Cytokine responses during mucosal infections: role in disease pathogenesis and host defence
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Mucosal pathogens use diverse and highly specific molecular mechanisms to activate mucosal inflammation. It may even be argued that their virulence depends on the inflammatory response that they induce. Some bacteria target epithelial cells and trigger them to produce inflammatory mediators but others cross the mucosa and activate macrophages or dendritic cells. Although systemic release of inflammatory mediators causes many symptoms and signs of infection, local chemokine production leads to the recruitment of inflammatory cells and lymphocytes that participate directly in the clearance of bacteria from mucosal sites. In this way, mucosal inflammation is a two-edged sword responsible for disease associated tissue destruction and crucial for the antimicrobial defence.

Understanding of these pathways should create tools to enhance the defence and interfere with disease.

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Abbreviations
γIFN interferon-γ
IL interleukin
LPS lipopolysaccharide
TNF tumour necrosis factor

Introduction
It is not the presence of microbes in our tissues that makes us sick. Vast numbers of bacteria may colonise different sites in the body, and viruses may persist for long periods of time, while the individual remains perfectly healthy. It is the host response to the infecting agents that causes the disease symptoms and tissue damage.

The host response to a bacterial infection depends on the virulence of the pathogen. It is commonly accepted that the most virulent bacteria cause the most severe, acute symptoms and long-term effects, but we know relatively little about how specific bacterial components trigger acute disease manifestations in the host.

Inflammatory processes provide a direct link between the microbe and the host in disease pathogenesis [1]. Infectious diseases are often the first to be activated by bacteria or viruses, and explain many aspects of acute disease. Inflammatory mechanisms are also crucial for the host defence against invading pathogens. Although current dogma emphasises specific immunity, inflammatory effector functions can be at least as potent in the early defence of the mucosal barrier against pathogenic microbes.

This review describes the mucosal cytokine response as a model for mucosal inflammation, disease induction, and the antibacterial defence at mucosal sites.

Mechanisms used by pathogens to elicit mucosal cytokine responses
Mucosal surfaces continue where the skin ends and complete the boundaries between the environment and the tissues inside the body (for review see [2,3]). The different cell populations in the mucosal barrier are equipped to sense and respond to the molecular contents in the lumen and to translate this molecular information into signals that can reach local or distant sites within the body.

According to the conventional wisdom, inflammation is best handled by specialised cells like macrophages, neutrophils and mast cells. We now know that epithelial cells respond to external danger and produce an array of mediators that transmit signals across the mucosal barrier [1,4–6]. Mucosal inflammation can be triggered by a direct interaction of pathogens with epithelial cells or with specialised inflammatory cells after the bacteria have crossed the tight epithelium to the lamina propria.

Both virulent bacteria and members of the indigenous microflora contain molecules that can trigger inflammation, but only the pathogens elicit a strong host response. This is quite puzzling, but at least two factors may explain this difference. First, pathogens have a broader repertoire of host-activating molecules than the commensals, or express molecular variants with a better specificity for the host response pathways. Second, the pathogens present the host-activating molecules more efficiently to the responding cell. This concept is illustrated by the host response to lipopolysaccharide (LPS). All Gram-negative bacteria contain LPS, but some structural variants are more efficient host response activators than others, and only some strains are able to efficiently present LPS in a way that triggers LPS-dependent responses (see below).

Bacterial activation of epithelial cell cytokine responses
Three pathways for the bacterial activation of epithelial cell cytokine responses are shown in Figure 1.

Attachment and transmembrane signalling
Attachment to epithelial cells is mediated by specific interactions between microbial surface ligands and host cell receptors [7]. Signal transduction pathways may be activated...
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Through fimbriae–receptor interactions or through the release of molecules that target adjacent cell surface receptors [8]. Downstream signalling causes transcriptional activation and production of inflammatory mediators in the epithelial cell. Toxins or other active molecules are presented to host cells more efficiently by the attached microbes than by the free-floating microbes [9].

