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Glycomics, glycoproteomics and the immune system

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Glycomics and glycoproteomics have become indispensible tools in the study of glycoconjugates. Mass spectrometry based methods are standardly used to study the proteome and/or glycome and these approaches are capable of providing both, qualitative and quantitative information using top down techniques. The human immune system marks a particular area of interest for glycomics and glycoproteomics research since a large number of key proteins in innate and adaptive immunity are glycoproteins. In numerous examples, the crucial influence of glycosylation on critical steps such as receptor interaction and binding has been demonstrated. In this review, we focus on different glycomics and glycoproteomics approaches and their application for studying protein glycosylation in the immune system.

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Introduction

The involvement of glycoconjugates in eliciting specific immune reactions has been accepted since the glycan nature of the ABO blood group types was recognized [1]. It has been reported earlier that alterations in the glycan profiles of immune cells play a role in regulating effector functions such as dendritic cell or T cell activation [2–4]. Slight modifications in the IgG N-glycan can completely reverse its function: addition of a single sialic acid molecule converts IgG from being a pro-inflammatory into an anti-inflammatory agent [5] and the addition of a core fucose residue on IgG Fc N-glycans can inhibit initiation of antibody-dependent cell cytotoxicity by obstructing its binding to the FcγRIIIa receptor [6]. Thus, to understand a complex system like the human immune system detailed knowledge is clearly required on the individual proteins and their particular posttranslational modifications such as glycosylation. Until recently, however, a detailed understanding of the glycome of the mammalian immune system was lacking. A deeper insight into the immune glycome is needed since glycan structures on immune cells interact with lectins such as the C-type lectins, S-type lectins (e.g. galectins), or I-type lectins (*e.g.* siglecs) (Figure 1). Typically, those lectins are expressed in a cell-type and activation-dependent manner and recognize glycans in a multivalent fashion thereby regulating immune cell functions [7,8]. New studies demonstrate the benefit of mass spectrometric glycomic strategies for the characterisation of the immune glycome [9]. Particularly, the combination of gene microarrays, quantitative real-time PCR, and mass spectrometry (MS) has contributed substantially to the glycan profiling of immune cells [10]. This review will focus on selected glycomics and glycoproteomics approaches published in the time frame from 2008 to 2011 that were employed successfully in studying key molecules of the immune system.

Glycomics — the global approach

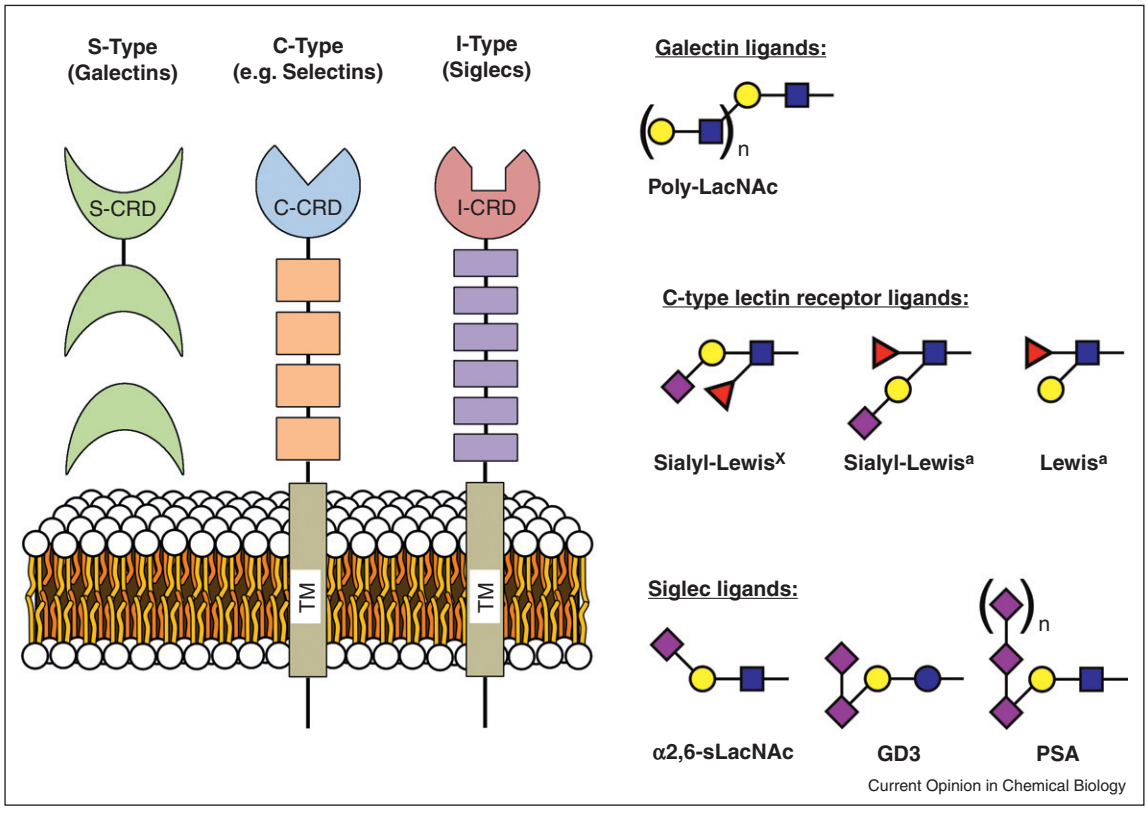
Glycomics involves all methods and approaches used to profile the glycome of a particular cell or cell compartment. A common feature of glycomics workflows targeting protein-bound glycans is the enzymatic release of N-glycans using peptide:N-glycosidase F (PNGase F) with subsequent isolation of the released oligosaccharides [11•,12,13]. The lack of an O-glycan analogue for PNGase F makes chemical release of O-glycans by reductive β-elimination or hydrazinolysis the methods of choice to release the O-glycome from the protein carriers [12,13,14•] before the released glycans typically are subjected to mass spectrometric analyses (Figure 2).

