Exploring the immunomodulatory potential of microbial-associated molecular patterns derived from the enteric bacterial microbiota

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The human intestinal lumen represents one of the most densely populated microbial niches in the biological world and, as a result, the intestinal innate immune system exists in a constant state of stimulation. A key component in the innate defence system is the intestinal epithelial layer, which acts not only as a physical barrier, but also as an immune sensor. The expression of pattern recognition receptors, such as Toll-like receptors, in epithelial cells allows innate recognition of a wide range of highly conserved bacterial moieties, termed microbial-associated molecular patterns (MAMPs), from both pathogenic and non-pathogenic bacteria. To date, studies of epithelial immunity have largely concentrated on inflammatory pathogenic antigens; however, this review discusses the major types of MAMPs likely to be produced by the enteric bacterial microbiota and, using data from in vitro studies, animal model systems and clinical observations, speculates on their immunomodulatory potential.

The intestinal epithelium plays an active role in innate immunity, and pattern recognition receptors (PRRs) are utilized to detect the presence of bacteria and their associated antigens. PRRs are germline-encoded, sensory molecules which recognize a range of highly conserved bacterial motifs, termed ‘pathogen-associated molecular patterns’ (PAMPs) (Medzhitov, 2001). However, the ability of PRRs to recognize these bacterial moieties is not limited to just pathogens, and so the term ‘microbial-associated molecular patterns’ (MAMPs) may be more accurate (Medzhitov, 2001; Sanderson & Walker, 2007) and will be used throughout this review. Epithelium-associated, enteric immune cells, such as macrophages, dendritic cells, T-cells and B-cells, differentially express two major groups of PRRs, the cell surface Toll-like receptors (TLRs) and the intracellular nucleotide-binding oligomerization domain (NOD) receptors (Hornung et al., 2002; Iwasaki & Medzhitov, 2004; Akira et al., 2006). Non-professional immune cells of the intestinal epithelium, such as enterocyte cells, also constitutively express the two groups of PRRs (Furrie et al., 2005; Gribar et al., 2008), thus vastly enhancing the recognition of MAMPs.

TLRs are type I integral membrane glycoproteins found within the plasma and endosomal membranes of mammalian cells (Takeda & Akira, 2005). TLRs consist of three distinct domains (Botos et al., 2011); a MAMP-binding extracellular domain, which contains a variable number of leucine-rich repeats (Bell et al., 2003); a transmembrane domain, which spans the host cell membrane, thus holding the receptor in place; and a cytoplasmic signalling domain, the Toll/IL-1R homology domain, which is responsible for
the intracellular transmission of the stimulatory signal (Akira et al., 2006). TLRs recognize a wide range of microbial moieties (see Table 1) and engagement by their respective ligand(s) triggers activation of intracellular signalling cascades leading to the induction of genes involved in antimicrobial host defence, such as those encoding proinflammatory cytokines and chemokines (Aderem & Ulevitch, 2000).

NOD receptors are a group of cytoplasmic receptors which are important for the recognition of intracellular bacteria. NOD-1, the first NOD receptor identified, recognizes a derivative of peptidoglycan, \( \gamma-D\)-glutamyl-meso-diaminopimelic acid (iE-DAP), found exclusively in Gram-negative bacteria (Girardin et al., 2003a; Chamaillard et al., 2003). Subsequently, the structurally similar NOD-2 was identified and found to confer cell responsiveness to the minimal bioactive peptidoglycan motif, muramyl dipeptide, found in both Gram-positive and Gram-negative bacteria (Girardin et al., 2003b; Inohara et al., 2003). Ligand binding to NOD-1 or NOD-2 leads to receptor oligomerization, which induces the recruitment of the serine/threonine kinase Rip2/RICK (Takeda & Akira, 2005). NOD-receptor-bound Rip2/RICK subsequently activates the NF-\( \kappa \)B-mediated expression of proinflammatory cytokines (Akira et al., 2006; Masumoto et al., 2006).

