Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens

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Abstract | How can probiotic bacteria transduce their health benefits to the host? Bacterial cell surface macromolecules are key factors in this beneficial microorganism–host crosstalk, as they can interact with host pattern recognition receptors (PRRs) of the gastrointestinal mucosa. In this Review, we highlight the documented signalling interactions of the surface molecules of probiotic bacteria (such as long surface appendages, polysaccharides and lipoteichoic acids) with PRRs. Research on host–probiotic interactions can benefit from well-documented host–microorganism studies that span the spectrum from pathogenicity to mutualism. Distinctions and parallels are therefore drawn with the interactions of similar molecules that are presented by gastrointestinal commensals and pathogens.

Mutualism
An interaction between two species such that both partners benefit in some way. For example, the gut microbiota receives nutrients from the host and provide the host with additional genetic and metabolic attributes, including the ability to harness nutrients that are otherwise inaccessible.

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The constituents of the human gastrointestinal microbiota exist in a continuum that ranges between mutualism and pathogenicity, fostered by residential and ingested microorganisms[1]. The microbiota is essential to human health, as it contributes to food digestion and the development and optimal functioning of the immune system, for example[2]. Interest in the beneficial functions of the human microbiota has resulted in the selection of specific species with putative health-promoting capacities for the treatment of conditions in which the microbiota — or its optimal functioning — is disturbed. These microorganisms, recognized as probiotics[3], are generally selected from *Lactobacillus* or *Bifidobacterium* species[4]. Clinical applications of probiotics include the prevention and treatment of gastrointestinal infections, inflammatory bowel diseases (IBD) and allergic diseases and use as adjuvants in vaccination[5].

The modes of action by which probiotics are thought to contribute to human health fall into three main categories[6]. First, certain probiotics can exclude or inhibit pathogens. This is currently the best studied probiotic mechanism and has been exhaustively reviewed elsewhere[6,7]. A second mechanism is to enhance the function of the intestinal epithelial barrier by modulating the various signalling pathways that lead to, for example, the induction of mucus[8] and defensin production[9,10], enhancement of tight junction functioning[11] and prevention of apoptosis[12]. The third method is to modulate host immune responses, resulting in both local and systemic effects[13]. Although there is substantial evidence from *in vitro* and animal studies for each of these categories of probiotic action, the results from clinical studies are far less convincing[14]. A better understanding of how probiotic bacteria interact with host cells is needed for their optimized application.

Cell wall molecules are key probiotic ligands that can interact with host receptors and induce signalling pathways, resulting in probiotic effects. Most probiotics studied today belong to the Gram-positive lactic-acid bacteria, in which the cell wall is typically composed of a thick peptidoglycan layer decorated with proteins, teichoic acids and polysaccharides[15] (FIG. 1a). However, some Gram-negative probiotics do exist, such as *Escherichia coli* strain Nissle 1917. Their cell wall is composed of a thin peptidoglycan layer, a periplasmic space and an outer membrane, which contains lipopolysaccharide (LPS)[16] that is itself further decorated with proteins and polysaccharides (FIG. 1b). The main cell wall macromolecules have a similar basic architecture between species, but various modifications, such as glycosylation (FIG. 1c), can contribute to the strain-specific properties of probiotics. In this Review, we discuss the known interactions between the probiotic cell surface macromolecules and host receptors. Metabolites of probiotics can also interact with various host receptors and induce signalling pathways[17], but these will not be discussed in depth. Throughout, we delineate the subtle differences that exist between probiotic and well-documented gastrointestinal commensal and

Adjuvant
Any substance that acts to accelerate, prolong or enhance antigen-specific immune responses when used in combination with specific vaccine antigens.

pathogenic microorganisms, with respect to the surface molecules that are present and the interactions that they mediate. Finally, we address the dynamics of the bacterial cell surface impacting on probiotic–host interactions.

Host receptors for probiotic molecules

PRRs and MAMPs. The host cells that have the most interaction with probiotics are intestinal epithelial cells (IECs), provided the bacteria gain access through the mucus layer (FIG. 2). In addition to IECs, probiotics can encounter intestinal dendritic cells (DCs) that are crucial players in innate and adaptive immunity. IECs and DCs interact with and respond to gut microorganisms by means of their pattern recognition receptors (PRRs). PRRs detect microorganism-associated molecular patterns (MAMPs), which are widespread and conserved

