hexuronate repeat comprises the polysaccharide backbone. HS contains approximately equal proportions of N-acetylated (*GlcNAc*) and N-sulphated ($GlcNSO_3$) disaccharides, whilst in heparin the amino sugar is mainly in the form of $GlcNSO_3$. In HS, N-acetylated and N-sulphated disaccharides occur mainly in separate sequences. The N-sulphated domains are enriched in IdoA residues and ester-linked (O)-sulphate groups. O-sulphation of HS chains is variable and is indicated by ($\pm S$). Heparin consists predominantly of trisulphated disaccharides of the type shown, in which O-sulphates are at C6 of $GlcNSO_3$ and C2 of IdoA (see text for details)

Chondroitin sulphate chains often consist of mixed disaccharide isomers, though haemopoietic cells commonly contain chains that are almost entirely sulphated at C4. Some chondroitin sulphate disaccharides are sulphated at both C4 and C6, and such disulphated units, called Di-E type disaccharides, are synthesised by mast cells, activated macrophages, eosinophils and human lymphocytes.

Dermatan sulphate is synthesised by polymer level epimerisation of a variable proportion of the GlcA units to the C5 epimer, iduronic acid (IdoA). In dermatan sulphate the GalNAc is mainly sulphated at C4 but the iduronate residue can also be sulphated at C2. Dermatan sulphate disaccharides may occur as disulphated units, called di-B type structures: (GalNAc, 4S β1, 4 IdoA, 2S).

Heparan Sulphate and Heparin

In heparan sulphate and heparin the disaccharide repeat units are rather complex due to variations in sulphation pattern and, in heparan sulphate, to the presence of extended nonsulphated

regions [37, 149]. The amino sugar component of heparan sulphate and heparin is glucosamine, which may be N-acetylated (GlcNAc) or N-sulphated ($GlcNSO_3$). Heparan sulphate contains about equal amounts of these two derivatives, GlcNAc always being in association with glucuronate whereas $GlcNSO_3$ is mainly linked to iduronate:

GlcNAc α 1,4 GlcA
|
(6S)

$GlcNSO_3$ α 1,4 IdoA
|
(6S) (2S)

In the heparan sulphate chain, these two basic types of disaccharide tend to be segregated into N-acetylated or N-sulphated sequences of variable length rather than being uniformly distributed [147, 148]. The major locations of the ester linked (O)-sulphates (indicated in parentheses above) are C6 of the amino sugars and C2 of iduronate. The degree of O-sulphation varies in heparan sulphates from different cell types [37], suggesting that these polysaccharides may have very specific roles to play in recognition events at the cell surface. O-sulphations are largely confined to the N-sulphated regions of heparan sulphate, creating domains of high charge density.

Rare but important isomers in heparan sulphate are 2-sulphated GlcA (GlcA, 2S) and 3-sulphated $GlcNSO_3$ ($GlcNSO_3$, 3S). GlcA, 2S is enriched in a heparan sulphate fraction found in the nucleus of a hepatoma cell line and its nuclear concentration has been correlated with the arrest of cell division [51, 52]. $GlcNSO_3$, 3S is an essential component of the anti-thrombin III pentasaccharide binding sequence in heparin and heparan sulphate which is largely responsible for the anticoagulant properties of the polysaccharides [74]. The sequence is:

$$-GlcNAc-GlcA-GlcNSO_3-IdoA-GlcNSO_3\,\alpha$$
$$\quad\;|\qquad\quad\;|\quad\;|\quad\;|$$
$$\quad 6S\qquad\quad 3S\quad 2S\quad 6S$$

The discovery of this sequence suggests that other important protein binding sequences may be present in heparan sulphate and heparin. The heparan sulphates display a broad repertoire of protein interactions which include growth factors, enzymes, extracellular matrix macromolecules and membrane receptors [36]. In the context of haemopoiesis, it is especially significant that heparan sulphate from inductive stromal cells binds the growth factors interleukin 3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF). The growth factor – polysaccharide complexes are mitogenically active [108], suggesting a possible role for heparan sulphate on stromal cells in sequestration and presentation of growth factors in bone marrow or lymphoid organs [35, 42].

