Molecular and Cellular Basis of Microflora-Host Interactions¹,²

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Abstract

Mucosal surfaces represent the main sites in which environmental microorganisms and antigens interact with the host. In particular the intestinal mucosal surfaces are in continuous contact with a heterogeneous population of microorganisms of the endogenous flora and are exposed to food and microbes. As a result, the immune system of the host has to discriminate between pathogenic and commensal microorganisms. This article reviews the types of sentinel cells that continuously sense the environment and coordinate immune defenses as well as the mechanisms of the innate and adaptive immune systems that are activated by bacterial and viral molecular patterns leading to inflammatory, allergic, or regulatory immune responses with special emphasis on probiotic bacteria. J. Nutr. 137: 756S–772S, 2007.

The first line of intestinal defense is provided by the mucus layer, which covers the epithelium and contains various protective and antimicrobial substances secreted by epithelial cells such as inflammatory responses. Therefore, innate mechanisms have evolved that assist in preventing invasion by commensals and eliminating potential intruders. When constitutive innate defenses are overflowed by pathogens, inducible immune responses are switched on. Innate and adaptive mechanisms are thus concomitantly and coordinately stimulated by mucosal sentinel cells responding to danger signals (1).

Enterocytes

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5 Abbreviations used: Ag, antigen; AP-1, activator protein 1; APC, antigen-presenting cell; ASK1, apoptosis-stimulating kinase 1; ATF, activating transcription factor; CCR, chemokine receptor; CpG-DNA, cytosine-phosphate-guanosine DNA; DC, dendritic cell; dsRNA, double-stranded RNA; ECSIT, evolutionarily conserved intermediate in Toll pathway; Eo, eosinophils; FAE, follicle-associated epithelium; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPCR, G protein-coupled receptor; HSP, heat shock protein; IL-1R, interleukin-1 receptor; IP-10, inducible protein 10; IRAK, IL-1R-associated kinase; IRF, interferon regulatory factor; JNK, c-Jun NH₂-terminal kinase; LAB, lactic acid bacteria; LAM, lipoarabinomannan; LFA, lymphocyte function antigen; LGG, Lactobacillus rhamnosus; GM; Golfin and Gorbach; LTA, lipoteichoic acid; MAL, MyD88-adapter-like; MALT, mucosa-associated lymphoid tissue; MAPK, mitogen-activated protein kinase; MDP, muramyl dipeptide; MEKK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase; MHC, major histocompatibility complex; MyD88, myeloid differentiation protein 88; NF-kB, nuclear factor-kB; NIK, NF-kB-inducing kinase; NK cell, natural killer cell; NOD, nucleotide-binding oligomerization domain receptor; PAMP, pathogen-associated microbial pattern; PBMC, peripheral blood mononuclear cell; PGN, peptidoglycan; PRR, pattern recognition receptor; sRNA, single-stranded RNA; TAB, TAK-1-binding protein; TAK, transforming growth factor-β activated kinase; TCR, T-cell receptor; TGF, transforming growth factor; TNFα, T cell; Toll-like receptor; TLR, Toll-like receptor; TOLLIP, Toll-interacting protein; TRAF, tumor necrosis factor receptor–associated factor; TRAM, TRIF-related adapter molecule; TRIF, TRIF domain-containing adapter inducing IFN-β.
complement components, mucins, enzymes, and defensins (2–4). The gut epithelium, apart from its participation in absorptive, digestive, and secretory processes, does not just act as a passive barrier but plays a major role in protection through interactions with the immune system (5). The small intestinal epithelium consists of 4 cell types, the absorptive enterocytes, the goblet cells, the enteroendocrine cells, and the Paneth cells in the crypts, whereas in the large intestine, Paneth cells are lacking. Each enterocyte has ~3000 microvilli at its luminal surface, highly organized into a brush border that facilitates the absorption of nutrients. Absorptive enterocytes are specialized for the transport of substances via channels and transporters and can internalize specific substances by nonspecific (pinocytosis) and receptor-mediated endocytosis. The epithelial cells are sealed at their apex by tight junctions, which act as channels for paracellular transport of specific substances (6).

**Pattern recognition receptors**

Intestinal epithelial cells produce various pattern-recognition receptors (PRRs) that recognize microbial motifs, referred to as pathogen-associated molecular patterns (PAMPs) (7). Because PAMPs are evolutionally highly conserved and invariable in microorganisms of the same class, mammals can recognize almost all microorganisms with a small number of PRRs. Two receptor families play an important role in the detection of PAMPs: Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain receptors (NODs) (8).

**TLRs.** The term Toll was originally related to a gene that plays an important role in the dorsoventral polarization in the ontogenesis of *Drosophila melanogaster* (9,10). In the 1990s, Gay and Keith recovered sequence homologies between the cytoplasmatic domain of Toll proteins and human IL-1 receptors (IL-1R), the Toll/IL-1 receptor (TIR) domains (11). Medzhitov et al. found the first human Toll homolog in 1997, which was later identified as TLR4 (12). TLRs are transmembrane proteins with an extracellular domain made of leucine-rich repeats and an intracytoplasmatic domain containing the highly conserved TIR domain. Variations in the leucine-rich repeat sequences generate receptors that can discriminate among many different PAMPs (13). Some TLRs are located on the cell surface (e.g., TLR1, -2, and -5), whereas others remain sequestered in intracellular compartments (TLR3, -7, -8, and -9) (14).