**Intracellular activation following invasion**

Invasion of the host cell by the bacteria or uptake of bacterial products into the cell may be required to induce a cytokine response [10]. *Shigella* and *Listeria* are classic examples of invasive bacteria that recruit cellular uptake mechanisms to enter the cells and to propagate themselves from cell to cell [11,12•,13,14]. Shiga toxin enters the cell [15] and activates the production of inflammatory mediators prior to the inactivation of protein synthesis that eventually kills the cells.

**Activation of nonepithelial cytokines**

Bacteria may cross the epithelial layer and be taken into ‘professional’ inflammatory cells such as mucosal macrophages. Release of cytokines by these infected, nonepithelial cells activates a secondary epithelial response or upregulates the response to infection [16,17]. Mucosal pathogens like *Salmonella* and *Shigella* do not primarily invade the epithelial cells but enter via the Payers patches and through the M cells, and infect macrophages and dendritic cells at this site.

**Attachment, transmembrane signalling and epithelial cell cytokine responses**

Using unpathogenic *Escherichia coli* as a model, bacterial adherence has been shown to enhance the cytokine responses of epithelial cells [5,18,19].

During *E. coli* infection, epithelial cell activation is fimbriae-mediated. *P*-fimbriated and type 1 fimbriated *E. coli* strains elicited higher cytokine responses *in vivo* and *in vitro* than isogenic, nonfimbriated strains [18,20,21]. Inhibition of epithelial cells glycolipid expression reduced the cytokine response to *P*-fimbriated *E. coli*, but had no effect on the cytokine response to type 1 fimbriated *E. coli*, which bind different glycoconjugate receptors [18]. Furthermore,
inhibition of E. coli adherence by receptor analogues inhibited epithelial cell cytokine responses [19,21].

More recently, the receptor specificity of the fimbriae has been shown to determine the transmembrane signalling pathway involved in epithelial cytokine responses [7]. P fimbriae recognise Galα(1-4)Gal-containing oligosaccharide sequences of varying chain length bound to ceramide in the outer leaflet of the lipid bilayer of mammalian cells. Ceramide has been shown to act as a second messenger in cell signalling and to activate downstream pathways involved in apoptosis and other cellular responses [22–24]. Agonists like TNFα, IL-1β and FAS bind to cell surfaces via their respective receptors and activate sphingomyelinas that cleave sphingomyelin to release ceramide [25–27]. We have recently shown that P fimbriated E. coli activate the ceramide signalling pathway, through the release of ceramide from the receptor glycolipids. Isogenic strains expressing type 1 had no effect on ceramide or glycosphingolipid integrity [8,28•].

Receptor fragmentation that occurs when the glycolipid is cleaved may represent a highly efficient strategy of host defence. First, the carbohydrate moiety of the receptor is released and makes the bacteria ‘lose their grip’ until novel receptors are expressed. The cleaved, soluble receptors may competitively inhibit further attachment of other P fimbriated bacteria to remaining cell-bound receptors and prevent experimental urinary tract infection in vivo [29]. Second, the release of the ceramide portion of the glycolipid receptor activates the receptor bearing cell to produce chemokines that recruit inflammatory cells to and clear bacteria from the local site (see below) [21,30,31].

LPS and epithelial cytokine responses

LPS is an integral component of the outer membrane of Gram-negative bacteria. Release of LPS in the circulation activates neutrophils, monocytes and macrophages to release pro-inflammatory cytokines such as tumour necrosis factor (TNF), interleukin (IL)-1β and IL-1α. The endotoxic activity of LPS resides in the lipid A portion of the molecule, and alterations in lipid A may change the biologic activity of LPS. For example, LPS from E. coli strains expressing non-myristoylated lipid A failed to activate monocytes and endothelial cells [32].