Heparin is the most highly sulphated of mammalian GAGs, and the main disaccharide unit is the trisulphated derivative [74]: $GlcNSO_3$, 6S α 1,4 IdoA, 2S. Only 10%–20% of the disaccharides in heparin are N-acetylated and, in contrast to heparan sulphate, the molecule has a relatively uniform level of sulphation [37].

Proteoglycans in the Haemopoietic System

Secretory Granule Proteoglycan – Serglycin

Serglycin is the major PG in the secretory granules of mast cells, basophils, natural killer (NK) cells, eosinophils and platelets [8, 9] (for review see [65]). The 20-kDa protein core is characterised by the presence of contiguous Ser-Gly repeats (24 repeats in the rat, 10 in mouse and 9 in human) and each serine is a potential glycanation site, resulting in the GAG chains being clustered in a short peptide region. This region is highly resistant to

proteolytic cleavage. The protein core is glycanated with different polysaccharides, including heparin in connective tissue mast cells, chondroitin sulphate enriched in E-type disaccharides in mucosal mast cells and basophils and chondroitin-4-sulphate in platelets. These data indicate that the protein core of serglycin is not the major determinant of the GAG substitution pattern. The PG may play an important role in complexation and packaging of basic proteases, cytolysins and histamines in the secretory granule matrix [133].

In the rat, a single gene encodes the peptide core of the *extracellular* serglycin of the yolk sac tumour cell and the core protein of the *secretory granule* serglycin of basophilic leukaemia cells [18, 142]. The transcription start site that has been predicted for the serglycin of the rat yolk sac tumour mRNA is ~ 220 nucleolides 5′ to the start site for transcription of the rat basophilic leukaemia cell serglycin [8]. It is possible that the eventual cellular destination of these PGs could be regulated by untranslated sequences within the 5′ regions of the transcripts, by their binding to specific subclasses of ribosomes which function to target proteins to different areas within the cell. By itself, the final translated core protein does not appear to dictate the eventual location of the PG. Alternatively, the expression of two differently sized serglycin mRNAs could result from the use of two distinct promoters, and may indicate that the serglycin gene is controlled by different tissue-specific regulatory sequences [18].

Invariant Chain

The class II antigens are major histocompatibility encoded molecules on the surfaces of lymphocytes, macrophages and monocytes. They are heterodimers composed of two transmembrane polymorphic glycoproteins, the α- and β-chains, and they play a central role in immune regulation by mediating the presentation of proteolytically processed foreign antigens at the cell surface. Class II molecules are often found in association with a third, nonpolymorphic protein called the invariant chain (Ii) [61]. The Ii can be substituted with a single chondroitin sulphate chain (Ii-CS) of variable length [17, 85, 116, 117], and the Ii-CS component appears to be tightly bound to the class II antigens since it can be coimmunoprecipitated with antibodies to the α- or β-subunits [115]. The function of the glycanated variant of Ii is inknown. Indeed, the function of Ii itself is not firmly resolved. The majority of Ii molecules are present as an intracellular pool of free protein monomers lacking condroitin sulphate substitution [81, 82, 116, 117]. However, the Ii-CS variant seems to be almost entirely bound to class II and the trimeric complex is principally located inside the cells, probably in a post-Golgi vesicle since chondroitin sulphate biosynthesis occurs exclusively in Golgi membranes. Ii may be essential for efficient transfer of class II to sites of intracellular processing of antigen [63]. The chondroitin sulphate chain may in some way facilitate this transfer and perhaps also prevent class II from associating with normal cellular proteins [111]. Class II dissociates from Ii-CS immediately before binding to antigen, the exchange apparently occurring in an acidic endosome (for further details see [65]).