To date, 11 mammalian TLRs have been identified, numbered 1–11 (Fig. 2), whose expression pattern is cell type specific (Table 1). TLRs are present on leukocytes that initiate or modulate the immune responses such as DCs and macrophages. They are also expressed in nonprofessional immune cells such as endothelial cells, fibroblasts, adipocytes, and epithelial cells (21,52). TLR2, -3, and -5 are associated with both human and mouse intestinal epithelium (2). TLR4 was found in the Golgi apparatus of murine epithelial cells of intestinal crypts (52) but not at the cell surface (53). Epithelial TLRs’ signaling provokes secretion of various mediators, including proinflammatory chemokines and cytokines, antimicrobial defensins, and tissue-remodeling enzymes (54,55). Exposure to bacterial products or proinflammatory cytokines can increase expression of TLRs, whereas IL-10 blocks this effect (8).

**Ligands.** Of the 11 mammalian TLRs only 9 have assigned ligands (Table 1) (56,25,48,49).

TLR2 recognizes lipoproteins and lipoteichoic acids (LTAs), which are integrated in a peptidoglycan (PGN) layer of gram-positive bacteria. The capacity of TLR2 to recognize many gram-positive motifs such as PGN, LTA (27,28), and lipopolysaccharides (LPS) (33) results from its ability to form heterodimers with TLR1 and TLR6 (see below) (29,32,57,58). TLR2 complexes have also been shown to mediate cellular responses to yeast cell walls, bacterial lipopeptides (39), as well as factors secreted by staphylococci (26), *Borellia* (36), *Neisseria* (35), or *Trypanosoma* (31).

TLR3 recognizes double-stranded RNA (dsRNA), a viral product that is synthesized during viral replication in infected host cells. Viral dsRNA expressed at the cell surface may be
derived from cells that were destroyed by cytopathic viruses (59). TLR3-deficient mice showed reduced immune responses to viral RNA (40).

TLR4 is mainly activated by LPS, which are the best-characterized PAMPs. LPS form a main component of the outer membrane of gram-negative bacteria (41). They induce many immune reactions, for example, the production of proinflammatory cytokines such as IL-12 and inflammatory effector substances such as NO. Initial transfection studies suggested that TLR2 was also a receptor for LPS, but this was an artifact of LPS contamination of TLR2 agonist preparation (60). TLR4 also recognizes viral proteins (43), mycobacterial compounds (38), and cryptococcal capsules (45).

TLR5 identifies the bacterial flagellin of gram-negative and gram-positive bacteria (47). In the human T84 colonic epithelial cells, TLR5 is restricted to the basolateral membranes (61). This implies that flagellin has to be transported across the epithelial cell to trigger immune responses. However, in vivo, TLR5 is equally distributed on the apical and the basolateral cell surfaces (1).

TLR7 and TLR8 are highly homologous to TLR9 (13). They recognize small synthetic antiviral molecules (50). TLR7 recognizes viral single-stranded RNA (ssRNA) (49) as well as the anticancer drugs loxoribine and bropirimine (51). Murine TLR7 and human TLR8 recognize guanosine- and uridine-rich ssRNA derived from HIV-1 (48).

TLR9 serves as a PRR for unmethylated cytosine–phosphate–guanosine (CpG)-DNA (62). Arthropods and other invertebrates, in contrast to vertebrates, do not possess methylated DNA (63), suggesting that nonsel pattern recognition mechanisms have evolved in vertebrates, enabling them to recognize invading pathogens (23).

TLR11 appears to recognize uropathogenic E. coli and protect the kidneys from ascending infections by these bacteria, but the molecular basis has not yet been identified (25).

Some TLRs need the presence of coreceptors to activate the signaling cascade. For instance, TLR4 interacts with CD14 and MD2 in the response to LPS stimulation (41) (Fig. 2). CD14 is a 50-kDa glycoprotein that is expressed as a glycosylphosphatidylinositol-anchored molecule (64). It also exists as a soluble molecule in serum (65) and milk (66). CD14 was identified as the binding site for LPS (67). MD-2 is a secreted protein associated with the extracellular domain of TLR4 and, possibly, TLR2 (68,69). MD-2 expression enhances TLR4-dependent activation of nuclear factor (NF)-κB by 2- to 3-fold (68). The details of how these molecules interact with LPS are not well understood as yet.

TLR2 is functionally associated with TLR1 and/or TLR6. The structures of these TLRs are highly homologous. The TLR2/TLR1 heterodimer recognizes triacylated lipopeptides, whereas the TLR2/TLR6 heterodimer recognizes diacylated structures.
of mycobacteria (14,29). Alteration in the physical interaction between the intracellular signaling domains of TLR1 and TLR2 triggers the signaling cascade as shown for mycobacterial LAM (33). TLR2 and TLR6 cooperate in mediating responses to yeast particles, gram-positive bacterial LTA and PGN, and LAM (33). TLR2 and TLR6 cooperate in mediating responses to yeast particles, gram-positive bacterial LTA and PGN, and LAM (33).

**Signaling pathways.** Stimulation of TLRs by PAMPs initiates a signaling cascade that, after different protein-protein interactions, conformational changes, protein kinase-mediated phosphorylation, followed by activation of transcription factors, finally leads to the secretion of cytokines and chemokines that mediate innate immune responses and induce the adaptive immune response (Fig. 2) (12).

In general, ligation of TLRs by their specific ligands (PAMPs) results in the recruitment of the adaptor protein MyD88 (myeloid differentiation factor 88) (70). MyD88 consists of an amino-terminal death domain and a carboxy-terminal TIR domain. MyD88 binds to TLRs through homotypic interactions between their TIR domains. The death domain of MyD88 interacts, in turn, with the death domain of the serine/threonine kinase MyD88. MyD88 then interacts with the death domain of the serine/threonine kinase IRAK1, which mediates the recruitment of another activation domain. IRAK4 phosphorylates IRAK1, which becomes auto-phosphorylated at its NH2 terminus, allowing the recruitment of another adaptor protein, TNF receptor-associated factor 6 (TRAF6) (73). TRAF6, in turn, associates with the mitogen-activated protein kinase (MAPK) kinase kinases transforming growth factor-β (TGFB) to its activated isoforms. The activated MAPK kinases then phosphorylate (Tyr) and activate the transcription factors, including the nuclear factor-kappa B (NF-κB) and the activator protein-1 (AP-1), which mediate the transcription of genes encoding cytokines, chemokines, and other important mediators of innate and adaptive immune responses.