Cell activation by LPS involves transmembrane signalling. Binding of LPS to the serum protein LBP enhances cell activation through membrane bound CD14, but CD14 is glycosylphosphatidylinositol (GPI) anchored,
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LPS is a structural analogue of ceramide, and has been suggested to bypass ceramide as an activator of Ser/Thr specific signalling mechanisms in CD14 positive cells [34].

Does LPS activate mucosal cytokine responses? LPS and fimbriae have been shown to synergistically activate inflammation in the urinary tract of mice [19]. Furthermore, the poor mucosal cytokine responses of LPS hyporesponder mice to Gram-negative bacteria suggests that LPS contribute to the activation of an inflammatory response. On the other hand, mucosal surfaces are under constant bombardment by LPS from the resident flora and still do not appear to be in a state of constant inflammation. So, how is this regulated?

Recent studies have suggested that epithelial cells differ from macrophages and inflammatory cells, in that they do not express surface CD14, and are refractory to purified LPS regardless of the polysaccharide structure. Inactivation of LPS in virulent isolates by LPS inhibitors, such as BPI and PolymyxinB, or by mutational inactivation of Lipid A had no effect on epithelial cytokine responses to whole bacteria. These observations demonstrated that LPS is a poor epithelial cell activator, and that LPS is not required for epithelial cell activation by P-fimbriated E. coli (M Hedlund et al., unpublished data).

Sansonetti and colleagues [35] have examined a different aspect of LPS interactions with epithelial cells. LPS was shown to be transported through the epithelial cells to infected mucosal sites. Adapted from [59].

Figure 3

Schematic model of neutrophil migration to infected mucosal sites. Adapted from [59].
the basolateral side. In this way the epithelium could deliver LPS to cells in the subepithelial compartment such as mucosal macrophages that may respond to LPS by producing the characteristic inflammatory mediators. This would explain the mucosal in vivo effects of LPS in the midst of nonreactive epithelial cells.

**Mucosal cytokine responses to pathogenic microbes explain local and systemic disease pathology**

The cytokines that are released in the mucosal compartment have local and systemic effects that elicit symptoms and signs of acute disease. The systemic effects include changes in temperature, blood flow and blood pressure through the activation of specific host response cascades in tissues distant from the site of infection. The systemic mediators should be stable in the circulation, and avoid circulating inhibitors (receptor antagonists or antibodies) that prevent them from reaching distant tissue sites. Release of mediators from the site of infection explains how mucosal microbes may cause systemic symptoms in the absence of microbial invasion.

The local effects depend on the cytokine repertoire of the first cell that responds to the pathogen, and on the establishment of a cytokine network between local cells (Figure 2). Local inflammation causes changes in vascular permeability, recruitment of inflammatory cells, accumulation of toxic products, activation of local neurons and disruption of tissue function.

Here, we describe some of the local and systemic cytokine effects and the induction of symptoms in the host, using IL-6 and IL-8 as examples.

**IL-6 has local and systemic effects**

Bacterial infections stimulate a mucosal IL-6 response [4,5]. IL-6 concentrations in the lumen increase following mucosal infections in animal models and in patients. In addition, epithelial cell lines and human mucosal biopsies secrete IL-6 following contact with bacteria in vitro [6,36–38,39].

IL-6 is a multifunctional cytokine with immunoregulatory and proinflammatory effects. In the mucosal compartment, IL-6 is thought to enhance the maturation of mucosal B lymphocytes into IgA producing plasma cells, although results from IL-6-knockout mice are ambivalent [40]. The local overexpression of IL-6 using adenovirus vectors to construct tissue-specific IL-6 transgenes led to the development of local lymphoid hyperplasia, and an increase in CD3+ lymphocytes in the bronchoalveolar lumen [41]. In humans with mucosal infections, significant correlations were found between IL-6 and IgA concentrations in secretions [42].