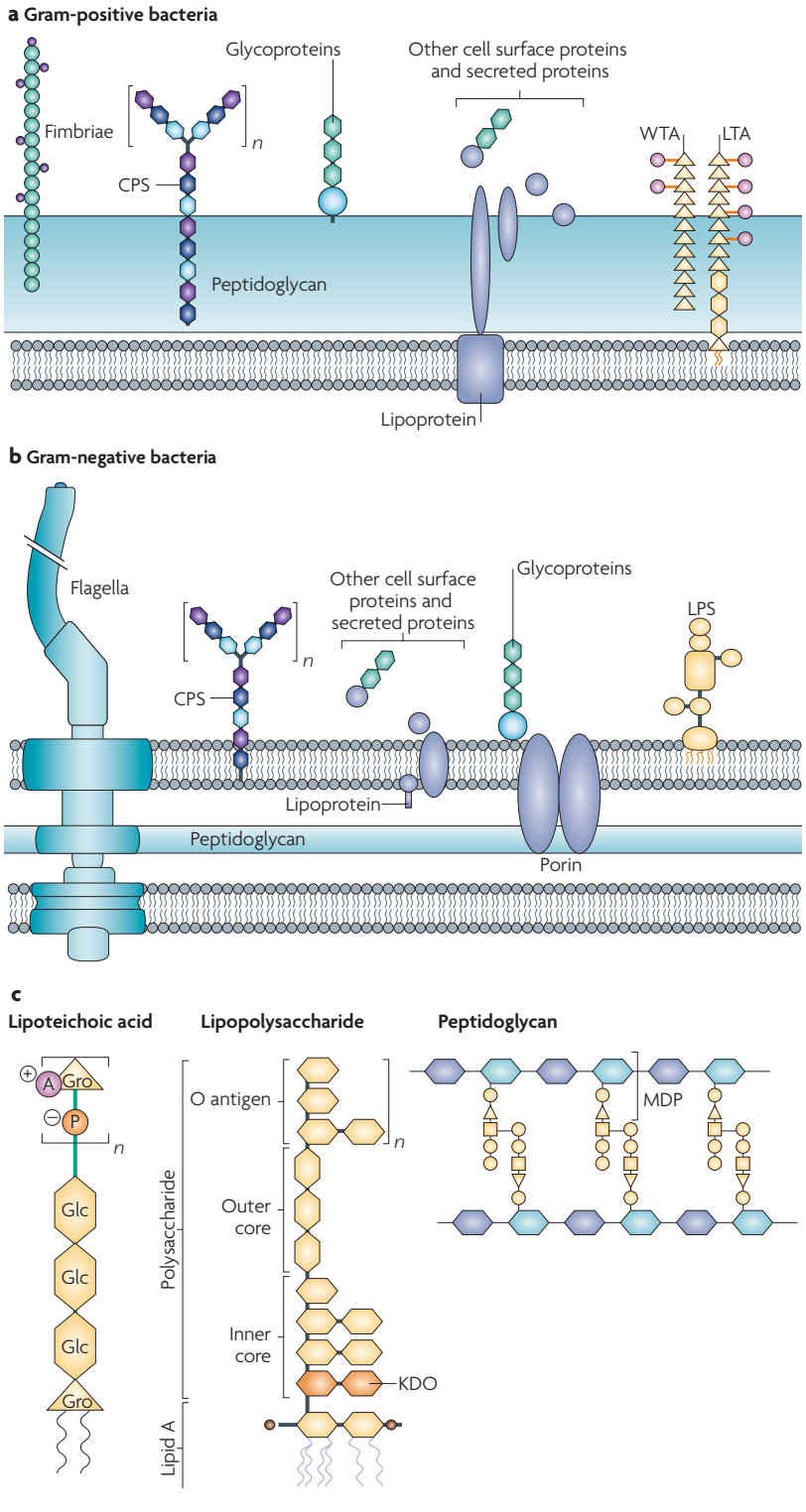

Figure 1 | **The probiotic Gram-positive and Gram-negative surface macromolecules and glycobiome. a** | Gram-positive bacteria. Peptidoglycan (PG) is the largest component of the Gram-positive cell wall in terms of dimension and molecular weight, although the sugar residues are usually not surface exposed[15]. Teichoic acids are the second major component of the cell walls of Gram-positive bacteria. These anionic polymers are generally made of repeating units of polyglycerol phosphate (Glc) or polyribitol phosphate covalently anchored to PG (wall teichoic acids; WTAs) or the cytoplasmic membrane (lipoteichoic acids; LTAs)[113]. Both WTAs and LTAs are often substituted with glycosyl residues or D-alanyl esters. In contrast to PG, LTA and WTA, the various cell wall-associated polysaccharide (CPS) molecules of Gram-positive probiotics do not have a common core structure. CPS molecules are generally long heteropolysaccharides of repeating subunits with different sugar moieties but can also be homopolysaccharides[114]. Glycan chains can also occur on cell wall-associated proteins, but the occurrence of these glycoproteins is not yet well documented[115]. Some proteins are secreted, such as the lectin-like moonlighting proteins that bind to sugar residues on host surfaces[116]. Some probiotic bacteria have long surface appendages composed of different subunits, termed fimbriae or pili[42] **b** | For Gram-negative probiotics, the main components of the cell wall macromolecules include lipopolysaccharides (LPS), CPS and various proteins, some of which are glycosylated. As in Gram-positive bacteria, the CPS molecules can be homo- or heteropolysaccharides with a highly variable structure. The best studied glycoproteins of Gram-negative bacteria are flagellins[94] and fimbrial structural proteins[115,117]. **c** | The structures of some of the cell surface molecules of bacteria. LTA is composed of a glycolipid membrane anchor and a long polyglycerol or polyribitol phosphate chain with substituents. All LPS molecules are composed of a hydrophobic lipid A molecule and a hydrophilic polysaccharide moiety consisting of a core region and a variable O antigen polysaccharide of repeating oligosaccharide units[16]. As the outermost part of the LPS, the O antigen is the major antigen, whereas lipid A interacts with PRRs. PG has a similar basic structure in all bacteria, being composed of glycan chains of repeating β-1,4-linked *N*-acetylglucosamine and *N*-acetylmuramic acid residues that are extensively crosslinked by two pentapeptide side chains linked to *N*-acetylmuramic acid. In lactobacilli, the consensus sequence is L-Ala/D-Glu/(L-Lys or meso-diaminopimelic acid)/D-Ala/D-Ala. Additionally, D-Asn is often used as a cross-bridge between D-Ala and L-Lys, and this residue can also be amidated[15]. Although most variations in PG occur at the stem peptides, *N*-acetylglucosamine and *N*-acetylmuramic acid can undergo different modifications, such as *O*-acetylation[15]. A, D-alanine ester or glycosyl substitutions; Gro, glycerol; KDO, 3-deoxy-D-manno-2-octulosonic acid; MDP, muramyl dipeptide; P, phosphate.

among microorganisms, often being located on bacterial surface molecules, and are not expressed by the host. PRRs have a broad specificity, so that a limited number of PRRs can detect a range of MAMPs, which results in a rapid response against any encountered microorganisms, including potential pathogens[18].

The best studied PRRs are Toll-like receptors (TLRs), which are transmembrane proteins present at the cell surface or on the membrane of endocytic vesicles or other intracellular organelles[18]. The extracellular domain of TLRs is characterized by leucine-rich repeats (LRRs) that are involved in ligand binding. Ligand recognition induces homodimerization or heterodimerization of the ectodomains, allowing the intracellular domains to initiate signalling. The cytoplasmic domain of TLRs contains the highly conserved Toll/interleukin-1 (IL-1)receptor (TIR) domain, which interacts with various adaptor molecules such as myeloid differentiation primary response protein (MyD88) to initiate signalling[19]. In this Review on surface molecules, we focus on the TLRs for which interactions with bacterial surface molecules have been documented: TLR2, which binds as a heterodimer with TLR1 or TLR6 (depending on the bacterial ligand), TLR4 and TLR5, all of which can be expressed by IECs and DCs, although at varying levels[19]. In addition, extracellular C type lectin receptors (CLRs)[20] and intracellular nucleotide-binding oligomerization domain-containing protein (NOD)-like receptors (NLRs)[21] are known to transmit signals on interaction with bacteria. Moreover, it is important to consider that surface-located PRRs do not function in isolation but often cooperate with co-receptors in multi-receptor clusters that are located in membrane lipid rafts[22].

Usually, the interaction between a MAMP and a PRR results in the induction of signalling cascades that mount a molecular response against the detected microorganisms; this response can include the production of immunomodulatory cytokines, chemokines, antimicrobial or

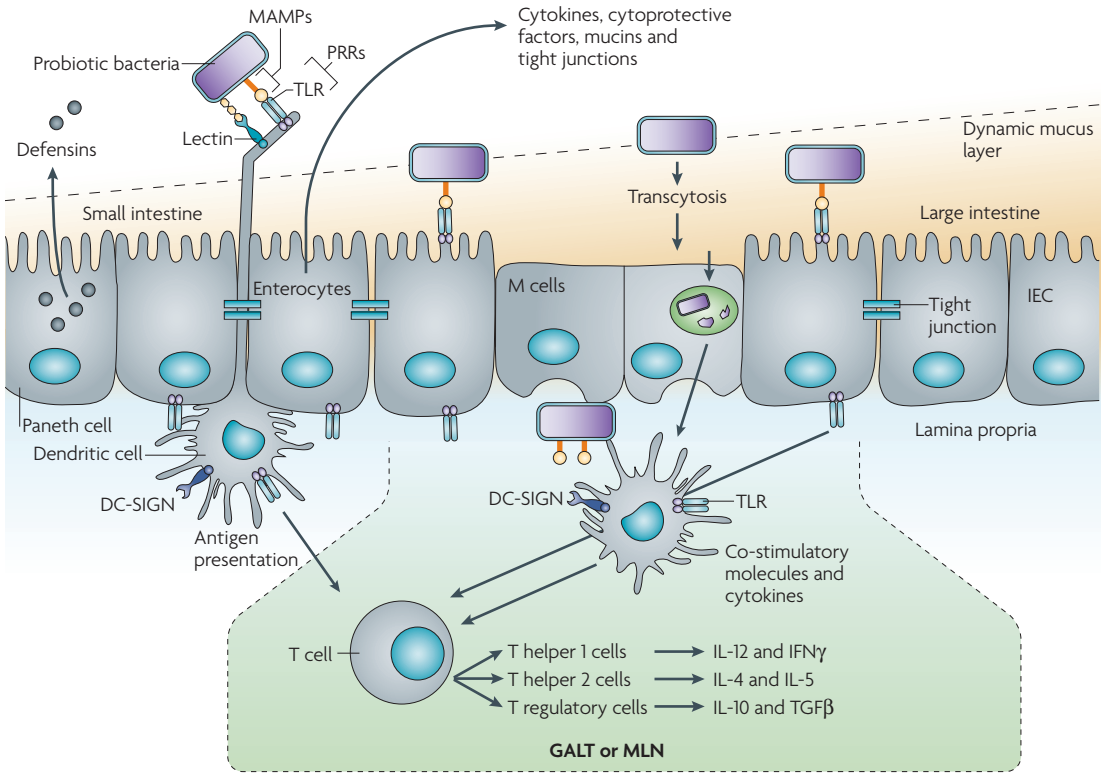

Figure 2 | **Interaction of probiotic bacteria with IECs and DCs from the GALT.** A fraction of ingested probiotics are able to interact with intestinal epithelial cells (IECs) and dendritic cells (DCs), depending on the presence of a dynamic mucus layer. Probiotics can occasionally encounter DCs through two routes: DCs residing in the lamina propria sample luminal bacterial antigens by passing their dendrites between IECs into the gut lumen[118], and DCs can also interact directly with bacteria that have gained access to the dome region of the gut-associated lymphoid tissue (GALT) through specialized epithelial cells, termed microfold or M cells[119]. The interaction of the host cells with microorganism-associated molecular patterns (MAMPs) that are present on the surface macromolecules of probiotic bacteria will induce a certain molecular response. The host pattern recognition receptors (PRRs) that can perceive probiotic signals include Toll-like receptors (TLRs) and the C type lectin DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN). Some molecular responses of IECs depend on the subtype of cell, for example, Paneth cells produce defensins and goblet cells produce mucus. Important responses of DCs against probiotics include the production of cytokines, major histocompatibility complex molecules for antigen presentation, and co-stimulatory molecules that polarize T cells into T helper or $CD4^+CD25^+$ regulatory T cells in the mesenteric lymph nodes (MLNs) or subepithelial dome of the GALT. IFNγ, interferon-γ; IL, interleukin; TGFβ; transforming growth factor-β.

