Microbes at the host surface

Sara K Linden[1] and Michael A McGuckin[2]

[1]Mucosal Immunobiology and Vaccine Center, Sahlgrenska Academy, University of Gothenburg, 405 30 Gothenburg, Sweden
[2]Mucosal Diseases Program, Mater Medical Research Institute and The University of Queensland, Level 3 Aubigny Place, Mater Hospitals, South Brisbane, QLD 4101, Australia.

The human body encounters pathogenic bacteria every day although establishment of infections after such contacts is rare. The skin and mucosal surfaces, which comprise the barrier between the body and the external milieu, are the first lines of defense, and colonization of these surfaces is normally the first step in bacterial disease. As a physical barrier, the skin is reinforced by dry, slightly acidic conditions, sloughing of cells and a resident microflora. The non-keratinized mucosal surfaces have functions such as secretion of digestive enzymes and absorption of nutrients that require the barrier to be 'semi-permeable' with ensuing demands on their design. In addition to a resident microflora and sloughing of cells, the mucosal surfaces are protected by a mucus layer. This gel-like layer is formed by highly glycosylated proteins referred to as mucins and is efficient in trapping microorganisms. The underlying cells relay signals to activate the immune system and mobilization of tissue and blood defense mechanisms is an important aspect of host defense. However, such mechanisms may be accompanied by tissue damage, and infectious diseases are often aggravated by an excessive host response to the invading pathogen. Invading pathogens also have weapons to fight the host with, for example mucin-degrading enzymes to disrupt mucus, toxins to disrupt epithelial integrity, and structures to adhere to the host and facilitate invasion. This review will focus on the interplay between the host and bacteria at the mucosal surfaces, which forms a barrier between the inside and the outside of the body.

Key words: Mucosal surface; bacteria; mucin; pathogen; host defence

1. The mucosal surface – a barrier against the outside world

The average person carries in the order of 10^{14} bacteria, which live in harmony with our mere 10^{13} host cells. The majority of these microbes protect the host from pathogens and some even provide nutrients and mature the host immune system. The mucosal surfaces, where these organisms normally live together, has a surface area of about 400 m^2. In addition to sloughing of cells and the presence of a resident microflora which competes for space and nutrients with arriving bacteria, the mucosal surfaces are protected by a mucus layer. This gel-like layer is formed by highly glycosylated proteins referred to as mucins and is efficient in trapping microorganisms. The mucus gel is constantly shed on the luminal side and replenished by secretion from the underlying epithelium, resulting in a continuous 'washing' of the mucosal surface. In virtually all mucosal tissues either the movement of luminal contents (eg. in the gastrointestinal (GI) tract) or directed movement via cilia (eg. in the respiratory tract) ensures that mucus is continually moving. In addition, the mucus gel contains protective proteins that defend against pathogens, and in the gastrointestinal tract bacteria are killed by acid and bile salts. The underlying cells mediate communication between the mucus gel and the immune system.

2. Mucins

Most pathogens cause disease by disrupting and/or penetrating mucosal surfaces, and in order to be successful they have to circumvent multiple elements of the host defense. The first barrier the pathogen encounters is the highly hydrated mucus gel that covers the mucosal surface and protects the epithelial cells against chemical, enzymatic, microbial and mechanical insult. The mucus gel is formed by high-molecular-mass oligomeric glycoproteins (mucins) and protection is reinforced by a number of 'defense factors' trapped in the gel matrix. Table 1 shows some examples of 'defense factors' present in the mucus layer. The thickness of the mucus layer is highly variable depending on, for example, tissue location. In addition, the data reported vary with the method used for the measurements. In a study performed on the rat GI tract *in vivo*, the mucus gel was found to consist of a firmly adherent layer with a thickness of 15-154 μm and a loosely adherent layer of 108-714 μm [1]. The presence of the two mucus layers may be explained by differences in mucin concentration [1]. In the colon, the firmly adherent mucus layer is so dense that it does not allow the luminal bacteria to penetrate and thereby protects the epithelial cells from contact with the large volume of bacteria present at this site [2]. The importance of this barrier is exemplified by the development of spontaneously developing inflammation and cancer of the colon in mice that are devoid of this mucus layer (MUC2 knock out mice) [3].

Table 1. Examples of 'defense proteins' present in mucus.