The systemic effects of IL-6 include the induction of acute phase responses (C-reactive protein [CRP] synthesis), and fever (hypothalamic temperature regulation). IL-6 is stable in the circulation, and can act as a messenger from local to systemic sites [43]. The effects on the temperature regulatory centre are thought to involve the local production of IL-1 and TNF but these cytokines are not stable and cannot travel from the local site of infection. In patients, positive correlations have been found between fever, CRP and local or circulating IL-6 concentrations [37,44].

**IL-6 and neutrophil recruitment**

Chemokines are commonly assigned to two groups, depending on the position of the first two cysteines and disulphide bridges within the molecule [45,46]. The ELR motif containing C-X-C chemokines are involved in neutrophil recruitment and activation [45]. The C-C chemokines are mainly active in interactions with monocytes and macrophages [47].

Mucosal infections trigger a broad chemokine response. *E. coli* stimulates epithelial IL-8 secretion, IL-8 receptor expression and IL-8 dependent neutrophil migration in vitro [21,48]. Kagnoff et al. [49] showed that colonic epithelial cells express a broad range of chemokines, including IL-8, GRO-α, GRO-β, GRO-γ, ENA-78, MCP-1 and RANTES [49]. Infection of human uroepithelial cells with *E. Coli* triggers the production of IL-8, GRO-α, -β, -γ, ENA-78, IP10, Mig, MCP-1, RANTES, MIP-1α and β (G Godaly et al. unpublished data). The spectrum of chemokine responses provides a basis for differential recruitment of reactive cell populations to mucosal sites.

The mechanisms of neutrophil migration to mucosal sites is outlined in Figure 3. Similar results have been obtained by several groups using an in vitro model based on the Transwell model system pioneered by Madara et al. [50] and most aspects of the model have been confirmed in human patients or experimental animal models [21,48,51].

**Mucosal chemokine responses to bacteria are modified by immunoregulatory cytokines**

The mucosal cytokine response to different pathogens is regulated by the specific immune system at the site of infection. T cells and immunoregulatory cytokines influence epithelial cell functions, including the expression of class II antigens, secretory component and intracellular adhesion molecule-1 (ICAM-1) [52,53]. Immunoregulatory cytokines stimulate epithelial cell cytokine production and modify the epithelial cell cytokine responses to bacteria [54–56]. IL-4 and *E. coli* synergistically enhanced uroepithelial cell IL-6 and IL-8 responses, whereas the combination of IL-4 or interferon-γ (IFN) enhanced IL-6 production but did not affect the IL-8 production. These effects provide a molecular basis for immunoregulation of epithelial cell cytokine responses through IFN and IL-4 produced by T helper cell type 1 (TH1) or TH2 cells, respectively.

**Inflammation and resistance to mucosal infection**

The resistance of human hosts to microbial attack is astonishing. Health is maintained in the midst of a seemingly
3. Mucosal Vaccines

7. Leffler H, Svanborg-Edén C: Papers of particular interest, published within the annual period of review, have been highlighted as: outstanding interest of special interest

1. Hedges S, Agace W, Svanborg C: Molecular mechanisms, we can direct the inflammatory response is a double-edged sword; it explains acute disease severity, but also the clearance of infection. Dissecting the molecular mechanisms, we can direct the inflammatory pathways and optimise the benefits for the host.

Conclusions

Mucosal surfaces serve as arbitrators between the environmental microbes and host tissues. Virulent microbes start the disease process by activating host responses that disrupt mucosal integrity. Numerous virulence factors contribute, either by promoting delivery of microbial products or by direct activation of the cells. The inflammatory response is a double-edged sword; it explains acute disease severity, but also the clearance of infection. Dissecting the molecular mechanisms, we can direct the inflammatory pathways and optimise the benefits for the host.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


In this paper, the authors propose that activation of the ceramide pathway by P-fimbriated E. coli differs from other activators of the pathway (e.g. TNF-α,...
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and IL-1β). Phorbol ester E. coli activate the release of ceramide from receptors for glucolipid, whereas the other activators stimulate ceramide release from sphingomyelin.