### Table 1: TLR Expression Patterns and Ligands

<table>
<thead>
<tr>
<th>TLR</th>
<th>Cells Expressed</th>
<th>Ligands and Their Origins</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1</td>
<td>Monocytes, polymorphonuclear leucocytes, T and B-cells, NK cells (8,15), mast cells (16)</td>
<td>Forms heterodimers with TLR2 (26)</td>
</tr>
<tr>
<td>TLR2</td>
<td>Myelomonocytic cells (8,15), murine bone marrow-derived mast cells (17), murine T&lt;sub&gt;H&lt;/sub&gt; cells (18), peripheral lymphatic epithelium of the small intestine (lymphatic vessels) (19), human intestinal epithelial cells (20,21), mast cells (16)</td>
<td>LTA, PGN (27,28) gram-positive bacteria</td>
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<td></td>
<td></td>
<td>Lipoproteins, lipopeptides (14,29), gram-positive bacteria</td>
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<td>Fungi conidia and hyphae, Aspergillus fumigatus (30)</td>
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<td></td>
<td></td>
<td>GPI-anchored proteins, Trypanosoma cruzi (31)</td>
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<td></td>
<td></td>
<td>Heat-killed bacteria, Listeria monocytogenes (32)</td>
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<td></td>
<td></td>
<td>LAM, Mycobacteria (33)</td>
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<tr>
<td></td>
<td></td>
<td>MALP-2, synthetic (29)</td>
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<td></td>
<td></td>
<td>Manuronic acid polymers, Pseudomonas aeruginosa (34)</td>
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<td>Membrane-associated proteins, Neisseria meningitidis (35)</td>
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<td></td>
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<td>Outer surface proteins A and B, Borellia burgdorferi (38)</td>
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<td>Pam3Cys, synthetic (29)</td>
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<td></td>
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<td>Phenol-soluble modulin, Staphylococcus epidermidis (26)</td>
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<td>Schistosome egg, Schistosoma mansoni (37)</td>
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<td>Soluble tuberculosis factor (STF), Mycobacterium tuberculosis (38)</td>
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<td>Zymosan, yeast (39)</td>
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<td></td>
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<td>dsRNA, virus (40)</td>
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<td></td>
<td></td>
<td>Polyl(C), synthetic (40)</td>
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<td></td>
<td></td>
<td>Lipoprotein, gram-negative bacteria (41)</td>
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<td></td>
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<td>β-defensin 2, host (42)</td>
</tr>
<tr>
<td>TLR3</td>
<td>DCs (8,15), murine T&lt;sub&gt;H&lt;/sub&gt; cells (18), human intestinal epithelial cells (20,21)</td>
<td>F Protein, RSV (43)</td>
</tr>
<tr>
<td>TLR4</td>
<td>Myelomonocytic cells (8,15), mast cells (16,17), murine T&lt;sub&gt;H&lt;/sub&gt; cells (18), peripheral lymphatic epithelium of the small intestine (lymphatic vessels) (19), human intestinal epithelial cells (20,21)</td>
<td>Heat-sensitive cell-associated mycobacterial factor, Mycobacterium tuberculosis (38)</td>
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<td>Hsp60, Hsp70, GP96, host (44)</td>
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<td>Uncertain, maybe LPS contamination (24)</td>
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<td>Manuronic acid polymers, Cryptococcus neoformans (45), Pseudomonas aeruginosa (34)</td>
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<td>Oligosaccharides of hyaluronan, host (46)</td>
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<tr>
<td>TLR5</td>
<td>Myelomonocytic cells (8,15), murine T&lt;sub&gt;H&lt;/sub&gt; cells (18), human intestinal epithelial cells (20,21), mast cells (16)</td>
<td>Flagellin, gram-negative and gram-positive bacteria (47)</td>
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<tr>
<td>TLR6</td>
<td>Mast cells (16,17), murine T&lt;sub&gt;H&lt;/sub&gt; cells (18)</td>
<td>Forms heterodimers with TLR2 (26)</td>
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<tr>
<td>TLR7</td>
<td>Plasmacytoid DC (15), murine T&lt;sub&gt;H&lt;/sub&gt; cells (18), B cells (22), mast cells (16)</td>
<td>siRNA, virus (48,49)</td>
</tr>
<tr>
<td>TLR8</td>
<td>Myelomonocytic cells (8,15), murine bone marrow-derived mast cells (17), murine T&lt;sub&gt;H&lt;/sub&gt; cells (18)</td>
<td>Imiquimod, R-848, loxoribine, brepinimine, synthetic (50,51)</td>
</tr>
<tr>
<td>TLR9</td>
<td>Plasmacytoid dendritic cells (15), B-cells (22), osteoblasts, fibroblasts (23), murine T&lt;sub&gt;H&lt;/sub&gt; cells (18), mast cells (16)</td>
<td>ssRNA, HIV-1 (48)</td>
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<td></td>
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<td>R-848, synthetic (50)</td>
</tr>
<tr>
<td>TLR10</td>
<td>B cells, plasmacytoid DCs (22)</td>
<td>Unmethylated cpg DNA/ODN, bacteria, viruses (23)</td>
</tr>
<tr>
<td>TLR11</td>
<td>Macrophages, epithelial cells (25)</td>
<td>Unknown (22)</td>
</tr>
</tbody>
</table>