**Enteric-derived MAMPs and their immunomodulatory potential**

As mentioned previously, MAMPs constitute highly conserved microbial motifs and the following sections review those factors which are likely to be produced by the intestinal microbiota. Additionally, the potential immunomodulatory role of each MAMP is discussed.

**CpG-DNA**

Bacterial DNA contains an approximately 20-fold greater frequency of unmethylated 2'-deoxyribo(cytidine-phosphate-guanine) (CpG) dinucleotides than vertebrate DNA (Ewaschuk et al., 2007), thus predisposing it to MAMP activity with mammalian host cells (Bauer et al., 2001). Methylated bacterial DNA loses its stimulatory potential (Ewaschuk et al., 2007), thus confirming that its MAMP activity is attributable the increased expression of unmethylated CpG motifs. Moreover, the stimulatory effects of bacterial DNA on mammalian immune cells can be mimicked by CpG-containing synthetic oligodeoxynucleotides (CpG-ODNs) (Dalpke et al., 2006).

Hemmi et al. (2000) demonstrated that TLR-9 confers responsiveness to bacterial DNA in host macrophages and B-cells, as their counterparts isolated from TLR-9-deficient mice were not susceptible to the physiological effects elicited by CpG-DNA. Human intestinal epithelial cell lines (HT29, Caco-2 and T84 cells) were subsequently shown to constitutively express TLR-9 mRNA, the upregulation of which was stimulated by pathogenic CpG-DNA (Akhtar et al., 2003). Furthermore, Akhtar et al. (2003) also showed an increased secretion of the proinflammatory IL-8 by intestinal epithelial cells in response to CpG-DNA. Nevertheless, it was subsequently suggested by Dalpke et al. (2006) that stimulation of TLR-9 would be difficult in vivo, thus limiting the physiological importance of TLR-9. However, their work was undertaken utilizing the macrophage model; therefore, only intracellular TLR-9 was considered. In stark contrast to this, Ewaschuk et al. (2007) described an upregulation of apical surface expression of TLR-9 protein in intestinal epithelial cells, in response to pathogenic *Salmonella enterica* DNA, thus suggesting sensitization of the epithelial cells to further challenge by CpG-DNA. Intestinal epithelial cells constitutively express

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**Table 1. Human TLRs and their known MAMPs**

<table>
<thead>
<tr>
<th>TLR</th>
<th>Location</th>
<th>Ligand</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>TLR-2</td>
<td>Plasma membrane</td>
<td>Peptidoglycan</td>
<td>Bacteria</td>
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<td></td>
<td></td>
<td>Phospholipomannan</td>
<td>Fungi</td>
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<td></td>
<td>Haemagglutinin</td>
<td>Measles virus</td>
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<tr>
<td>TLR-2/TLR-1</td>
<td>Plasma membrane</td>
<td>Lipoprotein</td>
<td>Bacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Triacyl lipopeptides</td>
<td>Gram-negative bacteria</td>
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<tr>
<td>TLR-2/TLR-6</td>
<td>Plasma membrane</td>
<td>Zymosan</td>
<td>Fungi</td>
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<tr>
<td></td>
<td></td>
<td>Diacyl lipopeptides</td>
<td>Mycobacteria</td>
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<tr>
<td></td>
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<td>Lipoteichoic acid</td>
<td>Gram-positive bacteria</td>
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<tr>
<td>TLR-3</td>
<td>Endosomal membrane</td>
<td>dsRNA</td>
<td>Viruses</td>
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<tr>
<td>TLR-4</td>
<td>Plasma membrane</td>
<td>Lipopolysaccharide</td>
<td>Gram-negative bacteria</td>
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<td></td>
<td></td>
<td>Mannan</td>
<td>Fungi</td>
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<tr>
<td>TLR-5</td>
<td>Plasma membrane</td>
<td>Flagellin</td>
<td>Bacteria</td>
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<td>TLR-7</td>
<td>Endosomal membrane</td>
<td>ssRNA</td>
<td>Viruses</td>
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<tr>
<td>TLR-8</td>
<td>Endosomal membrane</td>
<td>ssRNA</td>
<td>Viruses</td>
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<tr>
<td>TLR-9</td>
<td>Plasma/endosomal membrane</td>
<td>CpG-DNA</td>
<td>Bacteria</td>
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<td>Viruses</td>
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<td>Protozoa</td>
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<tr>
<td>TLR-10</td>
<td>Endosomal membrane</td>
<td>Unknown</td>
<td>Unknown</td>
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</tbody>
</table>
Peptidoglycan

