

EDITORIAL

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Sweet talking—cellular carbohydrates and epithelial repair

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The barrier defences of the respiratory tract are constantly challenged and for them to work at their best, optimal repair of the epithelial barrier after damage is essential. Effective regulation of repair is a complex process as differentiated epithelial layers may remain intact for long periods and yet need to respond quickly and autonomously to repair damage when it does occur. Depending on the extent of damage the epithelial response will also vary. Small wounds are repaired by simply pulling the neighbouring cells together using an actin 'purse string' [1] while more extensive areas of damage leaving the basement membrane exposed are repaired by co-ordinated dedifferentiation and spreading of the remaining epithelial sheet followed by proliferation and re-differentiation as required [2–4]. These processes require a high degree of organization, maintaining cell–cell and cell–substrate contact and retaining maximal epithelial function, while the cells may move many times their own diameter from their original position. As damage is local and unpredictable, the response is largely autonomous and mediated by local co-operation between cells. This is clearly illustrated by the ability of epithelial cells to form and co-ordinate the repair of damage in pure airway epithelial cultures [2, 5–7]. There is growing functional evidence, including the paper by Patchell and Dorscheid [8] in this issue that carbohydrate-based interactions are critical in the normal processes of epithelial repair in the airway [7, 9]. It is also becoming clear that this does not simply reflect a single carbohydrate-based interaction, but rather a broader range of interactions involved in the repair of the epithelium.

The importance of glycosylation

Specific carbohydrate modification of proteins provides a ubiquitous and highly regulated means of modulating cell–cell and cell–substrate interactions. Certainly, many interactions between epithelial cells or with cells of the immune system are based on glycoconjugate binding or are modified by changes in cell surface glycoconjugates. For example, Pace et al. [10] established that human galectin I-induced apoptosis by binding to a specific set of cell surface glycoproteins with a dramatic re-distribution of surface glycoproteins in those cells which subsequently died. Binding of leucocytes to endothelium at sites of inflammation provide a further example. The α 1,3 linked fucose-containing glycoconjugates sialyl Lewis A and sialyl Lewis X are ligands for the cell adhesion molecule E-selectin, expressed on vascular endothelial cells. Selectins tether the leucocytes to the endothelium [11] and are a pre-requisite for the subsequent cell crawling and extra-vasation is required for the movement to the site of inflammation. Glycoconjugates are also key components of the intercellular environment as carrier molecules for endocrine and paracrine factors and as a part of the extracellular matrix. This is particularly true of the situation after damage where a temporary acellular scaffold is formed rapidly after epithelial damage and provides an extracellular matrix for subsequent regeneration of a differentiated epithelium. This relies heavily on hyaluronic acid and proteoglycans, which can form a space-filling structural matrix at comparatively low concentrations because of their high charge and long chain length.

Lectins as tools to study glycoconjugates

Lectins are widely used in studies of glycoconjugates; they are proteins which specifically bind carbohydrates and have provided essential tools to identify sugar groups and to monitor changes in their expression on the cell surfaces. They are a large and diverse group, typically purified from natural products and selected on the basis of their binding

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to specific sugar linkages. The native biological functions of most lectins remain unknown but it is likely that they have a significant role in binding and cross-linking specific glycoconjugates. The potential importance is again illustrated by the selectins, some of the best-characterized animal lectins forming a sub-family of the large and diverse C-type (calcium-dependent) lectin family. Individuals lacking the ability to add terminal fucose residues to selectin ligands show leukocyte adhesion deficiency type II [12] reflecting reduced leucocytes trafficking to inflammatory sites resulting from altered rolling on the endothelium. In using lectins as tools we are dependent on our knowledge of their natural affinity and some bind to a broad range of carbohydrate structures. The lectin concavalin A isolated from Jackbean (*Canavalia ensiformis*), for example, binds both D-glucose and D-mannose [13]. Other lectins can be characterized down to the level of the specific sugar linkage to which the lectin has highest affinity and binding is often also dependent on other nearby sugars. For example, the C-type lectin from the Japanese horned beetle, *Allomyrina dichotoma* (AlloA) binds strongly to galactose in a β 1,4 linkage to N-acetylglucosamine when the galactose is linked α 2,6 to sialic acid while when linked α 2,3 to sialic acid, binding is abolished. Using lectins it has been possible to associate specific glycosylation profiles with disease and with prognosis. Changes in cell membrane carbohydrate antigens have been shown to play an important role in carcinogenesis and metastasis [14] reflecting altered cell associations. Sialyl Lewis A and sialyl Lewis X antigens are commonly expressed on human cancer cells and are thought to be involved in the binding of the cancer cells to the endothelium during metastatic tumour spread. The expression of long-chain α 2,8-linked polysialic acid has been shown to reduce cell-adhesion molecule-mediated interactions because of the high charge and large excluded volume, and can also interfere with binding of molecules on other cells [15]. Expression of α 2,8-linked polysialic acid on cells of small-cell carcinoma of the lung may contribute to the highly invasive and malignant properties of this tumour [16, 17]. Thus, glycosylation can lead to significant changes in the biochemical and biological properties of the proteins and cell surfaces. It may lead to modification of charge, excluded volume, tertiary structure, and the accessibility of peptide moieties on proteins, consequently affecting the adhesiveness of the modified proteins and of other proteins on the cell surface. Lectin binding to carbohydrate groups disrupts these changes and can further affect both the binding properties of the proteins and the ability of other proteins to bind to them.