Defensin
In mammals, defensins are one of the major families of antimicrobial peptides that have a key role in the protection of mucosal surfaces against microbial invasion. They are usually 30–42 amino acids long, have a cationic charge and contain six cysteine residues that participate in three intramolecular disulphide bonds. Most defensins function by binding to the microbial cell membrane, resulting in the formation of pore-like membrane defects.

Tight junctions
Lipid–protein complexes at the apical junctions of epithelial cells, forming a barrier that can selectively allow the passage of ions and electrolytes.

Membrane lipid rafts
Transient cholesterol- and sphingolipid-enriched microdomains found in eukaryotic cell membranes, compartmentalizing cellular processes. They serve as organizing centres for the assembly of signalling molecules and receptors, for example.

Table 1 | **Examples of MAMP–PRR interactions of probiotics and gastrointestinal pathogens***

MAMP	Probiotic				Pathogen			
	Species	PRR	Co-receptor	Refs	Species	PRR	Co-receptor	Refs
Flagellin	*E. coli* Nissle 1917‡	TLR5	Unknown	10	*S.* Typhimurium	TLR5	Gangliosides (asialo-GM1)	27,120
Fimbriae	*E. coli* Nissle 1917‡ (type 1 fimbriae)	TLR4	Mannose glycoproteins	38	*E. coli* (P fimbriae)	TLR4	Glycosphingolipids	38
	L. rhamnosus GG	Unknown	Mucus glycoproteins	41,42	*C. perfringens*	Unknown	Unknown	121
Secreted proteins	*L. rhamnosus* GG p40 and p75 protein	Unknown	EGFR	12,45	*H. pylori* HP0175 protein	TLR4	EGFR	46
	L. johnsonii EFTu and GroEL	Unknown	CD14	47,48	*H. pylori* GroEL	TLR2	Unknown	50
Glycan ligands	*L. acidophilus* SlpA	DC-SIGN	Unknown	51	*H. pylori* LPS Lewis O-antigen	DC-SIGN	Unknown	82
CPS	*L. casei Shirota*	Unknown	Unknown	55	*S.* Typhi Vi polysaccharide	Unknown	Prohibitins	60
LTA	*L. plantarum*	TLR2	CD14 and CD36	71	*L. monocytogenes*	TLR2	CD14 and CD36	122
LPS (lipid A)	*E. coli* Nissle 1917 hexa-acyl lipid A‡	TLR4 and MD2	Unknown§	76	*Salmonella* hexa-acyl lipid A	TLR4 and MD2	Unknown§	76
					B. fragilis penta-acyl lipid A	TLR2	Unknown§	23,76
PG	*L. plantarum* DAP–PG	TLR2–NOD1 (or NOD2)	CD14	83	*L. monocytogenes*‖	TLR2–NOD1 (or NOD2)	CD14	123

*This table is not a complete list of comparisons. The main goal is to provide examples of similarities between pathogens and probiotics. ‡The interaction of these MAMPs with PRRs of the probiotic *E. coli* Nissle 1917 are not directly proved but only suggested, on the basis of the high level of similarity with documented MAMPs of closely related *E. coli* strains[77]. For more details, see main text. §Known co-receptors for canonical hexa-acyl lipid A include the integrins CD11b (also known as ITGAM)–CD18 (also known as ITGB2), CD55, CD81, the heat shock proteins Hsp70 and Hsp90, growth/differentiation factor 5 (GDF5) and CXC-chemokine receptor type 4 (CXCR4). By contrast, penta-acyl lipid A does not recruit CD11b–CD18, CD81, GDF5 and CXCR4 in the activation cluster[76]. ‖The PG *N*-deacetylase enzyme of *Listeria* spp. results in immune evasion[123]. Asialo-GM1, asialo-gangliotetraosylceramide 1; *B. fragilis*, *Bacteroides fragilis*; *C. perfringens*, *Clostridium perfringens*; CD36, also known as GP4; CPS, cell wall-associated polysaccharide; DAP, diaminopimelic acid; DC-SIGN, dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin; *E. coli*, *Escherichia coli*; EFTu, elongation factor Tu; EGFR, epidermal growth factor receptor; GroEL, also known as GroL; *H. pylori*, *Helicobacter pylori*; *L. acidophilus*, *Lactobacillus acidophilus*; *L. casei Shirota*, *Lactobacillus casei Shirota*; *L. johnsonii*, *Lactobacillus johnsonii*; *L. monocytogenes*, *Listeria monocytogenes*; *L. plantarum*, *Lactobacillus plantarum*; *L. rhamnosus* GG, *Lactobacillus rhamnosus* GG; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MAMP, microorganism-associated molecular pattern; MD2, also known as LY96; NOD, nucleotide-binding oligomerization domain-containing protein; p75, cell wall-associated hydrolase; PG, peptidoglycan; PRR, pattern recognition receptor; S. Typhimurium, *Salmonella enterica* subsp. *enterica* serovar Typhimurium; SlpA, S layer protein A; TLR, Toll-like receptor.

cytoprotective factors and co-stimulatory molecules[18]. The MAMP–PRR-induced signalling cascades involve the nuclear factor-κB (NF-κB)–inhibitor of NF-κB kinase (IκBK) and mitogen-activated protein kinase (MAPK) systems, which are mediated by rapid, transient post-translational modifications of proteins, thereby transmitting surface signalling to the cell nucleus[21]. The final outcome of these MAMP–PRR interactions and induced signalling pathways depends on the type of interacting microorganism and the reactivity of the interacting host cell. In various clinical applications with probiotics, such as treatments for IBD and allergic diseases, the aim is to tailor these interactions, as is discussed below.

MAMPs of probiotics, commensals and pathogens. Although the MAMPs that are present on conserved classes of bacterial macromolecules have a similar basic structure, subtle structural variations exist between these macromolecules on different microorganisms, especially for MAMPs located on the cell wall (FIG. 1). These variations lead to differences in the interactions with PRRs and potential co-receptors. This means that a macromolecule from one species can be an agonist for a certain PRR, whereas a similar macromolecule from another species can be an antagonist of that same PRR[23]. However, structural differences between the MAMPs of pathogens, commensals and probiotics do not allow easy discrimination between these functional classes of microorganisms. Therefore, a broader holistic view of how MAMPs and PRRs are represented on the bacterial and host cells, respectively, is needed[24,25] (see below).

MAMP–PRR interactions

Flagella. The initial contact between intestinal microorganisms and IECs is thought to occur through the interaction between large flexible microbial surface structures (FIG. 1) and PRRs of the host cells. This is best documented for the flagella of gastrointestinal pathogens. Flagellin is sufficient to recapitulate the pro-inflammatory response of IECs to *Salmonella enterica* subsp. *enterica* serovar Typhimurium[26] (TABLE 1). In addition, the PRR for these flagellins from both Gram-negative and Gram-positive pathogens was identified as TLR5 (REF. 27). Both the conserved flagellin MAMP and the interaction site on TLR5 have been characterized[28,29]. Some commensals, such as various *E. coli* strains, also have flagella that can induce

pro-inflammatory signalling[30]. However, *Bacteroides* spp., which are the principal commensal bacterial species, do not produce flagella, limiting their interaction with TLR5 (REF. 31). Recently, flagella were shown to correlate with probiotic effects (TABLE 1), although the examples are still scarce. For example, flagellins of the probiotic *E. coli* Nissle 1917 were shown to induce the expression of human β-defensin 2 (BD2, also known as DEFB4) [10] (FIG. 3), an inducible antimicrobial peptide synthesized by IECs to counteract adherence and invasion by pathogens (BOX 1).

