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Review

Mucosal physical and chemical innate barriers: Lessons from microbial evasion strategies

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ABSTRACT

The innate immune system has evolved since millions of years under a selective pressure. Among the different host mechanisms selected and conserved as a first line of defense, the gastrointestinal mucus layer constitutes an efficient physical and chemical barrier against invading microbes. Mucin glycoproteins and antimicrobial peptides are the major components of the mucus barrier, and evidences prove that they form an effective protection against most microbes. However, successful pathogens have evolved evasion strategies to circumvent this defense barrier. Here, we discuss the interactions between pathogens, mucins, and antimicrobial peptides, and the mechanisms that pathogens have developed to evade the innate defense systems of the intestinal mucosal barrier.

1. Introduction

The gastrointestinal mucosa has evolved several innate mechanisms of defense in response to its susceptibility to microbial assault due to its exposure to the external environment. Intestinal epithelial cells form a physical lining that acts as a barrier between the exterior environment and the remainder of the host. Moreover, these cells secrete constitutively, or in response to the presence of microorganisms, multiple defensive molecules into the mucosal fluid, such as mucins, antimicrobial peptides and immunoglobulins. Aggregated in a complex viscous structure named mucus, these mediators of the innate immunity form a physical and chemical barrier that has efficient antimicrobial activity, and the ability to regulate microbial colonization and invasion processes [1].

Host–pathogen interactions are a result of mutual inhibition, evasion and adaptation strategies that have evolved over their coexistence [2]. Microbial pathogens recognize environmental signals within the mucus and have developed a diversity of strategies to avoid or subvert this barrier, in order to attain the underlying epithelium, where they can initiate their infectious process. The host response to these pathogens encompasses changes in the production rate, components and properties of the mucus. Although the barrier is effective at keeping commensal microorganisms in check, pathogenic viruses, bacteria, fungi, and parasites have developed a wide range of mechanisms for circumventing it. Here, we review most of strategies elaborated by bacterial pathogens to evade mucus and antimicrobial peptides defenses.

2. Microbial evasion of mucus

The gastrointestinal mucosa relies on epithelial cells tightly linked *via* intracellular junctions thus forming a contiguous barrier, which is resistant to microbial invasion. The epithelial surface is coated with a layer of mucus, which continually moves along the gastrointestinal tract to clear trapped microbes. Mucin glycoproteins produced by mucus-producing cells, harbored by the epithelium, are the major macromolecular constituent of mucus and are at the origin of its viscous properties (Fig. 1). In addition to forming a relatively impenetrable gel, which acts as a physical barrier, mucus provides a matrix for a wide range of antimicrobial molecules, including antimicrobial peptides.

Epithelial mucins are a family of large complex glycoproteins containing a dense array of glycans typically comprising more than half of their mass. The extensive glycosylation of mucins protects them from most proteolytic enzymes produced and secreted by resident microbes. Oligomerization of mucins is likely to produce either extended filamentous structures or, more probably, web-like molecular structures, which to a certain extent block the

Abbreviations: LPS, lipopolysaccharide; MAPK, mitogen activated protein kinase; NF-κB, nuclear factor kappa-B; SIC, streptococcal inhibitor of complement.

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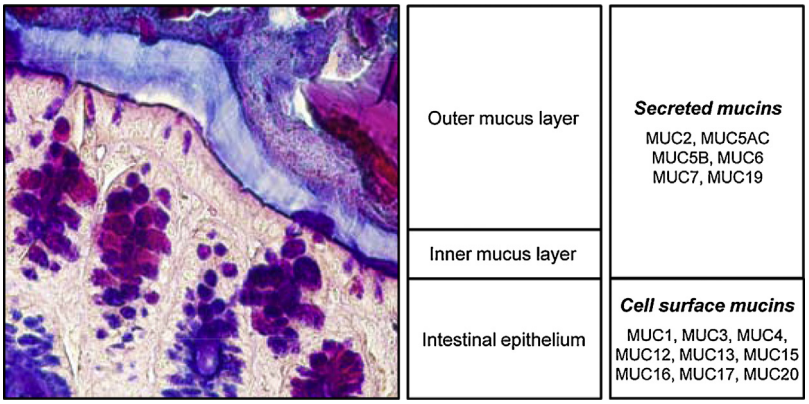

Fig. 1. Structure of the intestinal mucus layer. Histological section of mouse intestinal tissue stained by alcian blue and periodic acid Schiff (AB/PAS) reagents. The inner layer stains irregularly blue, whereas the outer layer stains blue/purple with a stratified arrangement. Mucus-producing cells appear in blue/purple in the epithelium. Magnification 40×.

penetration by microbes [3]. The extended conformation of mucins caused by dense glycosylation enables the molecules to occupy large spaces equivalent to those of bacteria [4]. Besides their capacity to form a physical barrier, mucins have direct and indirect roles in the defense against microbial infection. Mucin glycans are able to bind microbes, and in some cases, they either have direct antimicrobial activity or carry other antimicrobial molecules [5].