1 Abbreviations: CpG-DNA, cytosine–phosphate–guanosine DNA; DC, dendritic cell; dsRNA, double-stranded RNA; GPI, glycosylphosphatidylinositol; HSP, heat shock protein; HIV, human immunodeficiency virus; LAM, lipoolarabinomannan; LTA, lipoteichoic acid; MALP, macrophage-activating lipopeptide 2; NK cell, natural killer cell; ODN, oligodeoxynucleotide; PGN, peptidoglycan; poly(I:C), polyinosinic–polycytidylic acid; RSV, respiratory syncytial virus; ssRNA, single-stranded RNA; T<sub>H</sub> cell, T helper cell; TLR, Toll-like receptor.
growth factor (TGF-β)-activated kinase (TAK) 1, TAK-1-binding protein (TAB1), TAB2, or nuclear factor (NF)-κB-inducing kinase (NIK). This leads through 1 or more intermediate steps to the phosphorylation and activation of the IkB kinase (IKK). IKK phosphorylates the NF-κB inhibitor IkB, inducing its degradation and the release of NF-κB, which migrates into the nucleus and binds to genes containing NF-κB sites. NF-κB triggers the transcription of a wide variety of cytokines, chemokines, acute-phase proteins, and cell adhesion molecules (74). For instance, IL-1β, IL-6, and IL-8 as well as the costimulating molecules CD80 (B7.1) and CD86 (B7.2) contain κB sequences in their promoters. In most cells, NF-κB activation inhibits apoptosis. In addition to NF-κB, activation of TAK1 results in the activation of 2 MAPK pathways: Jun N-terminal kinase (JNK) and p38 (7,75). JNK can induce a variety of transcription factors including c-Jun, activating transcription factor 2 (ATF-2), Elk-1, and the nuclear factor of activated T cells. The p38 triggers Elk-1 and ATF-2 (76). Furthermore, it stabilizes several proinflammatory mRNAs, as a kind of posttranscriptional regulation of gene expression (77).

To date, 3 additional adapter proteins to MyD88, initiating a MyD88-independent pathway, have been discovered: MyD88 adapter-like (Mal), also known as TIR domain-containing adapter protein (78), TIR domain-containing adapter inducing IFN-β (TRIF), also called TIR-containing adapter molecule-1 (79), and TRIF-related adapter molecule (TRAM), also named TIR-containing protein (80). The activation of TLR4 by LPS from E. coli results in a MyD88-independent, Mal-dependent signaling pathway that leads to the early induction of interferon (IFN)-β. IFN-β activates STAT1-containing DNA-binding complexes in macrophages, leading to the activation of a subset of proinflammatory genes, for example, inducible nitric oxide synthase and inducible protein 10 (IP-10) (78,81). Mal can also collaborate with MyD88 in pathways triggered by TLR2 and TLR4 (82,83).

Engagement of TLR3 and TLR4 triggers a pathway that uses the adapter protein TRIF, which activates the gene-transcription factor interferon regulatory factor 3 (IRF-3), leading to IFN-β secretion. This messenger also subsequently activates the transcription factor STAT1 (79,84).

The third TIR domain-containing adapter TRAM provides specificity for the MyD88-independent pathway of TLR4 and also activates IRF-3 (80).

Further differences in TLR signaling are emerging. For example, TLR2 and TLR4 signaling is controlled by Toll-interacting protein (TOLLIP) which interacts with the TLRs’ TIR domains, resulting in the suppression of IRAK activity (85).

TRAF6 can associate not only with TAK1, TAB1, TAB2, and NIK but also with the apoptosis-stimulating kinase (ASK1, “evolutionarily conserved signaling intermediate in Toll pathway” (ECST)) (7), or “MAPK/extracellular signal regulated kinase kinase kinase” (MEKK) 3 (86). ASK1 activates the JNK–pathway, which induces activation of the transcription factor activator protein (AP)-1. ECST interacts with and activates MEKK1. MEKK1 induces activation of AP-1 as well as of NF-κB (7).

In a recent study, it was shown that MEKK3 is an essential signal transducer in TLR4 signaling. MEKK3 forms a complex with TRAF6 in response to IL-1 and LPS. Furthermore, MEKK3 is needed for IL-1- and LPS-induced activation of JNK and of p38. This pathway is used for LPS-induced IL-6 production (86).

Release of immunomodulating molecules. Depending on the nature and doses of PAMPs, TLRs trigger the expression and production of different cytokines, chemokines, and inflammatory effector molecules. They also induce up-regulation of costimulatory molecules on DCs and regulate the polarization of CD4 T helper (T4) lymphocytes into T4d1, T4d2, or T4d3/T1r subsets. TLRs can be divided into subgroups according to their ability to induce type I interferons (87). TLR3, -7, and -9 induce the production of IFN-α and IFN-β; TLR4 induces only IFN-β, and TLR1, -2, and -6 heterodimers are unable to induce secretion of type I IFNs. Whether TLR5, -8, and -10 control type I IFNs is not yet known (80,87).

Cytokines function as a network for communication between immune cells controlling immune responses or tolerance (Table 2). TLR signaling via the MyD88-dependent pathway triggers distinct gene expression patterns (80) with, on the one hand, secretion of proinflammatory (so called TLR1-type) cytokines such as IL-1, IL-6, IL-8, IL-12, and TNF-α. Some cytokines can induce the production of acute-phase proteins or chemokines, provoke feverish reactions, and up-regulate expression of adhesion molecules on postcapillary venules, thereby triggering the recruitment of inflammatory cells, including neutrophils and monocytes, into tissues. These reactions are essential for eradicating infectious organisms and terminating infection (87).