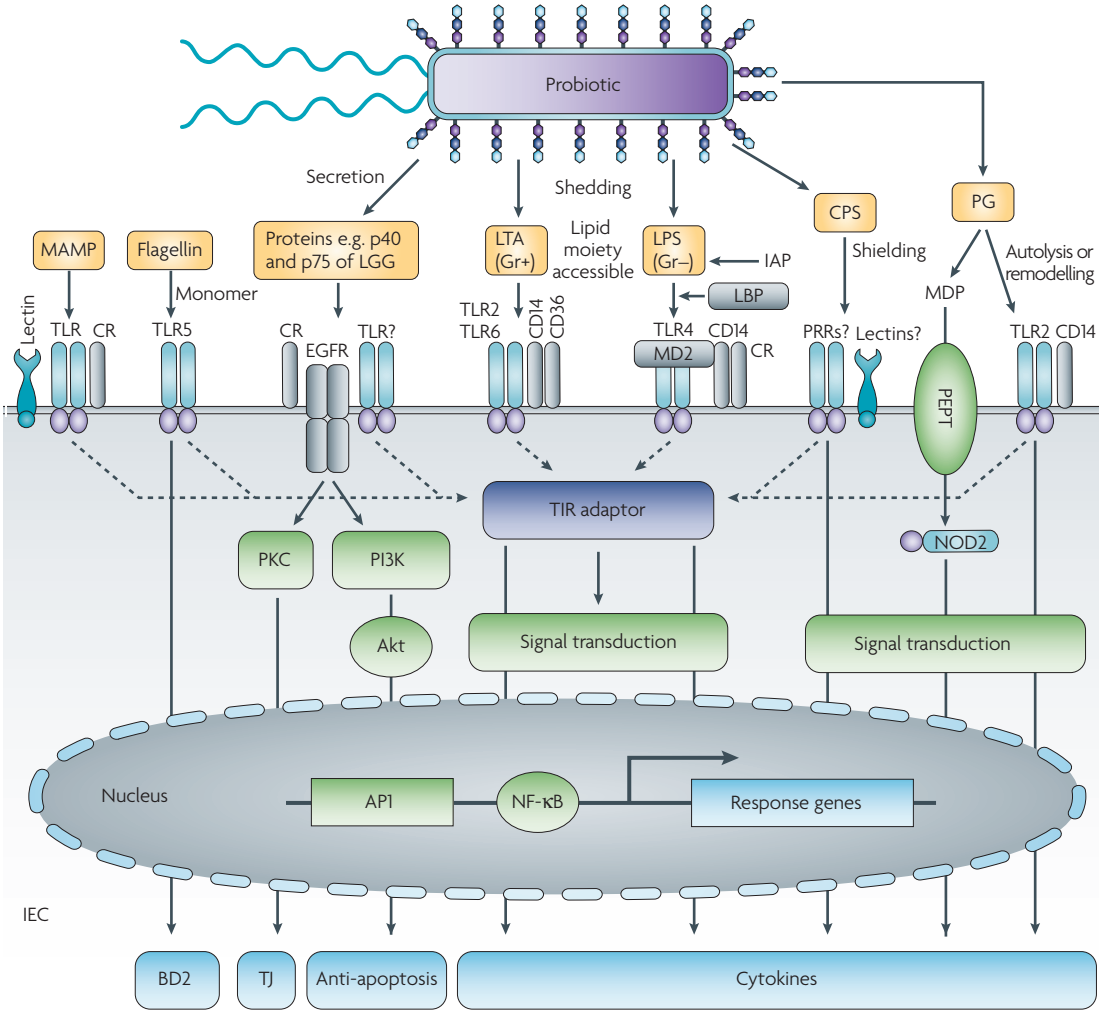

Figure 3 | **Probiotic MAMP–PRR interactions in IECs and associated signalling events.** Probiotic bacteria can interact with intestinal epithelial cells (IECs) using various surface molecules. Demonstrated effects of these interactions include: flagellin-mediated induction of human β-defensin 2 (BD2; also known as DEFB4) by *Escherichia coli* Nissle 1917 (REF. 10); *Lactobacillus rhamnosus* GG p40- and p75 (cell wall-associated glycoside hydrolase)-mediated anti-apoptotic and tight junction (TJ)-protecting effects[12]; and induction of cytokines by lipoteichoic acid (LTA)–Toll-like receptor 2 (TLR2)[71], lipopolysaccharide (LPS)–TLR4 and peptidoglycan (PG)– nucleotide-binding oligomerization domain-containing protein 2 (NOD2) (see main text for details). The accessibility of these microorganism-associated molecular patterns (MAMPs) for the host pattern recognition receptors (PRRs) varies considerably. For example: flagellin needs to interact as monomers with TLR5; p40 and p75 can mediate their effects after secretion; LPS and LTA need to be shed from the bacterial cell wall for the lipid moiety to become accessible; and PG needs to be hydrolysed (autolysis or remodelling). In addition, various co-receptors (CRs) that are associated with PRRs in lipid rafts can fine-tune MAMP–PRR signalling. Moreover, IECs have developed specific features for MAMP–PRR signalling. In healthy subjects, IECs are generally hyporesponsive for LTA and LPS from gut microorganisms, owing to several control mechanisms, including downregulation of TLR2 and TLR4 expression. The apical intestine alkaline phosphatase (IAP) enzyme reduces LPS–TLR4 signalling by detoxifying LPS[78]. PG interacts only with NOD2 after its ligand, muramyl dipeptide (MDP), is taken up by the apical peptide transporter PEPT1 (also known as SLC15A1)[87]. The interaction of probiotic cell wall-associated polysaccharide (CPS) molecules with PRRs or associated lectin-like co-receptors in IECs is not well understood, but some, such as the K5 capsule of *E. coli* Nissle 1917, have a documented cytokine-inducing capacity[56]. CPS molecules could also modulate the interaction of other MAMPs with their respective PRRs by shielding effects. EGFR, epidermal growth factor receptor; Gr+, Gram-positive bacteria; Gr–, Gram-negative bacteria; LBP, LPS-binding protein; LGG, *Lactobacillus rhamnosus* GG; MD2, also known as LY96; NF-κB, nuclear factor- κB; PI3K, phosphoinositol 3-kinase; PKC, protein kinase C; TIR, Toll/interleukin-1 receptor.

How can flagella be both important virulence factors and important probiotic factors? Various host and microbial factors modulate the flagellin–TLR5 interactions and, consequently, the triggered signalling pathways and host responses. Indeed, the TLR5-interacting MAMP becomes accessible only when flagellin is delivered as a monomer[28,29]. This will determine the amount of flagellin that is able to interact with TLR5 and the level of the host response that is induced. In addition, the presence of virulence factors that promote transcytosis of flagellin across the epithelium, such as *Salmonella* pathogenicity island 2 (SPI-2), can increase the availability of flagellin for TLR5 (REF. 32), because TLR5 has been shown to be localized at the basolateral pole in IECs[33]. This localization is suggested to allow discrimination between commensal bacteria residing in the gut lumen (that result in a low level of flagellin–TLR5 interaction) and pathogenic strains that reach the basolateral membrane after invasion (and that therefore result in a higher level of flagellin–TLR5 interaction)[26,33]. However, the basolateral compartmentalization of TLR5 has recently been challenged[34] and therefore cannot be seen as the only important discriminator between flagella from commensals (and probiotics) and pathogens. It is not only the amount of flagellin that can interact with TLR5 that is important; the specific structural features of the flagellin monomer need to be taken into account as well. For instance, several pathogenic bacteria, such as *Helicobacter pylori*, carry mutations in the conserved flagellin MAMP residues in order to evade immune recognition by TLR5 (REF. 28). Moreover, the picture becomes more complex if a broader view on MAMPs and PRRs is applied. In addition to interacting with TLR5, some flagella are reported to interact with TLR2 through binding of the co-receptor ganglioside asialo-gangliotetraosylceramide 1 (asialo-GM1), which is enriched in lipid rafts[35], showing that other MAMP interactions can further fine-tune the final outcome of flagellin–TLR5 interactions (see below for a discussion of the impact of cell wall-associated polysaccharide (CPS) on flagellin–TLR5 interactions).

Fimbriae. In contrast to flagella, which are primarily used for motility, fimbriae are dedicated to adhesion to host cells (often to glycosyl containing structures), making them even more interesting but, unfortunately, less documented ligands for PRRs of IECs (TABLE 1). Fimbriae (also called pili) occur both in Gram-positive and Gram-negative bacteria, although the biosynthesis and structure differs substantially between the two groups[36,37]. Several fimbriae of commensal and pathogenic *E. coli* strains are known to bind TLR4 (REF. 38). The specific binding capacity of these fimbriae seems to determine the engagement of distinct co-receptors and adaptor proteins in lipid rafts, thereby modulating signalling and the final outcome[38,39]. P fimbriae of pathogenic *E. coli* strains engage TLR4 of epithelial cells with glycosphingolipids as co-receptors, inducing a signalling cascade that involves TIR-domain-containing adaptor protein inducing *IFNb* (TRIF; also known as TICAM1) and TRIF-related adaptor molecule (TRAM; also known as TICAM2) but not MyD88. By contrast, *E. coli* strains containing type 1 fimbriae, which bind to mannosylated glycoproteins, activate TLR4 in a MyD88-dependent fashion[38]. The cell surface of the probiotic *E. coli* Nissle 1917 contains type 1 and F1C fimbriae that are involved in biofilm formation and intestinal colonization[40], but their interaction with host PRRs and importance for probiotic effects are, to our knowledge, not yet documented. Some probiotic lactobacilli, such as *Lactobacillus rhamnosus* GG, also have fimbriae that mediate adherence to mucus glycoproteins[41,42], and this could enhance the interaction of these fimbriae with PRRs and the induction of associated signalling pathways. However, the role of these fimbriae in probiotic effects remains to be validated. This is in contrast to fimbriae of some Gram-positive pathogens, which were shown to have key roles in inducing pro-inflammatory responses[43].