Peptidoglycan (PGN) is an essential cell wall component in virtually all bacteria and is particularly abundant in Gram-positives, where it accounts for 30–70% of their cell wall mass (Schleifer & Kandler, 1972). It is a mesh-like polymer consisting of β(1–4)-linked N-acetylgalactosamine and N-acetylmuramic acid, cross-linked by short peptides and is responsible for the maintenance of cell morphology and the resistance to osmotic pressure of bacterial cells (Dziarski, 2003). As a consequence of its presence in virtually all bacteria, substantial abundance in Gram-positive bacteria and absence from eukaryotic cells, PGN presents a perfect target for the host innate immune system (Dziarski, 2003). PGN is only released in relatively low amounts during mitotic division; however, it demonstrates potent immunological activity in mouse and human macrophages, subsequently stimulating the significant release of proinflammatory cytokines (Schwandner et al., 1999; Takeuchi et al., 1999; Wang et al., 2001). Gram-positive pathogens demonstrate significantly increased release of PGN during infection (Dziarski & Gupta, 2005).

Initially, it was commonly accepted that TLR-2 mediated cellular sensitivity to PGN in human macrophages (Schwandner et al., 1999; Takeuchi et al., 1999; Wang et al., 2001), and that responsiveness was enhanced by the co-receptor CD14 (Schwandner et al., 1999; Iwaki et al., 2002); however, this proposed stimulatory pathway was subsequently challenged by Travassos et al., who claimed TLR-4, not TLR-2, conferred cellular responsiveness to purified PGN (Travassos et al., 2004). Nevertheless, a re-evaluation of the phenomenon by Dziarski & Gupta (2005) conclusively demonstrated that TLR-2 was essential for the stimulation of macrophages by PGN, and suggested the results observed by Travassos and colleagues were due to the destructive and incomplete nature of the purification methods they used. Constitutive expression of TLR-2 mRNA has previously been observed in both ex vivo colonic epithelial tissue and in vitro colonic epithelial cell lines (HT29, Caco-2 and T84 cells) (Melmed et al., 2003; Furrie et al., 2005), thus suggesting the potential for intestinal immune modulation by microbiota-derived PGN. In addition, mutations in the NOD-2 gene, the product of which confers host cell responsiveness to the PGN derivative muramyl dipeptide, is strongly associated with the pathogenesis of Crohn’s disease (Hugot et al., 2001; Ogura et al., 2001), thus further strengthening the notion that PGN could play a role in intestinal homeostasis. More recently, a study by Macho Fernandez et al. (2011) indicated that PGN and its derived muropeptides are active in the probiotic functionality of Lactobacillus salivarius Ls33 and, therefore, might represent a useful therapeutic strategy in the treatment of inflammatory bowel disease.

Lipopolysaccharide

LPS is an amphiphilic membrane phospholipid (Fenton & Golenbock, 1998), which is essential for cell viability and outer-membrane permeability of Gram-negative bacteria (Rietschel et al., 1994). It also plays a key role in protection of the bacterium against host immune defences, enzymic degradation and antibiotic attack (Holst et al., 1996). Since only the Sphingomonas genus is found to lack LPS (Alexander & Rietschel, 2001), its ubiquitous expression in other Gram-negative bacteria presents the mammalian innate immune system with a major target (Erridge et al., 2002).

LPS is a glycolipid macromolecule consisting of three domains: the distal hydrophobic O-specific chains, or O-antigens, which extend from the bacterial surface; the interconnecting core region; and the hydrophobic lipid A region which acts as the membrane anchor (Bishop, 2005). O-antigens present a major target for the host’s antibody response of the adaptive immune system as they represent the extreme outer limits of the bacterial cell (Erridge et al., 2002). Nevertheless, it is the glycolipid membrane anchor, lipid A, which represents the biologically active moiety of LPS, with both free and synthetic lipid A molecules shown to reproduce the effects of whole LPS (Galanos et al., 1985).