Syndecan

Syndecan (Fig. 2) is a transmembrane PG first identified in cultured mouse mammary epithelial cells [101]. It is widely expressed in adult epithelia and in condensed embryonic mesenchyme and is believed to play a central role in binding epithelial cells to structural proteins of the interstitial matrix [118]. These binding properties are determined by the heparan sulphate chains. The core protein of syndecan (32 kDa) contains a short C-terminal cytoplasmic region, a hydrophobic segment and five potential glycanation sites in the N-terminal ectodomain [119]. An interesting feature of the syndecan structure is the hybrid char-

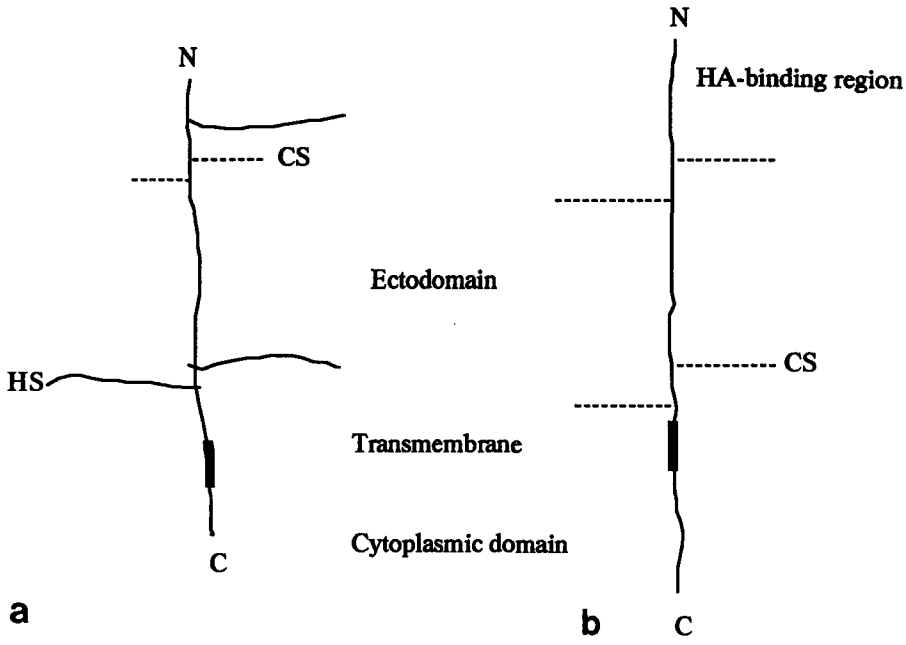

Fig. 2a, b. Transmembrane proteoglycans of the lymphocyte cell surface: **a** syndecan, **b** Hermes or CD44. Syndecan has a 32-kDa core protein substituted with heparan sulphate (*HS*) chains (*solid line*) on murine B lymphocytes. It is also expressed on epithelial cells as a hybrid proteoglycan containing both heparan sulphate and chondroitin sulphate (*CS*) chains. Hermes (CD_W44) is a lymphocyte homing receptor that participates in binding to molecular determinants (addressins) on high endothelial venules and to hyaluronic acid in the pericellular matrix. It may be synthesised as a glycoprotein with extensive N- and O-glycosylation or as proteoglycan in which the core protein (37 kDa) is further subsituted with glycosaminoglycan chains of the Cs type. Distinct functions are likely to be associated with the glycanated variant.
Syndecan and Hermes are transmembrane proteins, which hydrophobic peptide regions and relatively short cytoplasmic domains

acter of the glycanation profile, with both heparan sulphate and chondroitin sulphate chains present in the same protein. Heparan sulphate is the principal GAG but the number of GAG chains and the chain length can vary according to the tissue of origin [118]. Recently, syndecan was detected on the surfaces of murine B lymphocytes exclusively as a heparan sulphate PG [114]. Syndecan expression is closely linked to B cell differentiation. It is present on 40% of B cell precursors and on the majority of mature plasma cells. This pattern of expression fits well with the requirements for extracellular matrix interactions during B cell development.

Hermes – CD44

Hermes (or CD44) is a cell surface glycoprotein (85–95 kDa) involved in the recognition of high endothelial venules (HEV) by T and B lymphocytes [53, 54]. It is sometimes referred to as a homing receptor and appears to play a role in lymphocyte binding to peripheral, mucosal and synovial HEV [15]. Different determinants on the glycoprotein may be involved in specific molecular recognition. Hermes is also found on other haemopoietic cells and epithelial cells, including carcinomas [130]. Variations in glycosylation may influence the binding activity of the molecule. The glycoprotein is heavily N- and O-glycosylated and, interestingly, a variant of Hermes expressed on lymphocytes contains chondroitin sulphate chains [55] which increase the apparent molecular mass to 180–200 kDa (Fig. 2). There are four Ser-Gly dipeptides in the protein sequence that are potential glycanation sites. It is not known whether the gly-

canated variant of Hermes has a specific role to play in lymphocyte homing.