Other cell surface and secreted proteins. Fimbriae occur in only a few probiotic strains, but many probiotics have large surface proteins with highly repetitive structures that are involved in mucus adhesion[6]. Owing to these repeated domains and their role in adhesion, these molecules are also interesting candidates for further study of their interactions with PRRs or their modulation of PRR signalling through binding to associated co-receptors. For example, the mannose-binding lectin Msa of

Box 1 | **Interplay between probiotic bacteria and host antimicrobials**

To cope with the microbial challenges at the epithelial surface, the host innate immune system elaborates a battery of antimicrobial proteins ranging from enzymes such as lysozyme, small cationic microbicidal peptides (such as defensins, of which there are two main forms, α-defensin and β-defensin, and cathelicidins) to C type lectins[99]. These proteins have a broad-spectrum antimicrobial activity, being involved in both counteracting an attack by pathogens and controlling the autochthonous microbiota, and they therefore most likely also affect ingested probiotics. The antimicrobial proteins are expressed in multiple tissues in the body, including various intestinal epithelial cell types such as Paneth cells in the small intestine and enterocytes on the epithelial surfaces of both the small and large intestines. The expression of antimicrobial proteins can be governed by microorganism-associated molecular pattern (MAMP)–pattern recognition receptor (PRR) interactions. Human β-defensin 2 (BD2) is induced not only by pathogens such as *Helicobacter pylori*[100] but also by probiotics[9,10]. Flagellin was shown to be the *Escherichia coli* Nissle 1917 MAMP responsible for β-defensin induction[10]. Remarkably, whereas the symbiont *Bacteroides thetaiotaomicron* induces the mouse C type lectin regenerating islet-derived protein 3γ (RegIIIγ) that kills competing Gram-positive bacteria, the Gram-positive probiotic *Bifidobacterium longum* reduces RegIIIγ expression[101]. In addition, some constitutively expressed defensins, which are stored in secretory granules in Paneth cells, are released into the intestinal lumen on stimulation with bacterial products, including lipopolysaccharide and muramyl dipeptide[102].

Some of the interactions between the antimicrobial proteins and their targets are mediated by glycan structures. For instance, RegIIIγ and its human counterpart, HIP (also known as PAP or REG3A), bind to peptidoglycan, thereby directly mediating the selective killing of Gram-positive bacteria[103]. By contrast, defensins bind to negatively charged phospholipids in bacterial membranes of both Gram-negative and Gram-positive bacteria, thereby also targeting *Lactobacillus* species[104]. Cathelicidins have a similar mode of action, disrupting membranes by binding to bacterial membranes, LTA and LPS[105]. The effect of antimicrobial proteins on probiotic strains has not been investigated thoroughly, although it can be envisioned that these proteins will affect MAMP–PRR interactions. Given the distinct mechanisms of antimicrobial protein expression and the specific bacterial targets that these antimicrobials are binding to, it is clear that probiotic bacteria are both involved in and subject to the drive to keep homeostasis between the host and its associated complex microbiota.

Lactobacillus plantarum WCFS1 (REF. 44) could be involved in the induction of mucus expression in IECs[8], but this remains to be validated.

Contact between probiotic bacteria and host cells can also be mediated by secreted proteins (TABLE 1). For instance, two secreted proteins of the well-documented probiotic *L. rhamnosus* GG, designated p40 and p75 (cell wall-associated hydrolase), were recently found to promote IEC homeostasis, which could be important in the prevention of IBD[12]. *In vitro* and *ex vivo* experiments showed that *L. rhamnosus* GG or isolated p40 or p75 protein were able to inhibit tumour necrosis factor (TNF)-induced apoptosis in IECs[12]. These proteins stimulate the activation of anti-apoptotic Akt kinase in a phosphoinositide 3-kinase-dependent manner, probably mediated by activation of the epidermal growth factor receptor (EGFR)[12,45] (FIG. 3). This does not necessarily imply a direct interaction between EGFR and p40 or p75, however. For comparison, the binding of a secreted protein (HP0175) of the gastrointestinal pathogen *H. pylori* to TLR4 has been shown to result in transactivation of the EGFR in lipid raft signalling platforms in gastric epithelial cells[46]. The p40 and p75 proteins were also reported to protect tight junctions and attenuate barrier disruption in IECs. These effects are mediated by protein kinase C, extracellular signal-regulated kinase 1 (ERK1; also known as MAPK3) and ERK2 (also known as MAPK1)[11], indicating that these bacterial proteins can affect more than one pathway. In another probiotic strain, *Lactobacillus johnsonii* La1, the cell wall-associated moonlighting proteins elongation factor Tu (EFTu) and GroEL (also known as GroL) were shown to stimulate IL-8 secretion in IECs in a CD14-dependent mechanism[47,48]. CD14 is a co-receptor that is thought to function as a signal amplifier by moving TLRs into the kinase-rich environment of lipid rafts[49], but its role in EFTu and GroEL signalling needs to be further substantiated. Similarly, GroEL of *H. pylori* has been reported to induce IL-8 through TLR2 in human monocytes[50].

Besides interaction with IECs, probiotic surface proteins can also modulate DC function[13]. Recently, the S layer protein A (SlpA) of *Lactobacillus acidophillus* NCFM was found to regulate DC cytokine expression by interacting with the CLR DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)[51] (FIG. 4). This might depend on the glycosylation of SlpA, although this needs to be further documented. This is especially interesting, as DC-SIGN is known to interact with various structurally diverse glycosylated ligands of pathogens and thereby has a key role in the final host response against the encountered microorganism[52].

CPS. In contrast to pathogens, CPS molecules in probiotic bacteria (FIG. 1) are not well studied for their role in host–microorganism interactions[53]. In various pathogens, CPS molecules are key virulence factors that act by impeding macrophage and neutrophil-mediated phagocytosis[54]. The main role of pathogenic long CPS molecules is to shield other cell surface effector molecules and prevent them from interacting with host PRRs and complement factors. In *L. rhamnosus* GG, CPS was also found to shield surface molecules such as fimbriae[41]. CPS of *Lactobacillus casei* Shirota mediates the suppression of pro-inflammatory responses in macrophages[55] (TABLE 1). Recently, the K5 CPS molecules of *E. coli* Nissle 1917 were also reported to have an immunomodulatory effect in IECs[56] (FIG. 3). Whether these effects are mediated by shielding other surface molecules or by direct interaction with PRRs or co-receptors is not yet clear.

The CPS of the human symbiont *Bacteroides fragilis*, termed polysaccharide A, is known to have a key role in the development of the immune system in germ-free mice[57]. Colonization of germ-free mice by *B. fragilis* or treatment with purified polysaccharide A also protects against experimental IBD[58], although the mechanisms behind this are not yet fully understood. Polysaccharide A was shown to activate NF-κB signalling and cytokine production in DCs by TLR2-dependent mechanisms, modulating antigen presentation and $CD4^+$ T cell activation[59]. The fact that polysaccharide A, as a carbohydrate, interacts with TLR2 makes it possible that polysaccharide A simultaneously binds both CLRs and TLRs on DCs, possibly in lipid rafts or receptor clusters.

Vi CPS of the human pathogen *Salmonella enterica* subsp. *enterica* serovar Typhi suppresses pro-inflammatory responses mediated by flagellin–TLR5 interactions in IECs[60,61] (TABLE 1). The Vi CPS is only present in *S.* Typhi, which causes a severe systemic infection, and not in *S.* Typhimurium, which generally causes only localized gastroenteritis. The Vi CPS is thought to be an important discriminative feature between the disease outcomes of the two serovars, which both induce IL-8 production through flagellin–TLR5 interactions. Recently, the role of the Vi CPS was found to be linked to its capacity to bind to the prohibitin-like molecules that are enriched in lipid rafts and to reduce signalling through the MAPK pathway; this leads to immune evasion by suppression of early inflammatory responses[60]. Nevertheless, the structural features required for Vi CPS–receptor interaction and downregulation of IL-8 remain elusive. This is of interest, as several probiotics, such as the CPS-producing *L. rhamnosus* GG[41], have been shown to decrease flagellin-induced IL-8 production in Caco-2 cells[62], although the responsible probiotic factors remain to be determined.

