REVIEW ARTICLES

Glycobiology and medicine: an introduction

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Glycobiology deals with the nature and role of carbohydrates in biological events[1–3]. We can now bid farewell to the notion that oligosaccharides are a decorative irrelevance[4]. The past decade has witnessed an exponential interest in the associations of oligosaccharides with disease mechanisms, and substantial advances have been made in the design of carbohydrate based therapies and diagnostic techniques. This review, based on contributions to a meeting in 1996, offers an introduction to a flourishing area in clinical and basic research.

OLIGOSACCHARIDE SYNTHESIS

It is through their structural complexity that oligosaccharides are able to mediate biospecific information, one example being sialyl-Lewis^x tetrasaccharide, a crucial molecule in cell adhesion and trafficking[3,5,6]. The mechanism of their synthesis is therefore crucial. At the centre stage of the synthesis of glycoside bonds are the glycosyltransferases whose specificity for their substrates, the donor sugar nucleotides, and their acceptor is precise ('one enzyme-one linkage'[7]). The sequential action of glycosyltransferases produces oligosaccharide and glycan structures that reflect the constitution of the multiglycosyltransferase system[8] of a given cell type and implies that the targeting mechanism for glycosyltransferases along the secretory pathway is highly ordered[9].

The most important organelle involved in glycosylation is the Golgi apparatus (GA) originally described as *appareil réticulaire interne* by Camillo Golgi in 1898 and subsequently confirmed to be a morphological entity by electron microscopy[10]. Its biochemical properties have been explored by fractionation using galactosyltransferase (GTase) as a marker enzyme. Immunoelectronmicroscopical localization of this and other enzymes then clearly established the GA as the site of glycan chain elongation and termination[11,12]. The enzyme function within the cisterne is well ordered and GTase has been found predominantly on the trans side of the GA; a sequential arrangement of the glycosyltransferases increases their biosynthetic efficiency[13].

The catalytic function of the glycosyltransferases is essentially irreversible and results in generation of nucleoside-diphosphates, which are further degraded to monophosphates by phosphatases co-localized with glycosyltransferases. The nucleoside-monophosphate is then exchanged by an antiport mechanism across the Golgi membrane with newly made sugar-nucleotides synthesized in the cytoplasm.

The synthesis of a glycan specific for a given cell type thus depends on various factors[14]:

- Expression of functional glycosyltransferase at the proper location within the cell
- Synthesis and translocation of sugar nucleotides to the biosynthetic microenvironment of the Golgi lumen
- The proper trafficking of intracellular acceptor substrates
- Absence of toxic influences such as inhibitors of processing enzymes and glycosyltransferases

Knowledge of the cell biology of glycosylation has allowed development of a yeast expression system which produces large amounts of mammalian glycosyltransferases[15].

THE FUNCTION OF SUGARS

The elegant biosynthetic glycan-processing pathway in the cell allows, in principle, the same oligosaccharide to be attached to quite different proteins without having to code the information into the DNA of the individual proteins. However, the orientation of the attached oligosaccharide with respect to the polypeptide may greatly affect the properties of the glycoproteins. Further, different glycoforms of a protein can display quite different orientations of the oligosaccharides with respect to the protein, thus conferring different properties. A striking example is the structure of the Fc fragment of immunoglobulin G (IgG)[15]. The conserved N-linked complex oligosaccharides at Asn297 on each heavy chain of the CH2 domain occupy the interstitial space between the domains and also interact with the domain surface.

Loss of the two terminal galactoses from the oligosaccharide, as in the Fc fragment from patients with rheumatoid arthritis, results in a loss of interaction between the domain surface and the oligosaccharide. This permits displacement and consequent exposure of the oligosaccharides, giving them the potential to be recognized by endogenous receptor lectins[16]. This recognition of oligosaccharides is influenced by their accessibility, the

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number of copies of the oligosaccharides and their precise geometry of presentation[17]. These factors introduce a high degree of specificity and control.