In common with the other lymphocyte surface PGs, Hermes is a transmembrane protein and the N-terminal region in the ectodomain is homologous with the hyaluronic acid binding region present in the major PG of cartilage [41, 130]. Evidence has recently been presented which indicates that Hermes is a major cell surface receptor for hyaluronic acid [5]. This property is unlikely to directly determine HEV homing activity but may be required for transendothelial migration and subsequent cell movement within the lymph node matrix.

Proteoglycans of the Lymphoid Lineage

Syndecan and the glycanated variants of Hermes and Ii were identified in lymphocytes using specific antibodies. The majority of studies of lymphocytes have utilised metabolic radiolabelling to detect PGs and the contribution of these defined components to the overall PG content of the cells in unknown. PGs are normally labelled in their polysaccharide chains using [^{3}H]glucosamine and [^{35}S]sulphate as biosynthetic precursors.

T Lymphocytes

Murine T lymphocytes synthesise both chondroitin and heparan sulphate in vitro [12, 21, 46, 107, 154] and synthesis is enhanced by activation with concanavalin A, phytohaemagglutinin, IL-1 or IL-2, independently of cell proliferation [12, 46].

The PGs of a number of murine T cell lymphoma cell lines have also been studied. The lines EL-4 and RDM-4 incorporated [^{35}S]sulphate into PGs of M_r 100–150 kDa which contain GAG chains of 10–20 kDa. The secretory products were exclusively PG, whereas cell extracts contained both intact PG and free GAG chains [106, 154]. However, in sharp contrast to the normal uncloned murine T cells, heparan sulphate has been found as the major GAG species in all cellular compartments in these T cell lymphomas cultured in vitro.

The murine T lymphoma cell line Eb and its highly metastatic variant Esb have been studied for several parameters which may play a role in determining their different metastatic potential. The Esb variant constitutively expresses high levels of a β-glucuronidase which can degrade heparan sulphate chains and may be important for cell migration across basement membranes in which heparan sulphates are important structural elements [11, 48, 153]. Esb cells synthesize no detectable heparan sulphate. However, both the synthesis and the secretion of chondroitin sulphate is enhanced when compared to the nonmetastasising counterpart [125]. Whether these proteoglycan differences have any direct bearing on metastatic properties is unclear and further work is needed to clarify this point.

Mitogen-stimulated normal human T cells expanded in vitro by phytohaemagglutinin and IL-2 produce chondroitin-4-sulphate PG which is rapidly secreted into the medium [24, 140]. The molecular masses of the PG and GAG chains have been estimated to be 130 kDa and 25 kDa, respectively. Whilst the core protein has not been characterised or sequenced, the T cell chondroitin sulphate PG is largely resistant to proteolysis, suggesting that the core may have a domain of repeating Ser-Gly residues characteristic of the serglycin type.

Of the intracellular chondroitin sulphate of human T cells, a significant proportion (40%–50%) was found as free GAG chains [140]. These chains were not normally secretory products – a feature which has been noted for human B cells (Ranson, unpublished observations), monocytes [63] and multipotential stem cells [86]. A limited study of human T cell PGs by Levitt and Ho [70] did not identify a specific intracellular pool of GAGs but noted the presence of small quantities of

heparan sulphate in both the secreted and cell-associated fractions.

Human T cell subsets cloned by limiting dilution and expanded in vitro by phytohaemagglutinin, IL-2 and irradiated B lymphoblastoid and peripheral blood mononuclear cells have also been analysed [139]. CD4-positive (T helper) cells were found to be more active in the secretion of PG and contained a lower proportion of intracellular free GAG chains than T8-positive (T suppressor) cells following in vitro labelling (reviewed in [65]). How these differences in metabolism relate to the distinct functions of these T cell subsets is unknown. Evidence has been presented to suggest that T cell PGs can stimulate proliferation of murine B cells [71, 72].