LTA. One of the earliest studied MAMP-containing molecules of probiotic Gram-positive bacteria is lipoteichoic acid (LTA) (FIG. 1). LTA molecules are ligands for TLR2 in a heterodimer with TLR6, with CD14 and CD36 (also known as GP4) as co-receptors[63] (TABLE 1). Since LTA is ubiquitously present on almost all Gram-positive bacteria, IECs seem to have developed special mechanisms to tolerate the continuous presence of the many LTA molecules originating from commensals in the gut lumen, by expressing these LTA receptors and co-receptors at a limited level[64] and by upregulating specific inhibitors of TLR signalling such as the Toll-interacting protein TOLLIP[65]. In addition, as the two lipid chains of LTA are thought to mediate the interaction with the lipid-binding pocket

Moonlighting protein
A protein that has more than one role in an organism. Well-known examples in bacteria are cytosolic glycolytic enzymes, such as glyceraldehyde 3-phosphate dehydrogenase and enolase, which can function as adhesins once secreted outside the cell.

Prohibitin
A putative tumour suppressor molecule that regulates the mammalian cell cycle. Prohibitin and its related members are abundant in mitochondria but are also present in the cell membrane and the nucleus. In intestinal epithelial cells, prohibitin-like proteins are enriched in lipid rafts and are believed to be involved in signalling events.

of TLR2 (REF. 66), these chains must become exposed by shedding or lysis to induce signalling, thereby limiting the accessibility of these ligands. This suggests that LTAs from living whole-cell probiotic bacteria are probably not key PRR ligands for IECs. Nevertheless, isolated LTA molecules from *L. johnsonii* La1 and *L. acidophilus* La10 were reported to inhibit *E. coli*-induced and LPS-induced IL-8 release by IECs (FIG. 3), possibly by competitive binding with the soluble form of the co-receptor CD14 (REF. 67).

Interaction of LTA and TLR2 seems to be promoted in phagocytic cells such as macrophages and DCs (FIG. 4) compared with the levels of interaction in non-phagocytic cells such as IECs[68] (FIG. 3). Isolated LTA molecules from *L. casei* YIT9029 and *Lactobacillus fermentum* YIT0159 have the capacity to induce TNF expression in macrophages in a TLR2-dependent manner[69]. Highly purified LTA from *L. plantarum* KCTC10887BP seems to function as an antagonist of *Staphylococcus aureus* LTA-induced TNF production in monocytic cells, in a TLR2- and CD14-dependent manner[70]. Chemical deacylation experiments showed that the acyl chains are essential for agonistic LTA-induced TNF production and antagonistic LTA-induced tolerance, whereas chemical de-alanylation showed that D-alanylation is not important[70]. On the other hand, a mutational approach in *L. plantarum* suggests that D-alanylation of LTA mediates pro-inflammatory interactions through TLR2 (REF. 71). A strain with a mutation in the D-alanyl-lipoteichoic acid biosynthesis protein gene (*dltB*) was more anti-inflammatory than wild-type bacteria in peripheral blood mononuclear cells (PBMCs), and the effects were mediated through TLR2. However, the role of D-alanylation in the pro-inflammatory capacity of probiotic-derived LTA could not be completely confirmed in *L. rhamnosus* GG by the same experimental approach[72]. Of note, mutations affecting LTA substitution can have pleiotropic effects on other cell surface structures such as cell surface proteins, CPS and peptidoglycan (FIG. 1), thereby affecting the interaction with several PRRs.

LPS. In comparison with LTA in Gram-positive bacteria, the outer-membrane glycolipid LPS (FIG. 1) is the best studied activator of innate immunity from Gram-negative bacteria. As for LTA, the lipid moiety interacts with the innate immune receptor, implying that LPS also needs to be shed from the cell surface for TLR engagement[16]. The lipid A fraction of LPS interacts with TLR4 and MD2 (also known as LY96) and two important co-receptors, LPS-binding protein (LBP) and CD14 (TABLE 1). LBP extracts LPS from the outer membrane of Gram-negative bacteria or from vesicles that are released from them. CD14 promotes the delivery of smooth LPS with abundant O antigens to the TLR4 complex[16].

IECs are hyporesponsive for abundant LPS from commensal Gram-negative bacteria in the gut lumen[73], as they are for LTA (FIG. 3). This hyporesponsiveness is achieved by various mechanisms, including: decreasing the expression of TLR4, MD2 and CD14 (REF. 74); compartmentalization of TLR4 expression to certain intestinal regions such as the crypts[75]; and only responding to specific LPS structures. Diphosphorylated hexa-acyl lipid A seems to be the main agonist of TLR4 signalling[16]. Many gut pathogens, such as some *Salmonella* serovars and some pathogenic *E. coli* spp., have hexa-acylated lipid A. By contrast, penta-acylated monophosphoryl LPS of commensal *B. fragilis* is sensed mainly by TLR2 (TABLE 1) and can even inhibit recognition of mucosal LPS by TLR4 (REF. 23). Interestingly, these small differences in LPS structure have been shown to result in the formation of different activation clusters in lipid raft domains. For instance, in comparison with canonical hexa-acyl LPS, the TLR4 antagonist penta-acyl lipid A was shown to recruit fewer co-receptors in lipid rafts, resulting in inhibition of NF-κB activation, whereas MAPK signalling was still observed[76]. However, the number of acyl chains is not the only discriminator between pathogens and commensals; for example, commensal *E. coli* strains[16] and the probiotic strain *E. coli* Nissle 1917 (REF. 77) also have hexa-acylated LPS. In addition, the O chain composition of LPS as well as the length and phosphorylation level of and type of substitutions in the acyl chain (FIG.1) all affect the outcome of LPS–TLR4 signalling[16]. Interestingly, intestinal alkaline phosphatase has been shown to have a key role in detoxifying the LPS of commensal bacteria by dephosphorylating lipid A and preventing excessive inflammatory responses of IECs against the abundant LPS present in the gut lumen[78]. As this enzyme is only expressed apically by IECs, LPS from invading pathogens still activates pro-inflammatory signals.

In contrast to IECs (FIG. 3), phagocytic DCs seem to be more responsive to LPS (FIG. 4). It was shown by microarray analysis that the DC response against *E. coli* SD54 almost completely overlaps with the response induced by its hexa-acyl LPS[79]. LPS is also a strong inducer of DC maturation and subsequent T cell polarization on antigen presentation. Although several LTA-containing probiotics, such as *Lactobacillus salivarius*, only induce partial DC maturation, hexa-acyl LPS from *E. coli* induces full maturation of DCs[80]. Nevertheless, the *in vivo* importance of LPS for probiotic modulation of T cell polarization remains elusive, as the LPS–TLR4 interaction was recently found not to be responsible for the induction of interferon-γ-producing T helper 1 cells and the suppression of the allergen-induced T helper 2 cell responses that are mediated by *E. coli* Nissle 1917 in airways[81]. By contrast, LPS from *H. pylori* was reported to modulate the T helper 1 cell/T helper 2 cell balance through its Lewis O antigen-mediated interaction with DC-SIGN[82].

PG. Peptidoglycan (PG) is usually embedded in the cell wall and covered by various other surface molecules (FIG. 1). Before PG can be detected by PRRs, PG fragments must be released by remodelling, autolysis or lysozyme action in the gut. PG is a ligand for TLR2, with CD14 as a co-receptor[19] (TABLE 1). Diaminopimelic acid-containing PG fragments are more efficiently recognized by TLR2 than lysine-containing fragments[83]. Moreover, certain modifications of the carboxylic acids of glutamine and diaminopimelic acid (for example, amidation), such as

occurs in *L. plantarum*, reduce TLR binding, which is suggested to be a strategy of certain microorganisms to avoid the induction of pro-inflammatory cytokine expression by TLR2 (REF. 83). The variation in PG structure could be a discriminative feature between probiotics, as the stem peptides can differ substantially[15] (FIG. 1). Nevertheless, the importance of PG–TLR2 interactions for probiotic effects remains to be established. Moreover, PG detection is more likely to occur intracellularly through NOD1 or NOD2 than extracellularly through TLR2 (REF. 84). NOD1 detects γ-D-glutamyl-meso-diaminopimelic acid[85], which is mostly present in Gram-negative bacteria such as *E. coli* Nissle 1917 but is also found in some *L. plantarum* strains[15], whereas NOD2 recognizes the muramyl dipeptide (FIG. 1) of both Gram-positive and Gram-negative bacteria[85]. Amidation of the α-carboxylic acid of isoglutamic acid was recently found to decrease NOD1 sensing and NF-κB activation,