On the other hand, some PAMPs, for example flagellin, induce TLR2-type cytokines such as IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 (83). Some PAMPs induce antiinflammatory cytokines such as IL-10 and TGF-β, produced by regulatory T-cells (Treg), which lead to tolerance. The MyD88-independent pathway in TLR3 and TLR4 induces type I interferons, which initiate an autoregulatory feedback loop (Fig. 2) and activate IFN-inducible genes such as iNOS. NO is a local mediator that regulates several physiological processes in the human body. In the immune response, NO functions as an antimicrobial effector molecule and regulates various vascular and cellular responses. In the inflammatory process, NO has both proinflammatory and anti-inflammatory properties depending on the type and phase of response (94). At physiological levels, NO protects intestinal mucosa by regulating mucus and bicarbonate secretion and by maintaining mucosal blood flow. NO inhibits leukocyte infiltration, lymphocyte proliferation, and mast cell activation (95).

Nucleotide-binding oligomerization domain receptors. NODs are a family of cytosolic proteins. At least NOD1 (also named CARD4) and NOD2 (also named CARD15) act as PRRs and trigger a signaling cascade leading to inflammatory responses. Moreover, NOD proteins are involved in the regulation of caspase activity and apoptosis (96).

Most NOD-family members contain 3 distinct functional domains: an amino-terminal effector-binding domain, a centrally located NOD, and a carboxy-terminal ligand-recognition domain. Almost all human NODs contain leucine-rich repeats in their carboxyl termini. NODs recognize intracytoplasmatic PAMPs. NOD ligands are able to interact with NODs once bacteria that can lyse the phagosome, such as Shigella, reach the cytosol (97).

NOD1 is expressed ubiquitously. Signaling through NOD1 is required for activating NF-κB in an infection with gram-negative enteric bacteria able to bypass TLR activation (98), indicating that TLRs and NODs act synergistically.

NOD2, expressed by monocytes/macrophages and DCs and induced in intestinal epithelial cells by TNF-α (99,100), is a general sensor for muramyl dipeptide (MDP), the smallest bioactive PGN pattern in all gram-positive and gram-negative bacteria. MDP induces proinflammatory cytokines such as TNF-α and IL-1β via the NF-κB pathway. Additionally, it enhances the
secretion of different cytokines such as IL-6 and IL-12 in monocytes and DCs, as well as the expression of costimulatory molecules. MDP synergizes with LPS in the production of cytokines (96). NOD2 polymorphism is associated with Crohn’s disease, suggesting that bacteria (e.g., Bacteroides species) may play a role in the pathogenesis. The mutations result in the defective secretion of IL-10 associated with a pro-inflammatory phenotype (101). Moreover, Watanabe et al. showed that intact NOD2 signaling inhibits the TLR2 driven activation of NF-κB. The mutations in NOD2 or NOD2 deficiency lead to increased TLR2-mediated NF-κB activity, favoring a TH2-type response (92).

**M cells**

Membranous/microfold epithelial cells (M cells) are located in the follicle-associated epithelium (FAE) overlying mucosa-associated lymphoid tissues such as Peyer’s patches (103). They sample luminal macromolecules and microorganisms and deliver them intact to the underlying lymphoid tissue by transcytosis, enhancing Ag presentation (107). Immature DCs, myeloid (monocyte-derived), lymphoid, and plasmacytoid DCs are found in the organized gut-associated lymphoid tissue and in the mucosal epithelium and lamina propria. Langerhans cells are restricted to the stratified epithelium of the oral cavity, the esophagus, the anus, and the stratified epithelium of the genital tract. The various subsets express distinct cell surface markers (Fig. 3) (8).

Many pathogens including bacteria, viruses, and parasites exploit M cells to invade and infect the host (105). Also some lactic acid bacteria (LAB), for instance Lactobacillus casei, can cross the epithelial barrier by entering through M cells. In this way, they may directly come into contact with immunocytes (106).

### Intestinal antigen-presenting cells

DCs and macrophages are the main professional antigen-presenting cells (APC) found in mucosal tissues including the digestive tract, the airways, and the genital tract. Among the various DC subsets, myeloid (monocyte-derived), lymphoid, and plasmacytoid DCs are found in the organized gut-associated lymphoid tissue and in the mucosal epithelium and lamina propria. Langerhans cells are restricted to the stratified epithelium of the oral cavity, the esophagus, the anus, and the stratified epithelium of the genital tract. The various subsets express distinct cell surface markers (Fig. 3) (8).

They originate from blood precursors and become recruited to mucosal surfaces in response to homeostatic chemokines (CCL20) produced by the epithelium (107). Immature DCs act as sentinels, continuously sampling the mucosal surfaces for Ags. Activation by microbial products terminates endocytosis, enhances Ag presentation, and triggers a switch in the chemokine receptor program (108), allowing the cells to migrate into draining lymph nodes, where they can present the Ags to naïve B and T cells. DC maturation results in the secretion of cytokines and the up-regulation of costimulatory molecules (105).
Figure 3  Antigen presentation to T cells. Abbreviations: Ag, antigen; APC, antigen-presenting cell; CD, cluster of differentiation; DC, dendritic cell; ER, endoplasmic reticulum; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICAM-1, intracellular adhesion molecule 1; IFN, interferon; LFA, lymphocyte function antigen; MHC, major histocompatibility complex; NOD, nucleotide-binding oligomerization domain receptor; TCR, T-cell receptor; TLR, toll-like receptor; TNF, tumor necrosis factor. Internalized Ags are processed and presented by MHC molecules to T lymphocytes. The TCR recognizes processed Ags. Exogenous Ags are presented by MHC class II and generally activate CD4+ T cells, and endogenous Ags are presented by MHC class I, which activate CD8+ T cytotoxic cells. Exogenous Ags can also be loaded on MHC class I molecules in a process called cross-presentation. Pairs of costimulatory molecules play a crucial role in T-cell activation, such as ICAM-1–LFA-1; LFA-3–CD2; CD40–CD40L (CD154); OX40L–Ox40 (CD134); and CD80/86–CD28. Activation and differentiation of naive T cells require a first signal, mediated by the TCR, a second signal, provided by the costimulatory molecules, amplifying the first signal, and a third signal, in the form of chemokines and cytokines (e.g., IL-12) produced by activated DCs, contributing to the differentiation of activated T cells.