Protein	Function
Lysozyme	Digests bacterial peptidoglycans, *i.e.* breaks down the bacterial wall
Lactoferrin	Prevents bacterial growth by binding iron
Lactoperoxidase	Kills bacteria by generating superoxide radicals
Defensins	Introduce ion-permeable channels in the bacterial cell membrane
Trefoil factors	Involved in wound healing
Secretory IgA	Opsonisation of pathogens; prevents bacterial attachment to mucosal cells

Underneath the mucus layer, the cells present a dense forest of highly diverse glycoproteins and glycolipids, which form the glycocalyx. Again, the thickness is highly variable; for example the glycocalyx of cat and human intestinal microvilli tips is 0.1-0.5 μm thick, whereas that of the lateral microvilli surface is 30-60 nm [4-5]. In the electron microscope, the glycocalyx appears as filaments attached to the plasma membrane. The oligosaccharide moieties of the molecules forming the glycocalyx and the mucus layer are highly diverse and the average turnover time of the human jejunum glycocalyx is 6-12 hours [6]. Consequently, the mucosal surfaces presented to the outside world are constantly renewed and could potentially be adjusted to changes in the environment, including microbial attacks. Alteration in glycosylation has been proposed to influence cell adhesion, receptor activation, cell differentiation, and tissue morphogenesis.

2.1 Mucin Structure

Mucins can be divided into three distinct sub-families: (a) secreted gel-forming mucins, (b) secreted non-gel-forming mucins, and (c) cell surface mucins. All mucins have in common a high density of O-linked carbohydrates typically comprising over 70% of their mass. Each mucin is thought to form a filamentous protein carrying 100's of complex oligosaccharide structures [7], giving the mucin a 'bottle-brush' appearance. To date, at least 15 human mucins have been included in the family (MUC1, MUC2, MUC3A/B, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC12, MUC13, MUC15, MUC16, MUC17 and MUC20) and the expression profile of mucins varies between tissues with the gastrointestinal tract showing the highest and most diverse expression. Due to genetic variation in exons encoding the glycosylated domains the size of individual mucins can differ substantially between individuals.

The carbohydrate structures present on mucins are determined by the expression of specific glycosyl-transferases. Thus mucin glycosylation is governed by genetics, tissue specific enzyme expression and host and environmental factors influencing transferase expression. The terminal structures of mucin oligosaccharides are highly heterogeneous and vary both between and within species. This structural diversity may allow the mammalian host to cope with diverse and rapidly changing pathogens, as reflected by the observation that susceptibility to specific pathogens differs between people with different histo-blood groups [8]. This is exemplified by the observations that individual Secretor phenotype may determine the ratio of infection as well as course and severity of urinary tract infections, and acute gastroenteritis induced by Norwalk virus, and gastric diseases induced by *H. pylori* [9-11]. The terminal structures of mucin oligosaccharides further vary between and even within tissues in the same host. Because many bacteria and viruses have surface structures which bind to specific carbohydrate structures, the relationship between microbial adhesions and host ligands is likely to govern which niche in the body is infected, and also underlie the differential infection of mammalian host species.

2.2 Regulation and Modulation of the Mucin Barrier

The gel forming-mucins are produced by specialized cells in the epithelial surface and by glands in the mucosal tissues. Secretion occurs via both constitutive and regulated pathways [12]. In addition, both gel forming- and cell surface mucins show constitutive and inducible gene expression in mucosal epithelial cells. Expression of cell surface and gel forming-mucins can be changed by a large variety of factors, such as microbial products, neutrophils, inflammatory cytokines such as IL-1β, IL-6, IL-9, IL-13, interferons, TNF-α, nitric oxide and other uncharacterized inflammatory factors [13-25]. The mucin responsiveness to cytokines provides a functional link between mucins, innate mucosal immunity and adaptive mucosal inflammatory responses. The mucus niche is also affected by the microbes which reside in it. For example, adherence of probiotic bacteria upregulates cell surface mucin expression *in vitro* [26-27], perhaps representing an important part of the mechanism by which probiotic bacteria limit infection by pathogens. In contrast, the LPS of the pathogen *H. pylori* decreases mucin synthesis in gastric epithelial cells *in vitro* [28], representing a mechanism by which a pathogen can modulate the mucus barrier to their favour. The constitutive pathway continuously secretes sufficient mucin to maintain the mucus layer, whereas the regulated pathway affords a massive discharge as a

response to environmental and physiological stimuli. Stimulated mucin release can occur immediately and is accompanied by hydration resulting in a 100 to 1000-fold expansion in volume of the secretory granule contents [29].