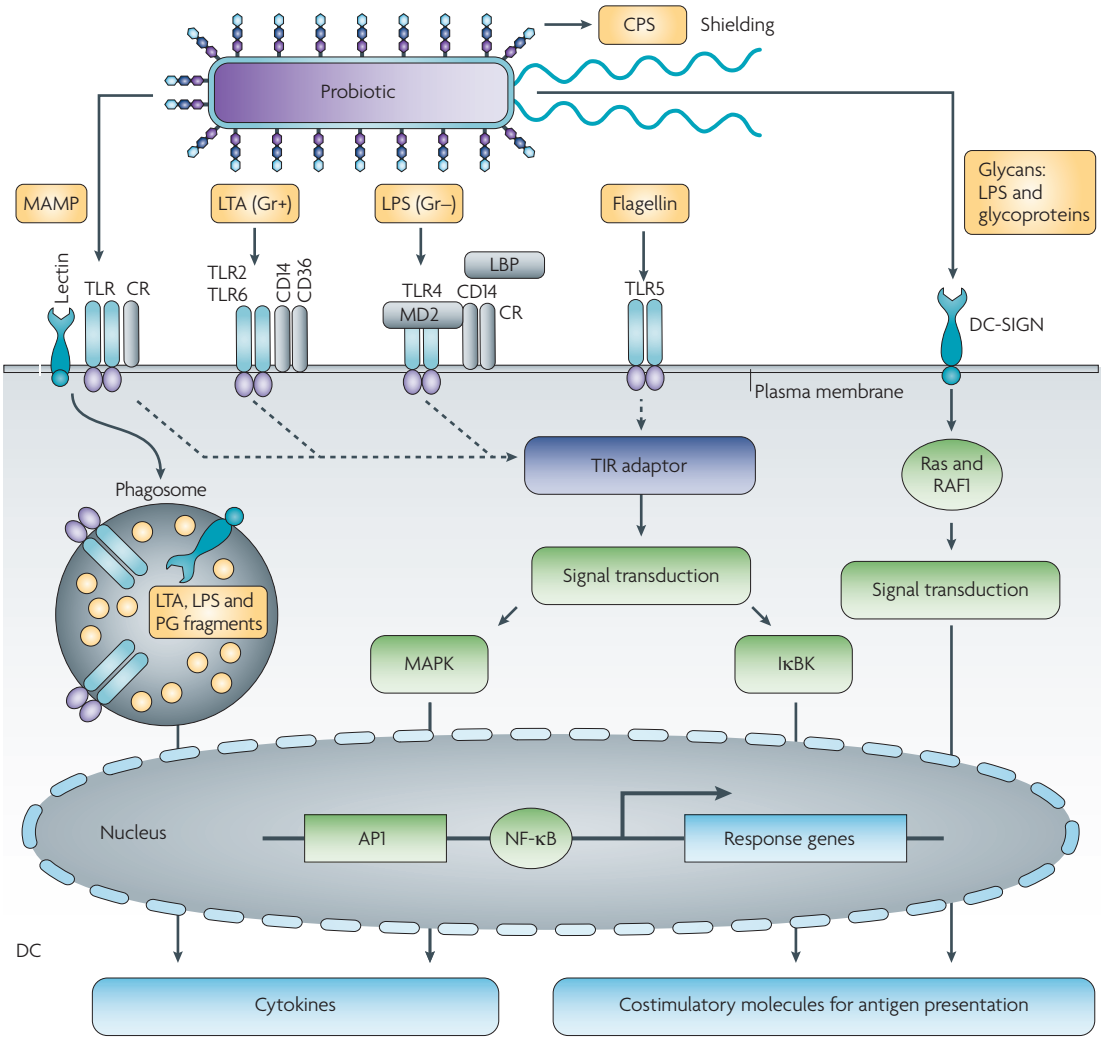

Figure 4 | **Probiotic MAMP–PRR interactions in DCs and associated signalling events.** Probiotic bacteria can interact with dendritic cells (DCs) using various surface molecules. Demonstrated effects of interactions include lipoteichoic acid (LTA)–Toll-like receptor 2 (TLR2)-mediated and lipopolysaccharide (LPS)–TLR4-mediated[16] induction of cytokines and co-stimulatory molecules for antigen presentation that can modulate T cell polarization. These interactions seem to be promoted by ingestion of the bacteria through phagocytosis and digestion in phagolysosomes, so that the interacting microorganism-associated molecular patterns (MAMPs) such as lipid A from LPS or the two acyl chains from LTA become accessible for pattern recognition receptor (PRR) recognition. In addition, the putative glycoprotein S layer protein A (SlpA) of *Lactobacillus plantarum* was shown to interact with DCs through the C type lectin DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)[51]. DC-SIGN is mainly a phagocytotic receptor, promoting uptake of the bacteria in phagolysosomes, but can also induce signalling that involves the small G protein Ras and the kinase RAF1, which mediates interaction with nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK)[20]. Cell wall-associated polysaccharide (CPS) could modulate these interactions by forming a shield that impedes phagocytosis[54]. CR, co-receptor; Gr+, Gram-positive bacteria; Gr–, Gram-negative bacteria; IκBK, inhibitor of NF-κB kinase; LBP, LPS-binding protein; MD2, also known as LY96; PG, peptodiglycan; TIR, Toll/interleukin-1 receptor.

Box 2 | **Dynamics of the host cell surface in response to bacterial encounter**

It has been well established that the surface glycosylation pattern of enterocytes changes during intestinal development, with a shift from sialylation to fucosylation that is evoked by dietary and hormonal factors[106]. It is likely that the expression patterns of glycans are functionally linked to the spatial and temporal complexity of the intestinal microbiota[93] as the composition of the microbiota changes during the first two years of life.

The example par excellence of bacteria-driven dynamic changes at the host cell surface might well be the induction of fucosylated glycans in the intestinal epithelium by the commensal species *Bacteroides thetaiotaomicron*, which colonizes the host at weaning[107]. In fact, this concerns a bidirectional interaction, as the availability of host fucose regulates the expression of a bacterial signal that induces the production of host fucosylated glycans. This stimulates the entrance and persistence of *B. thetaiotaomicron* in a competitive ecosystem and gives the host control over the composition of its microbiota[108].

This host glycosylation also affects microorganism-associated molecular pattern (MAMP)–pattern recognition receptor (PRR) interactions, as all Toll-like receptors (TLRs; which constitute the main PRRs) contain *N*-linked glycosylation consensus sites, including TLR2 (REF. 109) and TLR4 (REF. 110). This glycosylation is dynamic, as *Helicobacter pylori* has been reported to stimulate TLR4 glycosylation[111]. The effect of probiotics, or microbiota in general, on this glycosylation has not yet been investigated in detail.

Bacteria can also change the glycosylated surface of the intestinal epithelium by modulating mucin expression. Mucins are glycoproteins (with 70–80% O-linked glycosylation) that are secreted by goblet cells. The mucins assemble into a protective gel-like mucus layer, providing a bacteria-free zone adjacent to the epithelial surface. As such, the mucus layer is an integral part of the immunological ignorance strategy used by the host to keep a beneficial symbiosis in the host–microbiota relationship[112]. Some probiotics actively contribute to this epithelial barrier protection by inducing mucin 2 (MUC2) and MUC3 production[8], although the exact bacterial effectors remain to be identified. The induction of mucin synthesis can provide an additional niche for probiotics to transiently reside in the gastrointestinal tract and can promote contact with their receptors.

as another immune evasion strategy[86]. The interacting NOD ligands come into contact with IECs and DCs by different mechanisms. In IECs, the NOD2 ligand, muramyl dipeptide, is taken up by the apical dipeptide/tripeptide transporter PEPT1 (also known as SLC15A1) and subsequently mediates activation of NF-κB[87] (FIG. 3). In phagocytic cells such as DCs, PG peptide ligands are probably generated by ingesting whole bacteria and then digesting them in phagolysosomes[21] (FIG. 4). Some studies have suggested a role for intracellular PG–NOD interactions in certain probiotic effects[88,89], but to our knowledge the role of PG differences in these interactions needs to be further documented.