**TLR-mediated activation of antigen-presenting cells.** TLR-mediated signaling stimulates the maturation of DCs with the enhanced surface expression of major histocompatibility complex (MHC) molecules, T-cell costimulatory molecules (CD80 and CD86), and other activation markers as well as the induction of reactive nitrogen and oxygen intermediates (RNI and ROI) (58). Maturation increases the ability of the DCs to present Ags and activate T cells.

In mice and men, lymphocyte and DC cell trafficking is mediated by chemokine receptors. Expression of these receptors is modulated by TLRs. Engagement of TLRs by PAMPs down-regulates the expression of proinflammatory chemokine receptors and at the same time up-regulates homeostatic receptors such as chemokine receptor (CCR)7 that mediate the migration of the cells to draining lymph nodes (111–113).

In humans, the various DC subsets express different TLRs and consequently respond to distinct PAMPs (109). Furthermore, in response to PAMPs, the different DC subsets produce different chemokines that activate natural killer (NK) and naive T cells. TLR2-mediated signaling induces, for instance, the up-regulation of the surface expression of T-cell costimulatory molecules (CD80, CD86, and CD40) and the production of cytokines (7,114). These include, most significantly, TNF-α and IL-6, which are mainly produced by macrophages. Macrophages primarily produce IL-10, whereas DCs primarily produce IL-12. Furthermore, macrophages produce 10 times more NO than DCs. DCs may produce superoxide anion in principle but fail to react to LPS (113).

Depending on which TLR is engaged, T-cell differentiation into Th1 or Th2 type or even tolerance can be induced. Treg cells express different TLRs (18) and consequently can possibly be influenced directly by PAMPs. Altogether, these observations show that TLRs not only are crucial in the early phase of an infection when the innate immunity is activated but also link innate and adaptive immunity.

**Ag presentation to T cells.** Ag presentation requires a processing of the Ags with the generation of peptides that become loaded on MHC class I or class II molecules. Exogenous Ags are presented by MHC class II and generally activate CD4+ T cells, whereas Ags on MHC class I activate CD8+ T cytotoxic cells. The T-cell receptor (TCR) recognizes peptide Ags that are embedded in the peptide-binding part of MHC molecules. Generally, MHC I-restricted Ags are derived from endogenous proteins such as viral proteins, produced during viral synthesis. During intracellular processing in proteasome, peptides are generated that are then transported into the endoplasmic reticulum by “ATP-binding cassette” proteins required for the effective interaction with T cells, and probably also with B cells (87,109). In this way, an Ag that was recognized, for example, in the epithelium can be presented to T cells localized in the lymph nodes.

Immature DCs have been shown to migrate into the epithelium, opening the tight junctions that seal the epithelial intestinal cells and capturing Ags via their dendrites without disrupting the barrier function of the gut (Fig. 1) (110). DCs are also involved in the sampling of Ags and bacteria that are transported through M cells. The FAE constitutively produces the chemokine CCL20, responsible for the chemotactic migration of DCs into the subepithelial dome of Peyer’s patches (1).
transporters, the “transporter associated with Ag processing” (TAP) 1 and 2 molecules and become associated with the MHC class molecules. They are then transported via the Golgi apparatus to the cell surface. In contrast, MHC–II–restricted Ags are mainly derived from exogenous proteins collected in endosomes, which, after transport within intracellular compartments, are proteolyzed and bound to MHC class II molecules and reexported to the cell surface. A third pathway, termed cross-presentation, allows Ags internalized in phagosomes to be transported into the cytosol and processed into peptides by the proteasomes and transported back into the phagosomes where the peptides are loaded on MHC class II molecules. The protein and peptide transport machinery is derived from the endolysosomal reticulum that fuses with the phagosomes (115,116). This can lead to activation of naïve CD8+ T cells (117). Presentation of Ags occurs after the T cells and DCs have established immunological synapses mediated by adhesion molecules including DC ICAM-1 (intracellular adhesion molecule), which binds to LFA-1 (lymphocyte function antigen) on lymphocytes. CD2, expressed by T cells, is also found in the synapse in association with the TCR and participates in T-cell activation. CD2 is a receptor for LFA-3, expressed on all APCs. Additional costimulatory molecules include CD40, which interacts with CD40L (CD154) on T cells, and the B7 family molecules including CD80/86, that interact with CD28, as well as OX40L that binds to OX40 (also named CD134). Cytokines produced by DCs participate in T-cell activation and Th1-cell polarization (Th1 vs. Th2) (Fig. 3).

Thus, activation of T cells requires 2 signals, the TCR and the costimulatory signals, which are amplified by cytokines. In the absence of the second signal, the T cells become tolerant (anergy) (117,118).

Lymphocyte differentiation

Following infection by pathogenic organisms, the immune system has to select appropriate defense mechanisms. If the response is too weak, infection may not be cleared, and if it is too strong, inflammatory responses may lead to tissue damage (119). Although the mechanisms that control the type of adequate adaptive response remain poorly understood, TLRs and PAMPs appear to play critical roles in regulating Th1–thymocyte differentiation. Th1 responses are usually associated with inflammatory reactions, and Th2 cells with allergic responses and parasite clearance, whereas Threg cells are essential in modulating the immune response, preventing overreactors. Directing T helper (Th1) cells into the Th1 or Th2 direction seems to depend on the dose of Ag, on the type of PAMPs recognized by TLRs, and on the DC subset (myeloid, lymphoid, or plasmacytoid) that present the Ag (109). The different DC subsets express distinct TLRs as well as the costimulatory molecules (120) (Fig. 4). Short antigen stimulation leads to the development of Th0 cells, which produce a broad spectrum of cytokines. Differentiation of Th1 and Th2 cells requires prolonged antigenic stimulation (109,121). The costimulatory molecules CD80/86, CD40, and OX40L are thought to regulate Th1 differentiation (120). CD86 binding to CD28 is required for the development of Th1 responses (122), and CD40 engagement appears to be crucial for IL-12 production and Th1 polarization (123).