In a healthy individual, the basal systemic concentration of LPS in the human body can be in the range 3–10 pg ml⁻¹ (Alexander & Rietschel, 2001). Accordingly, the innate immune system can detect and, indeed, degrade such low concentrations of LPS in a phenomenon known as ‘LPS tolerance’ (Hoffman & Natanson, 1993; Ulevitch & Tobias, 1999), which has been shown to aid in defence against subsequent bacterial invasion by the parent strain (Hoffman & Natanson, 1993). However, larger quantities of LPS, often released by cell lysis during infection (Caroff & Karibian, 1999), can have a highly detrimental effect on the host, resulting in fever, increased heart rate, septic shock and, ultimately, death from multiple organ failure and systemic inflammatory response (Hoffman & Natanson, 1993; Caroff & Karibian, 2003). It is noteworthy that LPSs do not elicit their toxic effect by the killing of host cells, or even by the inhibition of host cellular function, but they are wholly dependent on the active inflammatory responses of the host (Rietschel et al., 1994).

The first stage in host recognition of LPS is the binding of the acute phase reactant, LPS-binding protein (LBP)
Lipoprotein

Lipoproteins (LPs) are proteins which contain lipid moieties covalently bound to an N-terminal cysteine residue (Braun & Wu, 1994). They represent a key component in the outer membrane of Gram-negative bacteria, particularly in members of the Enterobacteriaceae, such as Escherichia coli, which naturally secrete them at low levels into the surrounding media (Zhang et al., 1998). LPs are also present, albeit in much more limited quantities, in the cell wall of Gram-positives (Sutcliffe & Russell, 1995).

Brightbill et al. (1999) elucidated that host cellular responsiveness to bacterial lipoproteins in human macrophages is mediated via TLR-2. This was later confirmed by Wang et al. (2002) who demonstrated that pre-treatment of human monocytes with low concentrations of LP imparts a TLR-2 ‘tolerance’ that protects against subsequent treatment with higher concentrations of LPs. However, it was later discovered that TLR-2 actually forms a heterodimer with TLR-1 to confer cell responsiveness to bacterial LPs in murine macrophages (Takeuchi et al., 2002). Spirochaetal LPs from Treponema pallidum and Borrelia burgdorferi have been implicated in the pathogenesis of syphilis and Lyme disease, respectively (Sellati et al., 1998). Additionally, LPs elicit proinflammatory cytokine release in a range of human systems, such as whole blood (Karched et al., 2008), macrophages (Zhang et al., 1998) and neutrophils (Soler-Rodriguez et al., 2000); however, the immunomodulatory potential of either pathogen- or commensal-derived LPs with intestinal epithelial cells has not (to the authors’ knowledge) yet been explored. This could be an area of particular interest in future studies, given that E. coli are among the first bacteria to colonize the neonatal intestine (Hooper, 2004); therefore, the elevated presence of LPs in these bacteria could potentially have significant effects in the development of intestinal immunity.

Lipoteichoic acid

Lipoteichoic acid (LTA) is a membrane-associated, amphiphilic polymer, which extends from the cytoplasmic membrane, through the cell wall, to the outer surface of Gram-positive bacteria (Buckley et al., 2006). LTA is thought to aid in bacterial attachment to host cells (Granato et al., 1999), and is also immunologically active, having previously been demonstrated to elicit proinflammatory cytokine secretion from macrophage cells (Standiford et al., 1994). In contrast to this, LTAs from strains of potentially probiotic lactobacilli were unable to stimulate a proinflammatory response in the HT29 intestinal epithelial cell line, but actively inhibited E. coli- and LPS-induced IL-8 release in these cells (Vidal et al., 2002). Additionally, oral ingestion of LTA (isolated from Staphylococcus aureus), prior to induction of experimental colitis via dextran sulfate sodium, conferred protection in mice with colons depleted of commensal microbiota, subsequently reducing mortality, morbidity and severe colonic bleeding (Rakoff-Nahoum, 2015).
et al., 2004). From these contrasting studies we are unable to speculate what function LTA potentially plays in intestinal homeostasis, therefore it is evident that more research is required in this field. Also, there is some debate as to which of the TLRs confers host cell responsiveness to LTA. Schwandner et al. (1999) demonstrated that human embryonic kidney cells were activated via TLR-2; however, Takeuchi and colleagues disputed this, as their results showed that TLR-2-deficient mice were still responsive to LTA, whereas TLR-4-deficient mice were not (Takeuchi et al., 1999), thus suggesting TLR-4 confers responsiveness.

