Goblet cells as mucosal sentinels for immunity

MA McGuckin[1,2] and SZ Hasnain[1,2]

Mucus, the viscoelastic material produced by all mucosal surfaces, is gaining increasing recognition as being an integral component of both mucosal homeostasis and defense from infection.[1–4] In a recent paper in *Science*, George Birchenough *et al.*[5] from Gunnar C. Hansson's laboratory at the University of Gothenburg describe a previously unrecognized mechanism by which mucus-producing goblet cells sense and respond to microbial products to bolster mucosal defense.

First, a quick summary of what we know about intestinal mucus and some key prior studies relevant to the Birchenough paper. The major (but not only) constituents of mucus responsible for its biophysical properties are secreted mucin glycoproteins that are synthesized as large homo-oligomeric macromolecules by specialized goblet cells, stored within intracellular granules and secreted onto the mucosal surface. On secretion, the mucins are rapidly hydrated and form mucus, which generally has >90% water content. In the mouse, Muc2 is the predominant intestinal secreted mucin in health, and deficiency of Muc2 leads to spontaneous inflammation and susceptibility to infection.[6,7] The Hansson laboratory has been at the forefront of demonstrating how the intestinal mucus layer separates the underlying epithelium from luminal microbes and how depletion of this mucus layer may be centrally involved in intestinal inflammatory disease.[8,9]

Although it is relatively well-documented how goblet cells are responsive to various arms of immunity in terms of regulation of mucin synthesis and secretion,[3] over the last few years a more complex role for goblet cells in regulation of immunity has emerged. Rod Newberry's group from Washington University first demonstrated in *Nature* that some small intestinal goblet cells endocytose soluble material from the lumen and are capable of passing antigens to underlying $CX3CR1^+$ dendritic cells; they called these goblet-cell-associated antigen passages (or GAP cells).[10] In a 2015 paper, in *Mucosal Immunology* they showed that GAP cells could also occur in the colon, that they were repressed by TLR ligands in a goblet cell intrinsic Myd88-dependent manner and the absence of goblet cell Myd88 resulted in activation of both $CD103^-$ and $CD103^+$ dendritic cells and consequent mucosal inflammation.[11] In 2016, in *Gut* they extended this observation to show that antibiotic treatment of mice resulted in amplified GAP cell activity, translocation of commensal microbes across the epithelium, presentation of them to immunity and consequent inflammatory responses.[12] Another relevant study was published in *Cell* by Richard Flavell and Brett Findlay's groups showing that the Nlrp6 inflammasome regulates autophagy, which is required for secretion of mucin granules, and that mice lacking epithelial Nlrp6 had defective mucin secretion and barrier function.[13] It should be pointed out, however, that the diminished mucus barrier in $Nlrp6^{-/-}$ mice was not reproduced by Birchenough *et al.*[5]

The recent *Science* paper shows that in the mouse colon there are a minority of upper crypt goblet cells that sample luminal soluble material and rapidly secrete Muc2 in response to endocytosis of microbial products (including lipopolysaccharide (LPS), LPS subcomponent lipid-A, the triacylated lipopeptide P3CSK4 and flagellin, but not lipotechoic acid, bacterial DNA, muramyl dipeptide or g-D-glutamylmeso-diaminopimelic acid).[5] Working with mucus is technically difficult and many of these experiments were dependent on measuring the rate of growth in mucus thickness in explant cultures of intestinal epithelium. However, the authors were aided by their production of a transgenic mouse expressing a mCherry form of human MUC2 in goblet cells, which enabled static and real-time monitoring of goblet cells and Muc2 secretion. Muc2 secretion was dependent on the cognate Toll-like receptors (TLR) for the microbial products, and on Myd88 but not Trif. Active endocytosis of the microbial products increased intracellular ROS, which was required for activation of the Nlrp6 inflammasome and caspases 1 and 11, and which in turn triggered compound exocytosis of Muc2. The goblet cells that endocytosed microbial products appeared to: (a) be extruded from the epithelium as they underwent compound exocytosis; and (b) spread the signal to secrete Muc2 to adjacent goblet

[1]Inflammatory Disease Biology and Therapeutics Group, Mater Research Institute—The University of Queensland, Translational Research Institute, Woolloongabba, Queensland, Australia. Correspondence: M McGuckin (michael.mcguckin@mater.uq.edu.au)
[2]These authors contributed to equally to this work.