Some cytokines are released by both cell types, e.g., IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), and TNF-α, whereas Th1 cells secrete IL-2, IL-12, IL-18, IFN-γ, and TNF-β, and Th2 cells produce IL-4, IL-5, IL-9, IL-10, and IL-13 (Table 2). Th1 cells express distinct chemokine receptors that allow them to migrate and home to different mucosal sites. Th1 cells preferentially express CCR5 and CXCR3, and Th2 cells express CCR3 and CCR4 (112). Th1 cells provide help to CD8+ cells and activate macrophages, and Th2 cells enhance humoral immunity and activate eosinophils (Eo) as well as mast cells, triggering the release of the content of their vacuoles. Regulatory loops amplify Th1 or Th2 responses. Thus, IFN-γ produced by Th1 cells inhibits the differentiation of Th1 cells, and IL-10 secreted by Th2 cells reduces secretion of Th1 cytokines, suppresses macrophage activation, and possibly even influences cytotoxic T cells and NK cells. Some studies indicate that IL-10 blocks IL-12 expression by inhibiting NF-kB activation, but how these pathways interact remains unclear (122).

Threg cells constitute a third group of CD4+ T-lymphocytes comprising naturally occurring Threg cells and induced Threg cells. Naturally occurring CD4+CD25+ Threg cells represent 5–10% of the peripheral CD4+ T-cell population (124) and appear to maintain tolerance to self-Ags (125). They also play a role in regulating pathogen-specific responses. TCR signaling via APCs is required for the activation of CD4+CD25+ Threg cells (124). CD4+CD25+ Threg cells have suppression activity and act in a cell contact-dependent manner (126). Induced Threg cells are derived from regular CD4+CD25+ T cells and show variable expression of CD25 (124). In the gut, 2 additional immunomodulatory CD4+ T cells have been identified: Tr1 cells that mediate bystander suppression by secreting IL-10 and Th3 cells that produce TGF-β, and are believed to play a role in oral tolerance (125). The differentiation pathway of Th3 cells is probably induced by immature or semi-mature DCs and anti-inflammatory cytokines (IL-10 and TGF-β) in the absence of proinflammatory signals (124,127). For instance, lactobacilli from the gut flora can induce partial DC maturation but fail to trigger proinflammatory cytokines (90,127). The lack of CD40 up-regulation on DCs seems to induce Th1 cells as well (128).

The role of DCs in the induction of a Th1 immune reaction is better characterized. The uptake of Ags or the recognition of PAMPs in peripheral tissues leads to the maturation and migration of DCs to lymph nodes (87). Ag presentation by MHC II molecules on mature DCs in the presence of costimulatory molecules results in the production of IL-12 and the activation of naïve T-cells leading to a Th1 phenotype (120). Different TLR agonists can induce the same Th1–cell polarization, which reflects differential TLR expression by the different DC subsets. Thus, mouse plasmacytoid DCs, in response to CpG and myeloid DCs after LPS stimulation, induce a strong Th1 response reflecting high levels of TLR9 on plasmacytoid DC and high levels of TLR4 on myeloid DCs (109).

Protein allergens, in the absence of PAMPs, do not activate APCs (121). For instance, inflammation in the airways of TLR4-deficient mice exposed to allergens is reduced, probably because of decreased CD86 expression by DCs and a resulting Th2 response (129). DCs coincubated with LPS and histamine express less CD86 than DCs matured in the absence of histamine, suggesting that histamine induces a Th2 response by selective up-regulation of the expression of a costimulatory molecule (122).

Different TLR agonists induce different Th1 responses in human DCs, depending on whether the MAPK or NF-kB signaling pathway is activated. E. coli LPS, via TLR4, triggers a MyD88-dependent Th1 response in DCs with the production of IL-12p70, and flagellin, via TLR5, induces a MyD88-dependent Th1 response with the production of IL-12p40 but not IL-12p70 (37,93). Bacterial lipopeptides induce a Th1 polarization in a TLR2 and IL-12-dependent manner. Pathogens, by their PAMPs, contribute directly to the maintenance and activation
of long-term T-cell memory in both Ag-dependent and Ag-independent manners (130).

