

Carbohydrates are good for you!

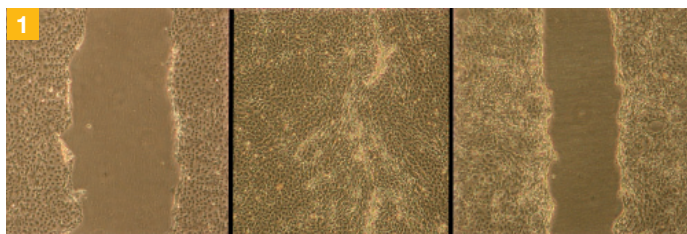
A closer look at the investigation of glycoconjugates in epithelial repair.

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The upper airways carry air to the lungs and are therefore the primary impact site for environmental particulates, including potential pathogens. The complex structure¹ of these airways provides a protective barrier including elements of both the acquired and innate immune systems². The epithelial cells also facilitate the clearance of material landing on the airway. Damage to this protective barrier, for example in asthma, can result in a loss of function or structural integrity and associated local mucosal activation³. A number of studies have shown that epithelial repair in normal airways involves the rapid and autonomous migration of the surrounding cells to cover the damaged area⁴. In this article we look at the microscopical investigation into the essential role of cell surface carbohydrates, called glycoconjugates, on these repair processes.

Molecular role of glycoconjugates

Epithelial repair mechanisms and the effects of disease are not yet fully understood. However, we do know that many of the important interactions between neighbouring cells and with itinerant cells of the immune system are based-on or modified-by cell surface carbohydrate moieties called glycoconjugates. It has been reported for example⁵, that human Galectin I (a member of a family of structurally related carbohydrate-binding proteins) induces apoptosis by binding to a specific set of cell surface glycoproteins. A well-defined group of glycoconjugates containing fucose (C6H12O5) are widely expressed in mammals with several key biological processes attributed to them. For example: the Lewis antigens, found on the surface of white blood cells, contain α 1,3 linked fucose while the H antigen, a blood group antigen, is a α 1,2 fucosylated precursor of the Lewis antigens.



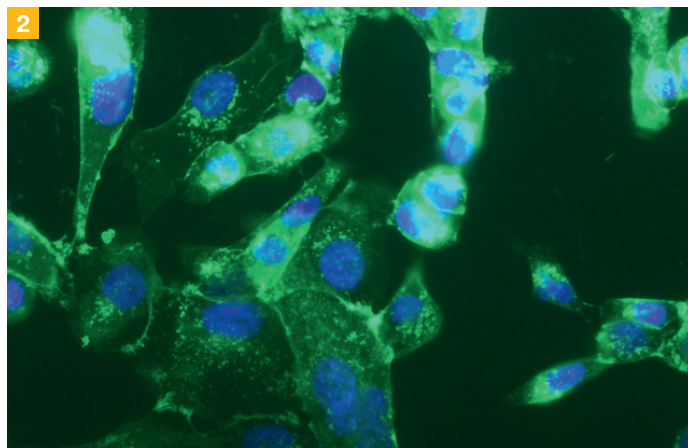
Cultures of cell derived from airway epithelium (1)

The conformation of the fucose linkage provides a further level of discrimination – the roles of glycoconjugates (complex molecules containing carbohydrates) can only be fully understood if their structures are known. During leukocyte binding to endothelium at sites of inflammation for instance, the fucose containing glycoconjugates – Sialyl Lewis A and Sialyl Lewis X are specific ligands for E-selectin (a cell adhesion molecule) which is present on vascular endothelial cells. Selectins then

tether the leukocytes to the endothelium⁶ and are essential for the cell crawling and extravasation required for their movement to a site of inflammation. The important role of fucose-containing glycoconjugates, has been further shown by the higher prevalence of asthma and wheezing in the absence of Lewis antigens⁷.

Investigating the extent of this role

Lectins are naturally occurring glycoconjugate binding molecules which provide highly selective tools to identify or block specific glycoconjugate motifs. The lectin from the mushroom *Aleuria aurantia* (AAL) has a high affinity and is very selective for α 1,6 linked fucose found in disaccharides, oligosaccharides and glycoproteins on the surface of non-secretory columnar and basal epithelial cells, but importantly does not bind to the basement membrane⁸.