Not surprisingly, microbial pathogens have developed dedicated strategies to thwart the mucus barrier. These include mechanisms to allow efficient penetration of the mucus, proteolytic enzymes that degrade mucins, pathways that allow evasion of the mucus barrier, disruption of the cells that produce mucins, and inhibition of mucin synthesis by mucus-producing cells (Fig. 2).

2.1. Penetration of the mucus barrier

One feature common to some enteric pathogens is the existence of flagella, which allow the bacteria to propel themselves within the viscous mucus barrier. As a consequence, pathogens with perturbed flagellar function have reduced pathogenicity, highlighting the crucial importance of motility in infectious processes [6]. Moreover, some pathogens modify mucus properties in their local microenvironment to increase their motility, such as *Helicobacter pylori*, which can increase the pH of its close environment, thus reducing the viscoelasticity of the surrounding mucus [7]. *Salmonella*, which is an invasive bacterium, needs to circumvent the mucus barrier to reach the epithelium [8]. As such, flagellar motility and chemotaxis have been found as important for induction of colitis in mice, to allow bacteria to come in close contact with the epithelium and swim against the mucus flow [9,10].

Most enteric pathogens are chemoattracted by amino acid and carbohydrate constituents of mucins, and use mucus as a signal to express genes involved in pathogenicity. For example, after exposure of *Campylobacter jejuni* to the purified human mucin MUC2, genes encoding the cytolethal distending toxin, the multidrug efflux system, or the flagellin A, are upregulated [11]. Alternatively, the mucus environment can be seen as an advantage for the pathogen, as exemplified by *H. pylori*, which is protected from the low acidity of the stomach by residing deep in the mucus layer [12].

2.2. Degradation of mucins

Enzymes, such as proteinases and glycosidases, produced and secreted by microbes have a role in destroying the mucus barrier. In most cases, mucus is effective at physically trapping microbes and allowing for their rapid expulsion. As long as the mucus-flushing rate exceeds mucin degradation, the microbe is rapidly eliminated. Degradation of the mucus layer by intestinal commensal bacteria is one beneficial form of mucin degradation. A small part of commensals have a crucial catabolic role in maintaining the thickness of the mucus without penetrating the layer. The genome of these bacteria encodes a high proportion of exoglycosidases, which degrade colonic mucins. Then, bacteria use the glycans as an easily available source of energy [13,14]. Complex populations of commensal mucolytic bacteria dominate the outer side of the mucus layer, and this may favor the host by excluding pathogens. In that way, some probiotics inhibit the adherence of pathogens to epithelial cells, such as enteropathogenic *Escherichia coli* [15].

In contrast, pathogens produce degrading enzymes that disrupt the balance between mucin secretion and degradation. Proteolytic cleavage of mucins causes disassembly of the oligomerized mucins, resulting in substantial decrease of mucus viscosity, dispersal of the layer, and diffusion and dilution of antimicrobial peptides. The enteroinvasive *Yersinia enterocolitica* bacterium has been shown to degrade intestinal mucins from rabbit. Compared to mutants, the virulent strain is more effective at degrading mucins, showing a correlation between mucinase activity and virulence [16]. *H. pylori* secretes a glycosulfatase known to target sulphated mucins. This enables the bacteria to migrate through the mucus layer of the stomach to adhere to gastric cells [17]. An example of both response to mucus and degradation of mucins comes from *Vibrio cholerae*, which switches on the *hapA* gene expression after exposure to intestinal mucus. This gene encodes a haemagglutinin protease, which has a mucolytic activity that is required for penetration through the mucin barrier [18].

2.3. Avoidance of the mucus barrier

An additional strategy commonly used by enteric pathogens is to avoid the mucus barrier. Intestinal M cells, which capture and present microbes to the underlying antigen-presenting cells, can be seen as a port of entry in the mucus barrier [19]. Indeed, the dome epithelium in which M cells lie lacks mucus producing cells, and therefore is not covered by a thick mucus layer. Moreover, apical surface of M cells has only sparse microvilli and an apparently thin glycocalyx.

Crossing of the barrier *via* M cells allows the initiation of non-inflammatory immune responses against commensal microbes living in the intestinal lumen. This highly controlled immune response is crucial to promote and maintain intestinal homeostasis [20–22]. However, the bad side of this mechanism is that M cells also constitute the major point of attachment and entry for a