B Lymphocytes

A brief biochemical analysis of B cell PGs has been reported [70] with both human B-lymphoblastoid cell lines and peripheral blood B cells being found to synthesise chondroitin and heparan sulphate PGs which were constitutively secreted in vitro.

Recent work in our laboratory on neoplastic and normal human B cells cultured in vitro has shown the principal PG synthesised by these cells to be chondroitin sulphate. Heparan sulphate has also been identified, and comprised between 2% and 20% of the total radiolabelled GAG material. An analysis of centrocytic non-Hodgkin's lymphoma cells revealed the presence of both intact heparan sulphate PG ($\sim$ 28 kDa) and intact GAG chains ($\sim$ 8 kDa) in secreted material. Heparan sulphate PGs of varying sizes ($<$ 30 kDa to 150 kDa) have similarly been noted in murine T cells and T lymphoma cells lines [46, 105, 154].

The presence of heparan sulphate species in human B cells is of considerable interest since these molecules may have important roles in lymphocyte function. Mention has already been made of the involvement of heparan sulphate in the binding of several growth factors and in the control of cell adhesion. They could therefore be important in the regulation of lymphoma growth and disease dissemination. Further work to investigate both the structure and the function of lymphocyte heparan sulphate is clearly indicated. It will be of special interest to know whether the heparan sulphate is associated with syndecan, which has so far only been studied in murine B cells.

The major PGs of human B lymphocytes are chondroitin sulphates, and these are significant secretory products [70] (Ranson and Gallagher, unpublished). Our own work indicates that the majority of malignant phenotypes and tonsillar B cells appear to synthesise chondroitin sulphates in which a proportion of the disaccharides are disulphated (type E). In some human B cell lymphomas, distinct chondroitin sulphate species can be identified on the basis of differing chain length and sulphation. This contrasts with the homopolymeric condroitin-4-sulphate seen in normal human T cells [140], though whether this distinction is maintained for neoplastic T cell is unclear.

In common with a number of other studies of lymphocytes and monocytes, cell-associated radiolabelled material in neoplastic and normal human B lymphocytes consisted mainly of free chondroitin sulphate GAG chains that are usually retained by the cell. The function of this intracellular pool remains elusive.

Natural Killer Cells

Natural killer (NK) cells comprise a heterogeneous population of CD3-negative lymphoid cells that manifest cytotoxic activity against certain tumours and virally transformed cells. In addition, they are thought to play a role in haemopoietic differentiation and B lymphocyte function [49].

The secretory granules of these cells contain condroitin-4-sulphate together

with cytotoxic effectors such as pore-forming protein (perforin/cytolysin), protease and factors which resemble tumour necrosis factor and lymphotoxin [16, 56, 80, 121, 145, 156]. The PGs contribute to the efficient concentration of these effectors in the secretory granule and probably act to stabilise the proteolytic activities of the enzymes within the granules [146]. By analogy to mast cell heparin, the NK granule PGs may also alter the ability of these enzymes to cleave some substrates following exocytosis [40, 144]. It has been suggested that chondroitin sulphate may serve to protect the NK cells from cytolytic effectors such as perforin [24], perhaps by inhibiting perforin polymerisation.

Whilst NK cell PGs have been suggested as candidate mediators of cytotoxicity [156], work using β-xylosides, which produced a reduction in PG synthesis by 50%, showed this to have no significant effect on the cytotoxic activity of cultured NK cells and cytotoxic T cells [24]. Cytotoxicity assays using murine NK cells and a variety of target cells have also shown that exogenous chondroitin-4-sulphate, chondroitin-6-sulphate and dermatan sulphate at concentrations of up to 1 mg/ml have no effect on NK cytotoxicity as assessed by in vitro models [155]. However, in this assay system, exogenous heparin at low concentrations was found to be inhibitory, and analyses of several heparin types suggested that there was a positive correlation between increasing negative charge density and inhibition of cytotoxicity. Perhaps heparin inhibits the activity of the cytotoxic mediators or prevents NK cell activation, since similar suggestions have been proposed in lymphocytes for mast cell-derived heparin [33, 123]. It is interesting to note that, in contrast to heparin's inhibitory effects on NK cells, it has been found to be inactive for cytotoxic T cell-mediated cell lysis, emphasising the fact that cytotoxicity can clearly be achieved by multiple mechanisms [156].