The finding that glycosylation may vary with disease also leads to the concept that therapeutic manipulation might beneficially alter the properties of glycoproteins[18,19]. The ability to manipulate and modify sugar structures also provides an important approach in understanding the different functions of oligosaccharides. For instance, control of glycosylation can be influenced by amino sugars such as N-butyldeoxynojirimycin (B-DNJ), which inhibits the processing enzymes α-glucosidases I and II. Treatment of human immunodeficiency virus (HIV) with this compound modifies the N-glycan conformation of the envelope glycoproteins, inhibiting syncytium formation in infected cells and reducing the infectivity of released virus. The binding of virus to CD4 receptors is not affected, but entry (fusion) is greatly altered. The effect is correlated with the amino sugars causing a conformational change in V1/V2 region of gp 120. Presumably the altered glycosylation prevents interaction with carbohydrate-specific protein folding chaperones (such as calnexin) in the endoplasmic reticulum[19].

In contrast to the HIV envelope glycoproteins, which contain about 30 glycosylation sites, the hepatitis B virus envelope proteins contain only one or two glycosylation sites. *In vitro* treatment of this virus with B-DNJ results in retention of virus particles within the cell. Mechanistic studies indicate alteration of unique oligosaccharides on the M surface antigen, affecting the conformation of a region of M preventing virion encapsulation[18]. Note that DNJ is also a glucosyltransferase inhibitor and hence can be used to control glycolipid production; this has implications for treatment of glycolipid storage disorders such as Gaucher's disease[20].

OLIGOSACCHARIDES AS LIGANDS FOR EFFECTOR PROTEINS OF THE IMMUNE SYSTEM

Programmed changes occur in the display of the carbohydrate chains of glycoproteins and glycolipids at the surface of cells from the earliest stages of embryonic development and cellular differentiation right through to maturation, as revealed by monoclonal antibodies[2,21]. These findings led to the hypothesis that oligosaccharides (among which are the blood group antigens and related sequences) have important roles as ligands in macromolecular interactions—for example, as 'area codes' decoded by endogenous carbohydrate-binding proteins that determine cell migration pathways, or as ligating elements in multicomponent receptor systems[2,21,22]. To reveal recognition codes in oligosaccharides, techniques have been designed analogous to blotting for nucleic acids and proteins which enable oligosaccharide probes (neoglycolipids) to be generated from desired structurally defined or chemically synthesized oligosaccharides, or mixtures of oligosaccharides released from glycoproteins and glycolipids or the surface of the whole cells. These are then used as solid phase probes in binding studies with soluble proteins or with whole cells that express oligosaccharide carbohydrate-recognizing proteins[23]. The technology incorporates a microsequencing strategy, since the neoglycolipids have unique ionization properties in mass spectrometry. Indeed a picture is emerging of ways in which exquisite biological specificities are mediated by oligosaccharides that are not unique but are presented in various ways on glycoproteins or glycolipids which, in specific compartments of the body, serve as counter-receptors for the carbohydrate-recognizing effector proteins of the innate and acquired immune system[3,6]. The challenge now is to discover ways of boosting or inhibiting these interractions, *in vivo*, in a monospecific non-toxic way, in infections, disorders of inflammation and malignant disease.

GLYCOSYLATION AND MALIGNANCY

In malignancy, monoclonal antibodies reveal striking changes in the display of oligosaccharides of glycoproteins and glycolipids at the surface of cells[2]. The altered glycosylation can be simply demonstrated by lectin-binding[24]. With wheat germ agglutinin (WGA), lectin agglutination of transformed cells (but not of their normal counterparts) can be demonstrated. Lately, agglutination of murine lymphoma cells with high versus low metastatic potential has been seen with the soya bean agglutinin (SBA); presumably it recognizes more exposed GalNAc[25]. Transformed cells have provided a controllable model to trace pathways and understand cell surface behaviour. Agglutination by different lectins shows a huge range of changes to oligosaccharides at the surface of transformed cells; some of these relate to proliferation and cell-cycle but a consistent change of transformed cells has been increased N-linked multi-antennary oligosaccharides. These may appear:

- only on glycolipids (such as gangliosides on melanomas)
- only on glycoproteins (such as N-linked multi-antennary branches and altered O-linked chains on mucins)
- on both glycolipids and glycoproteins (many epithelial cancers show common lacto-structures on a backbone of Galβ1-2GlcNAcβ1-Gal or type 1 blood group, or of Galβ-4GlcNAcβ1-3Gal or type 2 blood group)

Carbohydrate structures that are tumour associated in one cell population may be normal components of another cell type[2]; for instance Le^x (3-fucosyl-N-acetyl-lactosamine) is a normal component of polymorphonuclear leucocytes

and monocytes (CD15) but its presence is associated with unfavourable outcome in colorectal, lung, hepatocellular and breast cancers.