In addition, glycosylation changes occur during infection and inflammation, for example in individuals with cystic fibrosis or chronic bronchitis [30], as well as in *H. pylori*-infected individuals [31]. In rhesus monkeys, which share strong similarities with humans in mucin glycosylation and disease development during *H. pylori* infection [32], *H. pylori* infection induces changes of mucosal glycosylation that alter the *H. pylori* adhesion targets [9]. Such changes of host glycosylation dynamically modulate host-bacterial interactions [9].

Invading pathogens also have weapons to fight the host with, for example toxins and mucin-degrading enzymes which may disrupt the mucus layer. However, due to the efficient host defense mechanisms, most interactions are benign or short-lived. Within the human population there is considerable variation in the ability to resist infections and one individual may effectively avoid infection or efficiently eliminate certain pathogens while being susceptible to attack from others.

3. Pathogen strategies for invasion/escape of host defense

The outcome of a persistent colonization can be symbiosis, commensalism or parasitism. The ability of bacteria to adhere, invade, evade host defenses and cause tissue damage is largely due to their ability to produce colonization and virulence factors. Colonization factors can, for example, be involved in adherence, iron acquisition and motility. In addition, bacteria can have virulence factors that damage the host or undermine host defense, such as toxins and proteolytic enzymes. To escape the host defense, pathogens can use sequestration (*i.e.* formation of a physical barrier against the host), humoral evasion strategies (*e.g.* expression of poorly immunogenic antigens or antigenic variation, mimicry and masking) and cellular evasion mechanisms (*e.g.* killing of phagocytes, inhibition of chemotaxis or phagocytosis, occupation of a 'safe' intracellular space, resistance to intra-vacuolar killing and suppression of cellular responses by cytokine manipulation). Bacteria encounter a large variety of environmental situations, including changes in temperature, osmotic pressure, pH, oxygen and nutrition levels, and are highly competent in adapting to a changing environment.

3.1. Host-microbe communication

Eukaryotic cells and bacteria communicate through a wide variety of signals such as toxins, metabolites, hormones, antibacterial peptides, enzymes and surface structures. There are 2000-5000 signal transduction proteins on the average mammalian cell, and a large number of lipid mediators are also involved in cell-cell and intracellular communication. In addition, bacteria use complex cell-cell and intracellular pathways to communicate, and have the capacity to utilize eukaryotic signaling pathways during infection. One example of how a pathogen has 'manipulated' its host to its advantage is *Bacteroides thetaiotaomicron* that induces fucosylation of mucin oligosaccharides which the bacteria then uses as a nutritient [33].

3.2. Microbial Adherence to the Epithelium

To colonize mucosal surfaces and invade the host, microbes commonly exploit host cell structures. The molecular basis for bacterial adhesion to host cells can for example be hydrophobic interactions, cation-bridging (*i.e.* divalent cations counteracting the repulsion of the negatively charged surfaces of bacteria and host) and receptor ligand binding. Binding is usually of low affinity, but clustering of adhesins and receptors cause multivalency effects, leading to a strong combined binding effect. Fimbriae, outer membrane proteins and cell wall components (*e.g.* lipopolysaccharides) may all function as adhesins. Adhesion can affect the bacteria by stimulation/inhibition of growth as well as induction of other adhesive structures and proteins required for invasion or colonization. Numerous interactions between microorganisms and mucins have been demonstrated [34]. Bacteria may have multiple adhesins with different carbohydrate specificities, and modulation of surface receptor density, kinetic parameters, or topographical distributions of these receptors on cell membranes regulate adhesion. As an example, *H. pylori* binds to mucin oligosaccharides via at least four adhesins, which differ with anatomical site, mucin type, pH and gastric disease status [31, 35]. Thus, for *H. pylori*, binding to mucins can have differing consequences during colonization of the oral to gastric niches and during long term infection.

4. Mucins in the defense against pathogens

The increase in mucus secretion as a response to infection is evidence that mucus is an integral part of the host defense [36]. Formation of the mucus gel is important in itself, as it provides a physical barrier as well as a matrix supporting the retention of host anti-microbial molecules. However, the secreted mucins themselves are likely to function as decoys for adhesins that pathogens have evolved to adhere to the cell surface, because the mucins express many of the

oligosaccharide structures found on the cell surface and are constitutively produced in large amounts, constantly washing the mucosal surfaces.