Granulocytes

The staining characteristics of the intracellular granules of mature granulocytes (or polymorphonuclear cells) enables their classification into three broad populations: neutrophils, basophils and eosinophils. Most of the studies on granulocyte PGs have been performed on peripheral blood neutrophils using biochemical, autoradiographic and histochemical methods. Early work demonstrated that the intracellular granules contained chondroitin sulphate in association with basic proteins [93–96]. Chondroitin-4-sulphate was subsequently demonstrated in primary granules (lysosomes), with smaller amounts in secondary lysosomal granules [98, 141]. Chondroitin sulphate is a major secretory component of human basophils [84]. More recent studies have also identified both heparan sulphate and dematan sulphate in cell-associated and released material [72, 90], indicating that greater diversity in PG synthesis may be possible than had been suggested by earlier studies.

The PGs synthesised in vitro by peripheral blood neutrophils are primarily retained within the cells, being released into the medium upon adhesion and when neutrophils are exposed to microorganisms that stimulate phagocytosis [47, 72, 142]. In comparison to normal mature neutrophils, immature malignant human myeloid cells exhibit a greater rate of chondroitin sulphate and hyaluronic acid biosynthesis [87, 92].

The HL-60 cell line, derived from a patient with promyelocytic leukaemia, readily differentiates in vitro to more mature granulocytic cells with agents such as retinoic acid and dimethylsulphoxide [25]. On the other hand, phorbol esters and heparan sulphate-containing fractions of bone marrow lead to the development of some phenotypic features usually associated with macrophages [77, 79, 113]. Irrespective of the differentiation-induction agent, HL-60 cells synthesise only chondroitin-4-sulphate,

though there is a notable reduction in the rate of synthesis upon differentiation [14, 77, 78]. HL-60 cells produce the serglycin type of PG [89, 137], and similar gene products are to be found in PGs from other polymorphonuclear cells, namely, the rat basophilic leukaemia-1 cell line [8] and human eosinophils [112].

Pulse-chase experiments with the mouse myeloid stem cell line FDCP-mix suggest that the synthesised chondroitin sulphate is metabolised as two distinct pools: a short half-life pool representing PG which is destined for secretion, and material destined for intracellular degradation to free chondroitin sulphate chains and some smaller fragments [86]. A similar secretion and degradation pathway has been elucidated for the human monocytoid cell line, M1 [76].

Mast Cells

Mast cells are distinctive in having intense metachromatic cytoplasmic granules (due to the attachment of highly sulphated GAGs to the serglycin protein), a capacity to synthesise and store histamine, and in having high affinity receptors for IgE (for reviews see [31, 134]). Distinct subpopulations of mast cell exist in vivo which differ both in their anatomical site and their biochemical features (Table 1). To date, two main populations have been clearly defined: mucosal mast cells and connective tissue mast cells. Mucosal mast cells can change their phenotype to connective tissue mast cells when placed in the appropriate environment, and vice versa [57, 88, 128].

Mucosal Mast Cells

Mucosal mast cells are not normally present in sufficient numbers in tissues to allow the isolation and characterisation of their proteoglycans, but the use of helminth-infected rats has enabled the analysis of mucosal mast cells following in vivo labelling with [^{35}S]sulphate [32, 133]. The isolated ^{35}S-labelled macromolecules contain highly sulphated chondroitin sulphate chains enriched in type E and type B disaccharides [68, 133]. Mucosal mast cells derived from human colon also synthesise chondroitin sulphate E rather than heparin [30].