How carbohydrates are involved in epithelial repair

Studies on repairing epithelial cells have shown that biologically significant roles can be assigned to cell surface carbohydrates during wound repair. Dorscheid et al.

[18] demonstrated that pre-treatment with inhibitors of O-glycosylation did not affect the rate of wound repair but that inhibition of N-glycosylation with tunicamycin [18] resulted in a substantial reduction in wound repair, suggesting an important role for N-glycosylated glycoproteins. Adding lectins binding to N-acetylglucosamine significantly inhibits repair in an *in vitro* model of wound repair implying that these glycoprotein(s) are necessary for normal wound repair [7]. Further work has identified particular glycoproteins involved in epithelial wound repair. The use of integrin subunit antibodies in models of repairing epithelium has demonstrated an inhibition of repair to a greater or lesser extent when cells were cultured on basement membrane matrices such as laminins or collagen IV [19]. In addition, later work has shown the importance of the laminin binding glycoprotein dystroglycan for cell migration and spreading during wound repair and suggests that this is dynamically expressed in order to bind to a laminin matrix [20]. This process was inhibited with the use of the N-acetylglucosamine specific lectin wheat germ agglutinin suggesting the importance of this carbohydrate moiety in this system. It is interesting to note that this model system is driven by the addition of epidermal growth factor and it is known that in differentiated airway epithelia the exposure of baso-lateral sides of cells to such apical growth factors might be a natural consequence of damage and repair [21].

Patchell and Dorscheid [8] have shown that the AlloA lectin prevented wound repair in respiratory epithelial cell monolayers. It is interesting to note that in this study the authors see little or no binding of the AlloA or GS α -1 lectin in the absence of wounding, suggesting that the glycoconjugates are only gradually synthesized, or unmasked over the 24 h following damage. Given the requirement for speed in repairing damage, it seems unlikely that early *de novo* expression of the molecule occurs so it is most likely that the glycoconjugate which binds AlloA is either unmasked, imported or the activity for which it is required occurs relatively late in the repair process. By affinity purification, the authors were able to show that fetuin, a serum glycoprotein, bound the lectin and that addition of fetuin to the cultures stimulated wound closure. They suggest that fetuin is important in repair and this activity is blocked by the binding of the lectin. The deposition of an extracellular protein would be consistent with the expression only after repair and also the staining pattern seen in this study. The authors also indicate that the fetuin binding asialoglycoprotein receptor (ASGPR) is expressed in their system. ASGPR is an endogenous C-type lectin that binds to glycoproteins after the terminal sialic acid is removed to expose the penultimate galactose residues [22]. The presence of exogenous lectins that attach to these will likely block the normal fetuin/ASGPR interaction, preventing clearance from the wound area.

Conclusion

In conclusion, there is now mounting evidence that glycoconjugates play a significant role in the regulation of repair in airway epithelium. As glycosylation can be considered a ubiquitous feature of cell surface proteins this is not in itself a surprise. It rather provides a challenge to understand what part the sugars play in the coordinated communication necessary between cells in order to reform an organized and differentiated epithelium. It further provides a different range of physiological mechanisms potentially involved in pathological changes including interactions with microorganisms with corresponding potential for therapeutic intervention.

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