In surgical biopsy material one can readily detect abnormal glycosylation with monoclonal antibody or lectin histochemistry. However, positive staining may result from binding to many different structures and biochemical extraction/isolation of the ligands is neccessary for characterization. Then the presence of a structure does not mean it is biologically available, so functional studies are needed. Many of the cryptic antigens present in normal tissues become exposed in malignancy and biologically may be cancer specific. T and Tn antigens are such structures, associated with very different biological behaviours: T antigen is detected in a large proportion of cancers and has been associated with good prognosis; Tn is usually associated with aggressive cancers. Interpretation is difficult when, as often happens, many structures are present in a tumour but present on different cells. From a pathologist's perspective, the clinical outcome is determined by the cell population exhibiting the most aggressive characteristics. However, immune responses to these epitopes may modify cancer behaviour and, although low levels of natural antibodies to these structures exist in normal populations, active immunotherapy to structures as Tn and Sialyl-Tn is being attempted[26]. Other recognition systems for altered glycosylation, such as carbohydrate receptors on macrophages, or even diet derived lectins, may modify cancer behaviour, and the presence of carbohydrate receptors on cancer cells themselves[27] may lead to new ways for targeting specific chemotherapy to cancers.

GLYCOSYLATION AND AUTOIMMUNE RHEUMATIC DISEASE

Some observations in rheumatological disease suggest an association of glycosylation disorders with the disease processes[4,28–35].

Oligosaccharides are associated with arthritis

In rheumatoid arthritis the galactose content with immunoglobulin G is subnormal[36]. A synthetic abnormality involving the lymphocytic enzyme GTase may be responsible. This enzyme is reduced in both B and T lymphocytes in patients with rheumatoid arthritis[37].

Agalactosyl-IgG has a pathogenic role

Some IgM rheumatoid factors, derived from synovial tissue, are dependent on the lack of galactose within IgG for their binding. These rheumatoid factors are monoreactive and, as the galactose level declines, so their binding can increase threefold[38]. Mannose binding protein interacts with N-acetylglucosamine-bearing structures. Agalactosyl-IgG is such a structure and when bound by mannose binding protein the result may be complement activation. This is thought to trigger an inflammatory process[39]. Animal models of arthritis have been developed in which the inflammatory process is triggered by agalactosyl-IgG[40].

Galactosyltransferase

Glycosylation homoeostasis within rheumatoid arthritis lymphocytes is thought to be abnormal. In healthy individuals there is a positive feedback mechanism ensuring that galactosyltransferase levels increase as IgG galactose levels decline. In rheumatoid arthritis this does not occur[41]. Where does this breakdown in enzymatic control arise? Not only is the overall galactosyltransferase activity reduced within lymphocytes, but serum galactosyltransferase isoenzymes vary[42].

There are two peaks of enzymatic activity in serum after isoelectric focusing. In rheumatoid arthritis, unlike psoriatic arthritis and other rheumatic diseases, there is a significant acidic shift in the galactosyltransferase activity profile, with a unique peak forming at pH 5.05. These isoenzymes are thought to reduce efficiency in ability to galactosylate structures such as immunoglobulin G.

ARE THE GLYCOSYLATION ABNORMALITIES IN RHEUMATOID ARTHRITIS UNIQUE?