In mice, a similar mucosal mast cell population can be derived from murine bone marrow (so called "bone marrow-derived mast cells") [102, 104]. In isolation these mast cells preferentially synthesise chondroitin sulphate E [103, 136], but in coculture with fibroblasts they become histochemically and morphologically similar to connective tissue mast cells [27]. Analogous phenotypic changes

Table 1. Differences between rat mucosal and connective tissue mast cells

Property	Mucosal mast cell	Connective tissue mast cell
Histochemistry	Safranin negative	Safranin positive
Gramules	Few, small	Many, large
Life span	Short ($t_{1/2}$ 40 days)	Long ($t_{1/2}$ > 6 months)
T cell factor dependence	Yes	No
Proteoglycans	Chondroitin-4-sulphate Chondroitin-4,6-disulphate (type E) Chondroitin sulphate (type B)	Heparin
Histamine content	Low	High
Serine protease	Protease II	Protease I

can be induced by injecting bone marrow-derived mast cells into the peritoneal cavity of mast cell-deficient W/W^v mice [97].

Connective Tissue Mast Cells

The secretory granules which contain PG are a prominent morphological feature of connective tissue mast cells. In rodents these cells synthesise heparin proteoglycans of 750–900 kDa containing 10–12 GAG chains of 70–80 kDa [109, 157].

Although [^{35}S]sulphate is incorporated into heparin by connective tissue mast cells, small quantities of *unlabelled* chondroitin sulphate E can also be detected [58]. This may represent residual chondroitin sulphate synthesised during an earlier (mucosal mast cell-like) stage of development. However, it is clear that connective tissue mast cells retain a latent capacity for chondroitin sulphate E synthesis since they produce this GAG in the presence of β-xylosides [134, 135].

Although the mediators produced and released by mast cells have been well characterised, the precise biological functions of mast cells remain unclear in many instances. However, roles for mast cells in immediate hypersensitivity reactions and in host defence against parasitic infections have been well established [83, 122]. The highly acidic nature of the PG components of the secretory granule probably help to maintain charge neutrality with histamine and basic proteins and may facilitate the osmotic swelling of the secretory granule prior to it fusing with the plasma membrane [124]. PGs may continue to exert regulatory influences following mast cell degranulation. PG-protease complexes have been detected on the mast cell surface, and this will limit the diffusion of discharged enzymes and regulate their activities [4, 123, 126, 144].

Additional interactions of mast cell-derived heparin with complement components may occur, heparin having been found to inhibit the activity of late complement components in vitro [146] and to inhibit complement-mediated inflammation and cell lysis in experimental models [28, 29]. The proteolytic activity of the secretory granule enzyme chymase which degrades the complement component C3a is also promoted by heparin [40]. Since mast cell heparin is a potent anticoagulant, a role in regulating local coagulation in areas of inflammation has been proposed [75].

The common occurrence of mast cells at sites of tumour neovascularisation and on the mitogenic effects of mast cell heparin on some cultured endothelial and fibroblast cell lines has led to suggestions that mast cell heparin is involved in tumour angiogenesis [59, 110]. The release of a heparan sulphate-degrading endoglycosidase during mast cell degranulation may also facilitate the release of heparan sulphate-bound bFGF, an angiogenic factor, from the extracellular matrix [13].

Platelets

Chondroitin sulphate PGs have been reported primarily in platelet α-granules with small amounts on the cell surface [10, 132]. The α-granules contain several bioactive molecules including serotonin, fibrinogen, platelet factor 4, ADP, thrombospondin and platelet derived growth factor. Whilst complexes between platelet proteoglycan and platelet factor 4 have been investigated [10, 50, 69], and suggestions made that the PG enables an efficient packaging of platelet factor 4 in the α-granule, it is likely that the chondroitin sulphate interacts with more than one component of the α-granule [120].

The core protein sequence has been reported for human platelet PG [2, 99], and the N-terminal sequence is highly homologous to serglycin. Interestingly, a proposed site for proteolytic scission of the serglycin protein was shown to have been cleaved in a proportion of the platelet PG extract [99]. This raises the possibility that processing of the platelet core protein may occur inside the α-granule or

following its release from the cell. Such a modification may conceivably alter the functional properties of the PG and further studies are required to assess whether this processing step has biological importance.