Flagellin
Flagellin is the highly antigenic, monomeric subunit of bacterial flagella (Ramos et al., 2004). Flagella are rotary motor-like structures, which are expressed by the majority of motile bacteria in the intestine (Berg, 2003). Hayashi et al. (2001) determined that bacterial flagella possess TLR-5 stimulatory ability, and it was confirmed shortly afterwards that TLR-5 exclusively confers cellular responsiveness to extracellular flagellin (Gewirtz et al., 2001). Monomeric flagellin is naturally released by bacteria, either by leakage due to uncapping or by active depolymerization; however, it can also be sheared from the bacterial surface by host proteases or detergents present in the intestine (Ramos et al., 2004). Flagellin unquestionably plays an important and highly complex role in intestinal homeostasis, as it has been implicated as a major antigen in Crohn’s disease (Lodes et al., 2004; Targan et al., 2005) and, paradoxically, as a protective moiety against spontaneous colitis (Vijay-Kumar et al., 2007). Steiner et al. (2000) first showed that flagellin has the potential to stimulate an immune response from intestinal epithelial cell lines. However, it was subsequently demonstrated that in vivo flagellin must first be translocated from the mucosal to the serosal domain of the epithelial layer (Gewirtz et al., 2001), despite intestinal epithelial cell lines exhibiting both basolateral and apical TLR-5 expression (Cario & Podolsky, 2000). A significant level of translocation is normally considered a trait of pathogenic bacteria (Ljungdahl et al., 2000); therefore it can be hypothesized that the intestinal epithelium is able to distinguish between commensal and pathogenic flagellins simply by the physical exclusion of commensal bacteria. Epithelial responses to commercially available flagellin (isolated from the enteric pathogen Salmonella typhimurium) have been well characterized with HT29 and Caco-2 intestinal epithelial cell lines, as both were shown to secrete significantly increased levels of IL-8 in the presence of flagellin (Bannon, 2008). However, to date, the epithelial responses to non-pathogenic flagellins have little been considered.

Membrane vesicles (MVs)
MVs are small (50–250 nm diameter), spherical, bilayered membranous structures (Beveridge, 1999) produced by Gram-negative bacteria. MVs are not MAMPs in their own right, but rather represent a collection of MAMPs, as their composition, conformation and surface chemistry are small-scale reproductions of the intact outer membrane of Gram-negative bacteria (Beveridge, 1999; Schooling & Beveridge, 2006). LPSs, outer-membrane proteins, phospholipids and periplasmic proteins are all present in MVs (Beveridge, 1999; Kesty & Kuehn, 2004), and proteins such as transmembrane porins, murein hydrolases, transporter proteins, flagellin and other virulence factors have all been identified in MVs by proteomic studies (Lee et al., 2008). It is starting to become apparent that these small membranous structures have the potential to deliver bacterial products to eukaryotic cells (Kaparakis et al., 2010).