The answer to this is probably no. Similar changes arise in several other diseases, but unique IgG carbohydrate profiles are probably associated with each rheumatic disease[43]. For example, the inverse relationship between galactose and N-acetylglucosamine levels on IgG in rheumatoid arthritis is not found in primary Sjögren's syndrome despite substantial variation in the level of N-acetylglucosamine[44]. Immunoglobulin sugar printing[45] is therefore possible, whereby individual rheumatic diseases are associated with a specific carbohydrate map. Pilot data refer to rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, ankylosing spondylitis and primary Sjögren's syndrome. We now need to test these data in larger studies to determine whether they will aid diagnosis and allow further dissection of disease mechanism.

CARBOHYDRATE-BASED THERAPEUTICS

The carbohydrate biochemical mechanisms involved in initiation or maintenance of disease processes now include viral and microbial adhesion, initiation of cell-mediated inflammation, and cancer metastasis. In general, interferance with these mechanisms comes down to either blocking a carbohydrate binding protein (e.g. the selectins) or inhibiting a carbohydrate processing enzyme (glycosidase or glycosyltransferases). Very few such therapeutic approaches have yet reached the clinic and we need a better

understanding of the chemical basis of successful strategies by which carbohydrate receptors and glycosidases/glycosyltransferases can be blocked or inhibited.

An early event in the recruitment of leucoytes to sites of inflammation is the adhesion of carbohydrates on circulating leucocytes to E-selectin, a carbohydrate-binding protein expressed by endothelial cells in response to certain inflammatory cytokines. The importance of the E-selectin adhesion pathway has been shown *in vivo*, and evidence has been presented that specific carbohydrate structures do indeed function as ligands for E-selectin.

In general, these carbohydrates are acidic derivatives of the Lewis x and Lewis a trisaccharide structures, in which sialic acid or inorganic sulphate is attached to the C-3 position of galactose. In view of the low affinity with which these acidic carbohydrates bind to recombinant E-selectin *in vitro* (measured Kd are usually of the order of 0.1–2 mmol/L, depending on assay format), there is uncertainty as to whether a carbohydrate-based antagonist can be successfully generated. Efforts have focused on defining the endogenous carbohydrate ligand for E-selectin, defining a full structure-activity relationship and evaluating the use of multivalent ligand constructs as antagonists.

THE DESIGN OF DRUGS

Nuclear magnetic resonance (NMR) spectroscopy and computer graphics molecular modelling can be used to display the dynamic structure of protein glycosylation, oligosaccharides and glycophosphatidylinositol (GPI) anchors. These are targets for a new wave of diagnostic tests and rational drug design. Glycosylation is a significant post-translational modification of most proteins[45]. The added oligosaccharides have diverse roles in protein function in addition to bearing specific recognition motifs[46,47]. Oligosaccharides have many recognition roles—for example, they interact at the cell surface with carbohydrate binding proteins and in the extracellular matrix with proteoglycans[48,49]; as major components of the mucin glycoproteins which line the respiratory and gastrointestinal tracts, they are tumour-associated antigens and host receptors for micro-organisms[50]; and they form structural components of many potent antibiotics and of anticancer drugs that bind DNA. The glycan of GPI anchors links the carboxyterminus of proteins to membrane lipid. There is now evidence[51] that this functions to maintain different conformations of proteins when anchored to the membrane or when released from the membrane by enzymes specific for this purpose (phospholipases). NMR[52,53] and computer graphics[47,50–52] are the preferred methods of analysis for studies of intermolecular and intramolecular protein-oligosaccharide interactions since they tell us about the dynamics of solution and membrane conformation. Further, these methods complement X-ray crystallography, which does not display either flexible regions of macromolecules or heterogeneous molecules that cannot be crystallized in their native state. These characteristics are particularly true of protein glycosylation and are probably an important factor in their functionality.

Speakers at the meeting were

Dr John S Axford (St George's Hospital Medical School, London, England); Professor Eric G Berger (Institute of Physiology, University of Zurich, Switzerland); Professor Raymond A Dwek (The Glycobiology Institute, Oxford, England); Dr Ten Feizi (MRC Glycosciences Laboratory, England); Dr Elisabeth F Hounsell (University College, London, England); Dr Tony Leathem (University College, London, England); and Dr Raj Parekh (Oxford Glycosystems Ltd, England).

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