The chondroitin sulphate PG isolated from serum is very similar to the PG isolated from platelets [91] and it has been mooted that platelet-derived chondroitin sulphate PG may function as a natural inhibitor of the complement factor C1q [3, 91, 127]. Plasma membrane chondroitin-4-sulphate on human platelets has also been demonstrated to effectively shield distinct platelet activation receptors and to participate in membrane calcium flux [131, 132]. The protein core of the membrane-associated PG has not been identified.

Monocytes and Macrophages

Monocytes and macrophages form part of the phagocytic system and are important for antigen processing and presentation, enzyme secretion and tumour cell lysis. The cells are both highly secretory as well as motile [1]. The extensive secretory repertoire of these cells includes enzymes, complement components, clotting factors, growth factors and PGs [63]. Freshly isolated human peripheral blood monocytes synthesise and secrete chondroitin-4-sulphate PGs. After 5 days of culture on plastic the cells develop macrophage-like morphology and begin to secrete more highly sulphated chondroitin sulphates which contain about 20% of the E-type disaccharides [66]. End-differentiated macrophages derived from human peritoneal fluid also secrete these chondroitin sulphate E-containing proteoglycans in vitro [62]. The synthesis of this species of chondroitin sulphate appears closely coupled to monocyte/macrophage differentiation [64, 66, 150]. Plastic surfaces and phorbol esters induce this switch in chain sulphation, whilst interferon-γ and lipopolysaccharide are ineffective [151], implying that the nature of the differentiation signal may be important in the modulation of PG sulphation. Monocyte differentiation is inhibited by culture on fibronectin substrata and under these conditions the cells fail to synthesise type E disaccharides [67].

The major fate of synthesised PGs in monocytes cultured in vitro is secretion via a constitutive pathway, but the functions of the secreted materials remain to be elucidated. It is also unknown whether this pathway is active in vivo and whether it can be modulated.

Similarly, the role of cell surface PGs in monocyte function has not been extensively investigated although the presence of cell surface receptors for GAGs and other sulphated polysaccharides has been suggested [23]. Levels of cell surface GAGs and hyaluronic acid are increased upon macrophage adhesion or phagocytosis [22, 45, 60], and since these cells display adhesive interactions with other cells and with matrix components, cell surface PGs may be important elements in monocyte and macrophage function.

Summary

The most prevalent and best characterised PGs in the haemopoietic system are the serglycins. They are found in immunosecretory cells and bind to molecules such as serine proteases, histamine, platelet factor 4, and perforin to facilitate concentrated packaging of these effectors in storage vesicles. There is also evidence to suggest that the serglycins have roles in regulating the activities of these effectors following their release from the cell [6, 7, 126, 138, 146].

The biochemical properties of PGs suggests that they are suited to roles in regulating cell adhesion and cell migration. A range of anionic polysaccharides including GAGs are capable of modulating lymphocyte migration in vivo and lymphocyte adhesion in vitro [19, 20, 26]. In lymphoid malignancy, studies in murine lymphoma cells have shown that

the potential of the cells for PG secretion correlates with their metastatic potential [125].

Cell surface PGs in haemopoietic cells such as syndecan and the glycanated variant of CD-44 have recently been demonstrated and further examples will certainly follow. It is likely that this field of research will lead to a clearer understanding of the roles of PGs in haemopoietic cell development. The potential of PGs to bind, and present in active form, several haemopoietic growth factors has already been documented [43, 108], and additional examples of PGs sequestering fibroblast growth factor, transforming growth factor-β and neuronal growth factors have been cited [34].

PG-mediated cell adhesion may also be a crucial requirement for progenitor cell growth [38, 42, 44]. Whilst much attention has focused on heparan sulphates, recent work on membrane-associated chondroitin sulphates suggests that these polysaccharides may be important for the binding of haemopoietic progenitors to stromal cells [143] and for haemopoietic cell proliferation in bone marrow [129]. Although some of the adhesive interactions are likely to be relatively nonspecific, they may have some therapeutic potential in, for example, assisting haemopoietic recovery from chemotherapy, inhibiting tumour dissemination, or facilitating stem cell engraftment following bone marrow transplantation. Research into the biochemistry of haemopoietic PGs and GAGs should not only provide new understanding of basic science, but hopefully provide novel therapeutic approaches for the treatment of human disease states.

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