A number of roles and functions have been suggested for MVs, including periplasmic equilibrium maintenance (McBroom & Kuehn, 2007), antibiotic protection (Ciofu et al., 2000; Manning & Kuehn, 2011), quorum sensing (Mashburn-Warren & Whiteley, 2006), biofilm maintenance (Schooling & Beveridge, 2006) and gene transfer (Dorward et al., 1989; Yaron et al., 2000; Renelli et al., 2004). However, a direct role in the virulence of Gram-negative bacteria is the most strongly supported. Kadurugamuwa & Beveridge (1995) first suggested the virulent nature of MVs due to the enrichment of antigenic LPS molecules and the inclusion of host tissue-destructive enzymes in MVs isolated from the respiratory pathogen Pseudomonas aeruginosa. Enterotoxigenic E. coli MVs preferentially package heat-labile toxin in their luminal space, protecting it from extracellular enzymic activity and deliver it directly to the cytoplasm of target cells (Kesty et al., 2004). A similar system was also seen in Helicobacter pylori, with its MVs encapsulating and transporting its major virulence factor, H. pylori vacuolating toxin (Parker et al., 2010). The immunomodulatory potential of MVs was recognized when MVs isolated from H. pylori were shown to elicit IL-8 release in human gastric epithelial cells (Ismail et al., 2003). More recently, MVs isolated from H. pylori have been shown to elicit IL-8 responses in human gastric epithelial cell lines through the delivery of peptidoglycan to the intracellular PAMP receptor NOD1 (Kaparakis et al., 2010). Additionally, MVs from P. aeruginosa have been shown to be potent activators of the proinflammatory response, stimulating the secretion of IL-8 in human lung epithelial cells (Bauman & Kuehn, 2006) and MIP-2 and IL-6 from murine macrophages (Ellis et al., 2010). Nevertheless, the interaction of MVs with intestinal epithelial cells have, surprisingly, been little studied, with only a recent investigation demonstrating that MVs isolated from the enteropathogen Vibrio cholerae elicit IL-8 from Int407 intestinal epithelial cells, via a NOD-1-mediated pathway (Chatterjee & Chaudhuri, 2013). In addition, despite the large population of Gram-negative bacteria present in the intestinal lumen, the immunological role of MVs produced by non-pathogenic enteric bacteria is yet to be elucidated. However, a study by Shen et al. (2012) has recently suggested that capsular polysaccharide-containing MVs, isolated from Bacteroides
fragilis, can protect against inflammation in the 2,4,6-
trinitrobenzenesulfonic acid experimental model of colitis in mice. In contrast, we have observed, in vitro, that MVs
isolated from the commensal bacterium E. coli strain C25
stimulate a concentration-dependent increase in the
secretion of IL-8 from the intestinal epithelial cell lines
HT29 and Caco-2 (unpublished results).

Exopolysaccharides

Although not typically recognized as MAMPs, there is
growing evidence that exopolysaccharides (EPSs), which
are long-chain polysaccharides released into the surround-
ing media during bacterial growth, have an immunomod-
ulatory function. EPS-producing bacteria are increasingly
used in the food industry and, indeed, naturally reside
within the intestine (Badel et al., 2011). EPSs form a highly
viscous local environment (Roller & Dea, 1992), thus
enhancing bacterial nutrient- and water-entrapping abil-
ities (Poulsen, 1999). EPSs have also been suggested to play
a major role in bacterial attachment (Watnick & Kolter,
1999) and are thought to play a key role in bacterial
protection against bacteriophages, antibiotics, lysozyme
and metal ions (Looijesteijn et al., 2001; Durlu-Ozkaya
et al., 2007).

EPSs are separated into two categories; homosaccharides
and heterosaccharides (Laws et al., 2001). Homosaccharides,
such as cellulose, dextran and levan, are made up of only one
type of monosaccharide (Laws et al., 2001), whereas
heterosaccharides consist of multiple repeats of oligosac-
charides, which themselves are composed of 3–7 sugar
residues (Laws et al., 2001). These oligosaccharide pre-
cursors typically contain D-glucose, D-galactose and L-
rhamnose sugars (De Vuyst & Degeest, 1999) and
occasionally include amino-sugars, such as N-acetyl-D-
glucosamine and N-acetyl-D-galactosamine (Badel et al.,
2011). Heterosaccharides are mainly produced by mesophi-
llic and thermophilic bacteria, such as lactic acid bacteria
(Cerning, 1990; De Vuyst & Degeest, 1999) and bifidobac-
teria (Ruas-Madiedo et al., 2006, 2010).