MS has evolved as the major tool for elucidating the glycome due to its unique features like routine high sensitivity, speed of analysis and its ability to deal with complex mixtures of compounds. Furthermore, tandem MS spectra provide fragmentation data of selected signals that assist significantly in the assignment and characterisation of particular glycan structures. On the basis of the ionisation method used, glycomics approaches can be roughly divided in electrospray ionisation (ESI) based approaches (often combined with online liquid chromatography separation) and matrix assisted laser desorption/ionisation (MALDI) based methods.

Glycomics based on ESI techniques

Traditionally, ESI ionisation is coupled with online liquid chromatography (LC) separation offering the advantage

Figure 1



Lectins in the immune system and relevant carbohydrate ligands (examples). Immune cells express lectin receptors such as S-type lectins (galectins), C-type lectins (*e.g.* collectins, selectins, lymphocyte lectins), or I-type lectins (siglecs). These lectins recognize glycan structures present on either pathogens or host cells. Exemplary glycan structures recognized by galectins, C-type lectins, and siglecs are shown. CRD = carbohydrate-recognition domain; S-CRD = S-type lectin CRD; C-CRD = C-type lectin CRD; I-CRD = I-type lectin CRD; TM = transmembrane region. For legend to monosaccharide symbols as suggested by the Consortium of Functional Glycomics please see Figure 3.

of easily introducing an additional dimension of separation on top of the mass spectrometric detection. Porous graphitized carbon (PGC) has emerged as the most widely used chromatographic medium for non-derivatised oligosaccharides due to its unique features of separating isobaric glycans [15,16••,17,18]. PGC LC-separation provides the MS instrument with the possibility to acquire separate tandem MS spectra for individual glycans otherwise not separable by mass itself (Figure 3). Stadlmann *et al.* used this approach to compare the glycome of recombinantly produced and partially therapeutically used IgG molecules, showing that PGC LC ESI MS provides essentially unrivalled depth of data in a short time frame [17]. Furthermore, small amounts of Galα1,3-Gal epitopes were detected to be present on the N-glycans of Zenapax, a therapeutic humanized monoclonal antibody directed to the alpha subunit of the IL-2 receptor of T cells. In contrast to most mammals, humans are unable to express Galα1,3-Gal structures, resulting in naturally occurring high antibody titers directed against this epitope [19].

Other important immunoglobulins such as secretory IgA (sIgA) have been subjected to glycomics characterisation by the PGC LC ESI MS approach [20••]. SDS-PAGE separation before glycan release allowed for a detailed individual mapping of the different glycans from the secretory component, IgA and joining chain that together make up sIgA present in secreted fluids like saliva or breast milk. The three different proteins exhibit a significantly different individual glycome, with about 50% of N-glycans from the secretory component containing one or two Lewis X epitopes, respectively. In contrast, essentially no Lewis X type structures were found to be present on either the IgA moieties of the molecule or on the joining chain. These glycosylation differences likely reflect the different origin of the three proteins since the secretory component is synthesised by epithelial cells but IgA and joining chain are derived from plasma cells. In the biological context, however, the N-glycans of the secretory component might play an important role for mediating interactions between the microbiome and the mucosal immune system. Mathias and Corthésy recently