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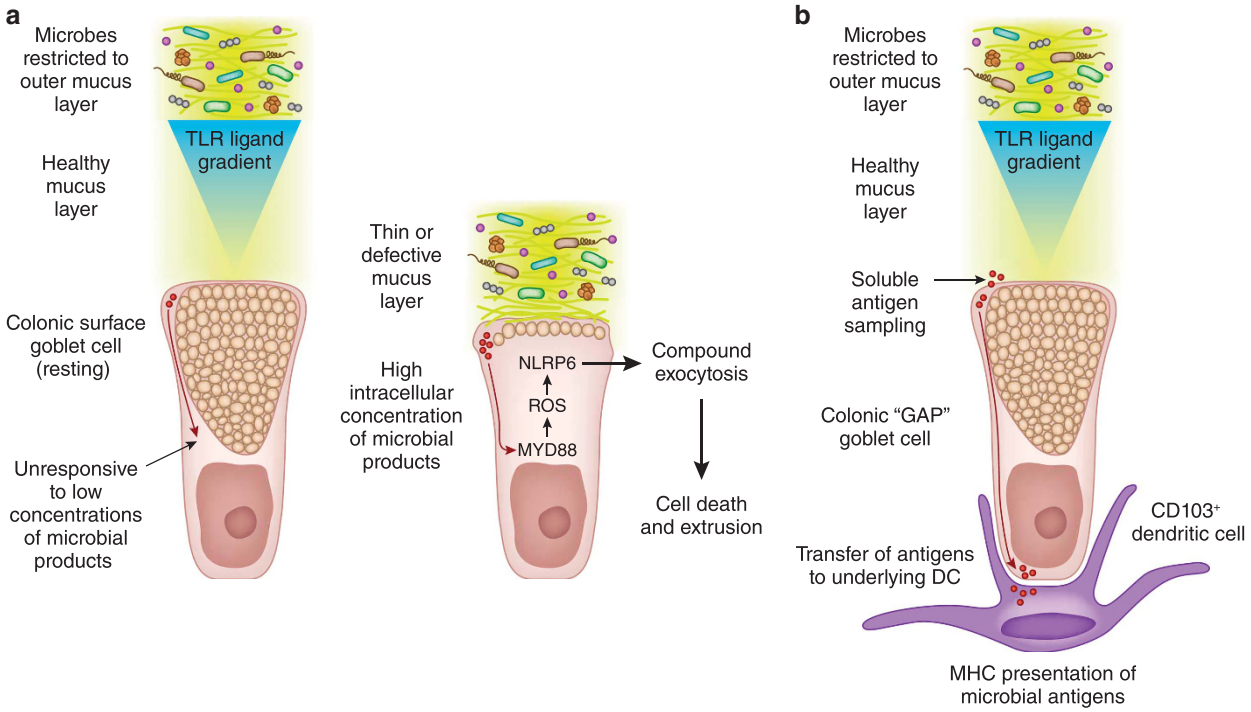

Figure 1 Diagrammatic representations of two differing descriptions of goblet cell function in microbial product sensing. (**a**) Representation of crypt surface goblet cell function as described by Birchenough *et al.*[1] A thick mucus layer usually covers the colonic epithelium separating microbes from the epithelial cells and establishing a diffusion gradient of microbial molecules. A subset of surface goblet cells continuously sample at the apical luminal surface, perhaps associated with low-level constitutive mucin granule secretion. In the case of an effective mucus layer, the low concentration of microbial molecules at the goblet cell surface is insufficient to activate the goblet cell. If the mucus layer is thin or defective in barrier function a high concentration of microbial molecules are taken up by the cell, and, when exceeding a threshold, activate Toll-like receptors and Myd88. Myd88 in turn switches on production of reactive oxygen species (ROS), which trigger Nlrp6 inflammasome-mediated activation of caspases 1 and 11. Compound exocytosis of mucin granules follows, resulting in rapid release of mucus, death of the goblet cell and its extrusion from the epithelium. (**b**) Representation of colonic goblet cells forming GAPs (goblet-cell-associated antigen passages) as described by Knoop *et al.*[11,12] Goblet cells that sample soluble antigens are present in the colon and sampling is enhanced by cholinergic signaling and inhibited by Myd88 signaling (sampling increases greatly after antibiotic therapy and in Myd88-deficient mice). Soluble antigen is passaged through the goblet cell from the apical to basolateral membrane where it is passed to closely adherent underlying $CD103^+$ dendritic cells. Disruption of the suppression of goblet cell sampling mediated by MYD88 results in immune activation in the draining lymph node and lamina propria, and local inflammation. Further research is required to explore how the models in **a** and **b** overlap and before we can fully understand the important role goblet cells have in barrier function and immunoregulation in the colon.

cells potentially via gap junctions.[5] The authors did not explore whether goblet cell extrusion occurred because the cell was undergoing caspase-1-mediated apoptotic or pyroptotic cell death, or some distinct process.