Kefiran, an EPS produced by a number of strains of
lactobacilli in the fermented milk drink kefir, has been
shown to possess a number of systemic physiological
activities; these include wound-healing properties, reduction
of blood pressure and cholesterol levels, and the retardation
of tumour growth in experimental models (Vinderola et al.,
2006). Kefiran also exhibits a potential role in intestinal
homeostasis, with an increase in luminal IgA and of both
pro- and anti-inflammatory cytokines, such as IFN-γ, TNF-
α, IL-6 and IL-10, observed in the small and large intestine
(Vinderola et al., 2006). In concordance with these
homeostatic effects, a study by Sengul et al. (2006)
demonstrated that EPS-producing bacteria were able to
significantly attenuate the inflammation of an experimental
colitis model, induced via intracolonic administration
of acetic acid, in rats. Additionally, this is supported further
by evidence at the cellular level, as murine macrophages
challenged with various EPSs (isolated from strains of
lactobacilli and bifidobacteria) demonstrate augmented
release of both pro- and anti-inflammatory cytokines, such
as TNF-α, IL-6 and IL-10 (Chabot et al., 2001; Bleau et al.,
2010; Wu et al., 2010). The mitogenic activity of EPSs
isolated from strains of lactobacilli and bifidobacteria is also
well characterized, with studies showing the promotion of
human, murine, porcine and bovine macrophage prolifera-
tion (Kitazawa et al., 1998; Chabot et al., 2001; Wu et al.,
2010).

With a large number of EPS-producing bacteria naturally
residing in the intestine, it is surprising that very little
research has been undertaken into the interaction of EPSs
with the intestinal epithelial layer itself. Previous studies
have investigated the potential of EPSs as antiproliferative
or anticytokic agents with intestinal epithelial cells
(Ruas-Madiedo et al., 2010; Liu et al., 2011), but, the
immunomodulatory effects of EPSs on these cells has
largely been neglected in the literature. However, Lebeer
et al. (2012), as part of a much larger investigation, have
reported that EPSs isolated from the known probiotic
organism Lactobacillus rhamnosus GG had no significant
effect on IL-8 mRNA expression in Caco-2 cells.
Additionally, a recent review article presented preliminary
data in which co-culture with EPS-producing strains of
bifidobacteria differentially modulated the secretion of
inflammatory cytokines, including IL-8 and IL-6, in the
Caco-2 intestinal epithelial cell line (Hidalgo-Cantabrana
et al., 2012).

The evidence presented above confirms that EPSs directly
associate with host cells in the intestine; however, the
molecular mechanisms by which they interact are not fully
understood. Chabot et al. (2001) suggested EPSs could
exert their action via the mannose receptor and a more
recent study by Ciszek-Lenda et al. (2011) demonstrated a
cross-tolerance between LPS and EPSs in macrophages,
thus indicating the possible involvement of a TLR-4-
mediated pathway. Nevertheless, another study, under-
taken by Lin et al. (2011), on a novel EPS (TA-1) isolated
from the thermophilic marine bacterium Thermus aqua-
ticus, provides the strongest candidate for an EPS receptor.
TA-1 was shown to stimulate the release of proinflamma-	ory cytokines TNF-α and IL-6 from murine macrophages
via a TLR-2-mediated pathway (Lin et al., 2011). This
is consistent with the fact that TLR-2 is a well-characterized
receptor for a range of microbial components (Takeda
et al., 2003; Akira et al., 2006).

Conclusions

Like their pathogenic counterparts, commensal bacteria are
able to stimulate an immune response from the intestinal
epithelial layer; however, the mechanisms of this low-level
inflammatory reaction are, as yet, largely unknown, but are
likely to involve PRR, such as TLRs. A number of possible
contributory factors, and their receptor(s), have been
discussed in this review. However, it is apparent from the
small number of studies undertaken thus far, which consider the MAMPs of the enteric bacterial flora, that much more research is needed in this field, in order to further uncover the complex relationship between the commensal microbiota and the intestinal innate immunity.

Acknowledgment

We thank the University of Huddersfield for providing a studentship to D.A.P.

References


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Edited by: S. Spiro