Cultures of cells derived from airway epithelium labelled with FITC tagged lectin (2)

Dr Lackie, a lecturer in the Infection, Inflammation and Repair Division within the University of Southampton's School of Medicine, and his co-workers have used this knowledge to conduct a series of experiments looking at wound repair following the addition of AAL. They used a simple protocol where a 'pulse' of FITC labelled AAL is 'chased' with unlabelled fucose to block binding of the lectin. This proved that AAL seriously inhibits repair while adding fucose, enabled the repair processes to happen as normal, providing the fucose is added in the first few hours. They also followed the fate of the AAL, showing that although it is internalised relatively quickly by the cells, its impact on the repair mechanism is enough to seriously disrupt successful repair.

Tools of the trade

One of the most essential tools for any cellular investigation and especially these epithelial repair studies is the microscope. Dr Lackie and co-workers use a number of different micro-

scopes including a standard Olympus CK2 inverted for examining cultures and an advanced motorised IX81 inverted for fluorescence-based time-lapse investigation, amongst others. The CK2 is fitted with an Olympus 3040Z digital camera so that the confluency, induced damage and repair to the monolayers can be imaged and recorded (as in Figure 1). The IX81 is fitted with an Olympus FView II high sensitivity, black and white digital camera to record the fluorescent images and is operated via the **cell^F** software programme. The system is also fitted with an automated stage and a Solent Scientific incubation system to provide environmental control, producing an excellent live cell imaging station for time-lapse experiments.

Time-lapse experiments using the IX81-based system enable the team to follow the molecular and cellular events involved in the repair process, as well as to assess the viability of the cells following repair. Dr Lackie commented, “Time-lapse imaging has allowed us to establish the time course of important processes such as the internalisation of the AAL and similar lectins, while confirming that, although the repair process may be inhibited by AAL binding, the inhibition does not affect cell survival rates.”

A modular ‘optical bench’

The modular design of the microscope ensures that all the components work together and that the **cell^F** software can provide the team with full control over the microscope, nose-piece, stage and imaging. More importantly, the software is designed to allow them to set their own experimental protocols and let them run automatically. Dr Lackie commented, “The microscope set-up enables us to complete multiple time-courses in parallel, which is useful from both time and experimental points of view. We can now maximise our throughput

to run between six and eight experiments simultaneously, providing us with a huge amount of data for each different parameter.” He continued, “When comparing results from the same ‘run’, this set-up also allows us to compare identical samples with different experimental treatments greatly reducing variability and increasing the value of our data.”

Conclusions

Dr Lackie and his colleagues, Dr Elizabeth Adam and Prof Stephen Holgate, have demonstrated⁹ the importance of $\alpha 1,6$ linked fucose in the repair of damage to epithelial cell layers. They showed that the addition of AAL to damaged monolayers of epithelial cells blocked, or significantly inhibited, the normal ability of the layer to repair. Any delay in repairing minor damage could significantly increase the already heightened risk of infection at sites of trauma. The AAL binding sites therefore appear to have an important role in the normal epithelial repair process. Furthermore, these observations have important implications, not only in understanding the epithelial injury-repair cycle, but also in the identification of novel drug targets for diseases such as asthma.

Integral to their ongoing research are a number of different microscopes, including an advanced Olympus research platform, offering them the fine control and flexibility required for the leading edge nature of their work. The value of these microscopic approaches has been recognised by the School of Medicine at the University of Southampton, who provided the funding to allow this equipment to be used as a core facility for research throughout the Medical School. The facility is housed in the Biomedical Imaging Unit at Southampton General Hospital.

3 Olympus IX81 inverted research microscope



ACKNOWLEDGEMENTS

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Figures

- 1** Cultures of cell derived from airway epithelium, grown until confluent and then damaged (a) after 18 hours the area of damage has repaired (b) except in the presence of lectin when repair is inhibited (c). Image courtesy of Dr Elizabeth Adam and Dr Peter Lackie.
- 2** Cultures of cells derived from airway epithelium labelled with FITC tagged lectin showing the internalisation of the FITC-lectin (green), nuclei are counterstained blue. Image courtesy of Dr Elizabeth Adam and Dr Peter Lackie.
- 2** the Olympus IX81 inverted research microscope

Dr Lackie's Research Group

The Lackie group have been looking at the cellular and molecular processes involved in epithelial repair. Their most recent research focus has been on actions of cell surface carbohydrate molecules – glycoconjugates, which appear to be very important in establishing and maintaining cell-cell interactions and are therefore essential in any repair process. Using a combination of techniques, including microscopical analysis, Dr Lackie and colleagues are investigating to what extent disruption of these carbohydrate based interactions hinders successful repair.

Information

Further information is available at:

www.microscopy.olympus.eu/microscopes/