Dynamics of probiotic cell surface molecules

When discussing the probiotic MAMPs that have the potential to engage PRRs and induce a signalling cascade, it is important to remember that the bacterial cell surface is a dynamic entity. Host developmentally controlled factors and environmental factors can modulate the expression and exposure of bacterial cell surface molecules. The first conditions that probiotic bacteria encounter and that have a tremendous impact on their cell surfaces are the acidity of the stomach and the detergent-like action of bile in the small intestine. These environments have been shown to influence PG biosynthesis and remodelling, LTA decoration with D-alanine residues, CPS expression and excretion of moonlighting proteins[6]. Nutrient availability, especially the carbohydrate resources in the intestine, probably also affect probiotic surface-related gene expression, as has been shown for the human symbiont *Bacteroides thetaiotaomicron*[90] (see also BOX 2).

In addition, the host innate and adaptive immune systems are thought to play a crucial part in modulating probiotic surface architecture. This has been well documented for pathogens and symbionts, of which successful colonizers of the human gastrointestinal tract seem to exhibit some level of immune evasion. This is achieved by modulating their surface molecules, either to minimize recognition by host PRRs and antibody responses or to enhance protection against antimicrobial responses such as the production of defensins, complement activation and phagocytosis. Surface glycans seem to have a key role in this dynamic modulation. For instance, CPS molecules are known to protect against complement activation and phagocytosis[54]. Certain microorganisms also upregulate CPS expression to protect against antimicrobial peptides such as cathelicidin (LL-37)[91]. Similarly, CPS molecules seem to have a dynamic role in the probiotic *L. rhamnosus* GG[41]. Whereas CPS molecules need to be downregulated for optimal adherence to IECs[41], they seem to be required for protection of *L. rhamnosus* GG against the antimicrobial factors of the lower regions of the gastrointestinal tract (S.L., J.V. and S.C.J.D.K., unpublished observations).

The adaptive immune system and the antibody response can also modulate the dynamics of the bacterial surface by epitope selection[92]. In *B. thetaiotaomicron*, immunoglobulin A responses were shown to modulate CPS expression to minimize activation of the innate immune system and intestinal pro-inflammatory gene expression[92]. This study also highlights that long-term gut residents must be able to modulate their immunodominant determinants continuously. Interestingly, *Bacteroides* spp. show a remarkable plasticity in displaying surface polysaccharides in comparison with closely related bacteria of the oral cavity[31]. For instance, *B. fragilis* can synthesize at least eight distinct CPS molecules and a large number of glycoproteins, which are all subject to phase variation[31]. The glycobiome of probiotic lactobacilli and bifidobacteria

seems to be more restricted: most strains produce only one or two CPS types; glycoproteins and phase variation have not been well documented. Nevertheless, the importance of the probiotic glycobiome needs to be further substantiated. Of note, the role of glycans in microbial interactions with PRRs in general is elusive, except for their interactions with CLRs. Glycans seem to dynamically modulate their binding efficiency with TLRs, for example by shielding other surface molecules or by binding associated co-receptors, without having a direct binding role. This is in contrast to the fact that glycan structures (such as O antigens, CPS molecules and glycans of certain flagella) are known to play a key part in inducing strain-specific antibody responses by the adaptive immune system[16,93,94].

Conclusions

The surface molecules of bacteria that can be regarded as probiotic (that is, health-promoting) rather than pathogenic are, at present, not easy to delineate. As exemplified throughout this Review, the final outcome of a host cell response against a microorganism (being pathogenic, probiotic or commensal) depends on the combination of the distinct MAMPs that can interact with the various PRRs and associated co-receptors that fine-tune signalling[22], as well as on the concentration of these MAMPs, their accessibility for the PRRs and the presence of other microbial effector molecules (such as toxins that are produced by pathogens) that can modulate host responses. In addition, two important factors that determines the responsiveness of host cells are the accessibility of the PRRs for the MAMPS (that is, the subcellular distribution, compartmentalization and expression levels of the PRRs in various host tissues) and host-derived direct or indirect negative regulators of PRR signalling. Current data suggest that pathogenic, probiotic and commensal bacteria can be roughly divided into three classes on the basis of the extent of the host response in IECs and DCs: pathogenic microorganisms (which are virulent and induce a strong host response), probiotics (which modulate certain IEC and DC functions and induce an intermediate response) and commensal bacteria (which exhibit homeostatic control of the response). As also suggested by Fischer *et al.*[38], pathogenic MAMP–PRR interactions seem to be enhanced by the presence of additional virulence factors that can bind to co-receptors or enhance the responsiveness of the host cells. For commensal bacteria, the interaction can be limited by the absence of certain MAMPs. For instance, commensal *Bacteroides* spp. lack flagella and have penta-acylated LPS, limiting their interactions with TLR5 and TLR4, respectively. Interestingly, probiotics of the genus *Bifidobacterium* seem to be more closely related to commensals than to lactobacilli in terms of their capacity to modulate host responses[5,95]. This is perhaps not surprising, when one considers the high numbers of bifidobacteria in the human microbiota (up to 3% versus less than 0.1% for lactobacilli)[96]. It will be interesting to relate possible differences in surface molecules between lactobacilli and bifidobacteria to host responses and probiotic effects.

Phase variation
The (epi)genetic reversible on-and-off switching of surface epitope production that may function to provide a pool of bacteria with an evolutionary advantage upon rapid environmental changes.

The available data on MAMP–PRR interactions for pathogenic, commensal and probiotic microorganisms show the complexity of this field. It is tempting to speculate that in the future, it will be possible to select surface molecules of probiotic bacteria depending on the host response that is aimed for in the probiotic treatment. For instance, it can be anticipated that the desired molecules will be different for patients with IBD than for patients with allergic diseases. In allergic diseases, the main target for probiotic applications seems to be the DCs, as they have the capacity to polarize T cell responses. Allergic diseases often result from exaggerated T helper 2 type immune responses. As such, for the prevention of allergic diseases, probiotic bacteria need to be able to beneficially modulate T cell polarization into an increase in T helper 1 cell and $CD4^+CD25^+$ regulatory T cell responses, by specially modulating DC functions. In addition, systemic induction of low-grade inflammation is thought to be important in probiotic effects against atopic eczema–dermatitis syndrome[97]. Similarly, probiotic applications that are aimed at enhancing the host immune response, such as those used for the prevention of traveller's diarrhoea or gastroenteritis, also seem to require probiotics with a mild immunostimulatory activity[98]. By contrast, for IBD treatment probiotic bacteria (and therefore surface molecules) that can counterbalance the pro-inflammatory pathology might need to be selected, taking the disturbed epithelial barrier into account. Additional care is warranted, as IBD patients show polymorphisms in PRRs that result in modified signalling pathways, such as that seen in NOD2 in many Crohn's disease patients[21].

The transition from *in vitro* mechanistic studies on bacteria–host cell interactions to the *in vivo* complexity of multicellular organisms will not be straightforward. The selection of optimal probiotic molecules for specific disease conditions requires further in-depth molecular studies dealing with both the cell surface of probiotic bacteria and the interacting host cells and their receptors. The steadily increasing possibilities of genomics-based and dedicated-mutant analyses along with the technological progress in the analysis of the probiotic surface proteome and glycobiome, complemented with parallel approaches for the host cells, will certainly advance the field. As highlighted in this Review, molecular research on probiotics can benefit from the concepts and tools developed in host–microorganism studies that span the spectrum from pathogenicity to mutualism (TABLE 1). An emerging theme is that host–microorganism interactions are not univocal but involve the complex interactions of various microbial surface molecules with various host receptors and adaptor molecules. The final host response is therefore determined by the coordinated action of the signals induced by the different receptors in different cell types. The identification and characterization of the bacterial molecules as ligands of these specific host receptors is key in understanding how not just probiotics but also the resident microbiota function. A detailed molecular comprehension should ultimately lead to a more focused and rational application of probiotics in functional food or as supporting therapy for specific disorders such as IBD, allergic disease and gastroenteritis.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

Entrez Genome Project:
http://www.ncbi.nlm.nih.gov/genomeprj
Bacteroides fragilis | *Bacteroides thetaiotaomicron* | *Escherichia coli* | *Helicobacter pylori* | *Lactobacillus acidophillus* NCFM | *Lactobacillus plantarum* WCFS1 | *Lactobacillus rhamnosus* GG | *Lactobacillus salivarius* | *Salmonella enterica* subsp. *enterica* serovar Typhi | *Salmonella enterica* subsp. *enterica* serovar Typhimurium | *Staphylococcus aureus*

UniProtKB: http://www.uniprot.org
BD2 | CD14 | CD36 | EFTu | GroEL | IL-8 | LBP | MD2 | MyD88 | NOD1 | NOD2 | p40 | p75 | SlpA | TLR1 | TLR2 | TLR4 | TLR5 | TLR6 | TNF | TRIF | TRAM

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