The widest diversity of cell surface mucin expression is in the mucosal tissues most at risk of infection such as the gastrointestinal tract, respiratory tract and eye. Many pathogens require direct binding to or penetration of mucosal epithelial cells to cause pathology. The cell surface mucins can be shed from the cell surface and one of the main functions of cell surface mucins may be to act as releasable decoy ligands for microbes attempting to anchor themselves to the glycocalyx, exemplified by the manner in which the cell surface mucin MUC1 acts as a releasable decoy for *H. pylori* [37]. Cell surface-mucins initiate intracellular signalling in response to bacteria, suggesting they have both a barrier and reporting function on all mucosal epithelial cells. Milk can limit bacterial and viral infections of the gastrointestinal tract and this has been attributed in part to the presence of large amounts of cell surface mucins, mainly MUC1 and MUC15, in the milk fat globule membrane [38-40].

Mucins have direct and indirect roles in defense from infection distinct from their ability to form a physical barrier and act as adhesion decoys. Not only do mucin oligosaccharides bind microbes, in some cases they either have direct antimicrobial activity or carry other antimicrobial molecules. A mucin oligosaccharide, α1-4-linked N-acetyl-glucosamine, which is expressed on some gastric mucins has been shown to directly interfere with synthesis of *H. pylori* cell wall components [41]. Similarly, the MUC7 mucin acts as a fungicide [42-43]. In addition, there is evidence for direct binding of anti-microbial molecules such as histatins and statherin by mucins which would help retain the anti-microbial molecules in the mucosal microenvironment where they can best protect the host. Secretory IgA (sIgA) is secreted via mucosal epithelial cells and needs to be retained in the immediate mucosal environment to maximise exclusion of pathogens. sIgA is retained at high concentrations in mucus where it can efficiently trap the pathogens. Regardless of whether anti-microbial molecules are retained in mucus by direct binding with mucins or by the biophysical properties of mucus, if mucin synthesis is aberrant or secreted mucins are degraded the anti-microbial molecules will have impaired retention and efficacy.

5. Circumvention of the Mucin Barrier by Mucosal Pathogens

Microbes have a wide variety of strategies to subvert or avoid the mucin barrier. Mucin barrier subversion strategies used by microbes include the production of enzymes capable of degrading mucin core proteins and mucin carbohydrates. Motility is also an important factor for bacterial mucosal pathogens to facilitate breaking through the physical mucus barrier. In fact, a vast proportion of mucosal bacterial pathogens have whip like structures called flagella, which they use to propel themselves through mucus [44-45]. *H. pylori* which have dysfunctional flagella have a greatly reduced ability to infect [46]. In conjunction with motility, enzymes that can degrade the mucus gel are produced by a broad range of bacterial pathogens to destabilize the mucus gel and remove mucin decoy carbohydrates for adhesins [47-51]. The widespread and critically required expression of neuraminidases by a wide variety of sialic acid binding mucosal viruses underlines the importance of elimination of mucin carbohydrates for their pathogenicity [52]. LPS from *H. pylori* decreases mucin synthesis [28], and the mucin carbohydrate binding adhesins BabA and SabA undergo phase variation and change expression during infection [53-54], which may allow them to evade the mucin based host defense mechanism.

Another strategy commonly used by mucosal pathogens is to avoid the mucin barrier. Intestinal M cells, specifically designed to capture and present microbes to the underlying lymphoid tissue, can be regarded as a hole in the mucin barrier. The dome epithelium in which they lie does not produce gel forming-mucins, and their apical cell surface has only sparse microvilli and a thin glycocalyx [55-56]. Consequently, even though M cells constitute only a very small percentage of mucosal epithelial cells, they are the major point of attachment and/or entry used by a large number of mucosal pathogens including bacteria (eg. *S. typhimurium*, *S. flexneri, Y. enterocolitica* and *V. cholerae*), viruses (eg. reovirus, HIV-1 and polio virus) and parasites (eg. Cryptosporidia) [55, 57-58]. Another strategy used by pathogens to avoid the cell surface mucin barrier is to disrupt the tight junctions between adjacent mucosal epithelial cells thereby exposing the vulnerable lateral membranes not protected by the glycocalyx. Such examples include *S. flexneri* [59], enteropathogenic *E. coli* [60], *Porphyromonas gingivalis* [61] and *H. pylori* [62].

In summary, the mucosal surface is thus a complex and dynamic environment, which constantly changes in composition in response to both bacteria and host factors. Although much progress has been made in recent years in the understanding of this environment, much more research is needed to fully understand the complexity of host pathogen interactions at the mucosal surface. As antibiotic resistance becomes a growing health problem worldwide, the understanding of this environment will become a more important tool to battle resistant infections.

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