The authors conclude that there is a goblet cell intrinsic system by which goblet cells positioned toward the top of colonic crypts sample microbial products and, if a pivotal concentration of TLR ligands is reached, initiate a cascade of intracellular signaling dependent on Myd88, ROS production and Nlrp6 inflammasome activation that results in compound exocytosis and extrusion of the goblet cell from the epithelial layer. In an evolutionary sense, this seems like a good system to rapidly deal with microbial pathogens that may have penetrated the mucus barrier and threaten infection of the epithelium without the need to engage underlying immunity. Although the goblet cell is killed in this process, the goblet cells near the top of the crypt are nearing the end of their short life. Furthermore, the extrusion of the cell ensures that if the TLRs are triggered because the goblet cell was infected itself (as has been shown in the mouse colon for bacterial pathogens[14,15] and even for commensals after antibiotic therapy[12]) that the infected cell is removed from the epithelium and the pathogens will be trapped in fresh mucus. The findings are also consistent with observations that colonic ischemia/reperfusion results in initial loss of mucus (unexplained) followed rapidly by microbial penetration deep into crypts and, in response, goblet cell compound exocytosis and shedding.[16] Interestingly, in this scenario the compound exocytosis was not restricted to goblet cells at the top of crypts. Although this seems at first like a straightforward system, like any new discovery, the findings raise many new questions that need to be addressed by future research.

ARE ALL GOBLET CELLS POTENTIALLY SENTINEL CELLS AND HOW IS THIS FUNCTION REGULATED?

Taking the work of the Newberry and Hansson groups together it is apparent that colonic goblet cells can endocytose soluble material from the lumen but that the basal level of this sampling is restricted to a minority of cells. As in all secretory cells, when goblet cells secrete mucin

granules there is a process of membrane fusion of the granule membrane with the apical cellular membrane, which is followed after secretion by recycling of the membrane and secretory machinery in an endocytic process. One possibility is that GAPs occur when goblet cells are undergoing constitutive secretion of Muc2 or other secretions, and the restriction to a minority of cells is simply a reflection of which cells are undergoing constitutive secretion at the time of the experiment. Both groups document a very marked increase in GAP cells in the absence of Myd88 introducing the concept that microbial products suppress this antigen-sampling system,[5,11] and that most goblet cells have the capacity to form GAPs/be sentinels. If the GAPs are indicative of basal constitutive secretory activity then this seems counterintuitive as one would predict that the host would want to bolster the barrier (secrete mucins and other host defense molecules) in response to microbial products.

Another conundrum presented by the data is that Myd88 signaling to the normal amount of microbial product in the lumen does not drive Muc2 secretion, but that higher concentrations drive marked secretion by compound exocytosis. Thus, it appears the concentrations of microbial products are pivotally important, which will be determined by the concentration at the luminal surface (mucus thickness, microbial penetration of mucus) and the rate of endocytic sampling. A further complexity that needs investigation is the apparent difference between surface and crypt goblet cells in their responsiveness to TLR ligands and cholinergic stimulation, and additionally, how the two different lineages of goblet cells in the proximal colon and small intestinal goblet cells function. How these functional differences are regulated at the molecular level also requires investigation. Future research should also address how this system operates during established infections and chronic inflammation, where the mucus layer is often diminished and chronic exposure to higher concentrations of microbial products or microbes themselves will occur. It is also important to understand how various arms of immunity affect responsiveness of goblet cells to microbial products, including the T_H1 responses that are associated with goblet cell depletion, and the T_H2 responses that drive goblet cell hyperplasia and copious mucus secretion.

IS THE SYSTEM ENTIRELY GOBLET CELL INTRINSIC?

Although Birchenough *et al.*[5] demonstrate that many aspects of this system are goblet cell intrinsic, there is a formal possibility that underlying immune cells are an integral component. Experiments were conducted in *Rag1*$^{-/-}$ mice to exclude lymphoid cells; however, no attempts were made to exclude a role for myeloid cells. In both the small intestine and colon, GAPs have been shown to transfer endocytosed antigen to closely integrated underlying dendritic cells, which then migrate to draining lymph nodes, present the antigen, and initiate immune responses.[10–12] Birchenough *et al.* describe spreading of Ca^{2+} signaling and mucus secretion to adjacent goblet cells, which could be blocked with gap junction inhibitory drugs. In their confocal microscopy images, after exposure to TLR ligands Ca^{2+} signaling can be seen in many non-Muc2-expressing cells. The underlying dendritic cells have the capacity to form gap junction connections with multiple epithelial cells and are therefore well positioned to participate in this process.

WHAT IS THE MUC2 CONTENT OF THE INNER MUCUS LAYER?

The mCherry MUC2 transgenic mice allowed visualization of mucus secretion and these data raise some fundamentally important questions about the nature of the mucus layer. When LPS is introduced to the explant cultures copious spreading Muc2 secretions can be seen. However, although the inner mucus layer appears intact in these explants (as shown by bead penetration studies) there is no fluorescent mCherry layer seen (**Figure 1d,e**).[5] This observation is consistent with the relatively poor Muc2 IHC staining of this layer reported previously by the Hansson group and others,[8] and raises questions about the Muc2 content of this inner mucus layer.

Goblet cells are found at all mucosal surfaces, and it is highly likely that the phenomenon of rapid secretion in response to microbial products will not be restricted to the intestine. The role of goblet cells as monitors of the extracellular environment, interactors with the microbiome and its products, and communicators with underlying immunity should be considered by all mucosal immunologists interested in mucosal homeostasis, defense from infection and mucosal inflammation.

DISCLOSURE

The authors declared no conflict of interest.

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