**Discrimination between pathogens and commensals**

Intestinal mucosal surfaces are in continuous contact with a heterogeneous population of microorganisms of the endogenous flora (up to $10^{11}$–$10^{12}$ per gram of luminal material in the colon) and are exposed to food and microbes. A major role of mucosal epithelia is the barrier function that is essential in preventing the colonization or invasion of the host by foreign microorganisms. Why commensals do not trigger proinflammatory host responses under normal conditions is not yet fully understood. The absence of inflammation could result from a lack of PAMPs produced by the normal flora or their dilution in the intestinal lumen. Most commensal anaerobic bacteria in the gut, including *Bacteroides*, *Bifidobacterium*, or *Eubacterium* species, are poorly characterized genetically and phenotypically (131), and thus, their PAMPs have not yet been identified. Enterocytes may be relatively unresponsive to TLR agonists and may not produce TLRs or functional signaling complexes. Normal, nondiseased human intestinal epithelial cells and lamina propria cells express TLR3 and TLR5 but little TLR2, TLR4, or their coreceptor MD-2 (20,21). Furrie et al. showed that TLR2 and TLR4 are expressed only in crypt epithelial cells, and expression was lost as the cells matured and moved toward the gut lumen (132). The lack of TLR2, TLR4, MD-2, membrane-linked CD14, or other molecules on epithelial cells probably explains the nonresponsiveness of the gut to LPS and commensal organisms (20,132,133), but the presence of TLR5 and TLR3 allows the epithelium to sense infections mediated by flagellated bacteria as well as components of enteropathogenic bacteria and dsRNA of enteroviruses (20,132). Additionally, intestinal epithelial cells express high levels of TOLLIP, which inhibit the TLR2 and TLR4 pathways, thus preventing chronic proinflammatory responses to commensals (133). Most microorganisms are concentrated in the lumen or trapped in the thick mucus layer covering the intestinal mucosa. A necessary step in successful colonization is the ability of bacteria to adhere to host surfaces. Generally, binding to intestinal host cells is essential for bacteria to resist the fluid flow of luminal contents and the peristalsis of intestinal contraction (134). Some microorganisms, in particular pathogens, are equipped with virulence factors that facilitate their penetration of mucus layers, their specific binding to epithelial surfaces, and their resistance to microbicidal components such as secretory immunoglobulin A, which is released in the intestinal lumen to prevent epithelial colonization by...
bacteria and viruses. Furthermore, immune reactions may require the internalization of PAMPs, as shown for the microbial PGN from the invasive enterobacteria Shigella flexneri that is recognized by the cytosolic PRR NOD1 (97,100). Other receptors that are not discussed in this article, such as dectin-1 and neonatal Fc receptors, can also recognize microbes and activate inflammatory or noninflammatory signaling (102,119).

Salmonella: a typical invasive pathogen. Salmonella are gram-negative bacteria that cause food-borne infections in humans and animals leading to gastroenteritis or typhoid fever depending on the species. More than 1000 serotypes are known.

S. typhimurium can cross the epithelial barrier through M cells, enterocytes (134), or via intraepithelial DCs that send dendrites between the epithelial tight junctions to sample bacteria directly (110) (Fig. 1). S. typhimurium induces NF-κB-dependent inflammatory responses. Flagellin is 1 of the major virulence factors that triggers proinflammatory cytokines in gut epithelial cells (47,135). TLR5 recognizes monomeric flagellin, the subunit of the flagellum of flagellated gram-positive and gram-negative cells. TLR5 is expressed on monocytes, immature DCs, and epithelial cells (47,107). Flagellin autoassembles into 11 protofilaments that form a 20-nm-large hollow cylinder. The mechanism responsible for the release of monomeric flagellin in the bacterial environment is not fully understood (136). It has been proposed that flagellin, which is secreted into the intestinal lumen, does not induce inflammation because the TLR5 is restricted to the basolateral site of the enterocytes (61). Restriction of TLR5 to the basolateral cell surface was observed in human carcinoma-derived enterocytes (T84) (61) but not in the normal human colon enterocytes (107). There are probably other mechanisms that induce nonresponses to flagellin, as suggested by the report of Mize and Snipes, who demonstrated that exposure of human monocytes, THP1-cells, Jurkat, and COS-1 cells to gram-negative flagellin results in a subsequent state of flagellin unresponsiveness or tolerance (137).

Flagellin stimulates the production of a large number of proinflammatory (IL-8, IL-12, IL-18, NO, defensins) and chemokine (CCL20 and CCL23) genes in Caco-2, T84 cells, and human peripheral blood mononuclear cells (PBMCs) (55, 138,139). Following flagellin stimulation, Caco-2 and T84 cells induce both CCL20 and IL-8 mRNA accumulation, but only CCL20 is synthesized and released in the medium, explaining the selective recruitment of immature DCs (107). IL-8 secretion that is induced by Salmonella is mediated by the p38 MAPK pathway, whereas commensal bacteria are unable to induce this pathway (140). Flagellin leads to the maturation of human DCs associated with the up-regulation of CD83, CD86, CR7, and MHC II molecules and promotes MD88-dependent TLR2-type responses (93,141). Intraperitoneal injection of bacterial flagellin to mice induced systemic IL-6 secretion (47).

Probiotics. Probiotics are microorganisms with beneficial health effects on the host (142). Probiotic bacteria used in food are mainly strains of gram-positive LAB, in particular lactobacilli and bifidobacteria. Strain- and host-specific adhesion of LAB to intestinal cells has been shown in vitro and in vivo. So the Lactobacillus rhamnosus strain Gorbach and Goldin (LGG), for instance, has a strong affinity for enterocyte binding sites of Caco-2 cells (143). In vivo, bifidobacteria interact with mouse epithelial cells of the small intestine and are internalized into the epithelial cells of the large intestine via the FAE cells (144). LAB use distinct pathways of gut internalization to make contact with the immunocytes in the underlying tissue.

The probiotic L. casei, for instance, is internalized by M cells and by FAE cells of Peyer’s patches. In contrast, the yogurt bacteria S. thermophilus, L. acidophilus, and L. delbrueckii ssp. bulgaricus interact with the immunocytes of Peyer’s patches through the FAE (106).

By these interactions, various kinds of strain-specific immunomodulations can be brought about as observed in many trials (Table 3).

As shown in macrophages, LAB can differentially induce cytokine production in a concentration-dependent manner (143). Not only the individual titers of produced cytokines but also the cytokine patterns seem to be dose-dependent, as the ratio between the cytokine titers varied with bacterial concentration. Christensen et al. found that a concentration of 10 mgL L. casei induced a high level of IL-12, IL-6, and TNF-α but almost no IL-10; 100 mgL induced the same level of IL-12 but a much greater level of IL-10 (90). Pathogens, such as streptococci, are generally more potent inducers of proinflammatory cytokines than LAB (89). But there are also differences between LAB strains. This may be caused by variations in the cell wall components, e.g., LTAs and PGNs of different LAB strains (143).

L. casei, in contrast to L. reuteri, induces IL-12, TNF-α, MHC II, and CD86 in DCs. L. reuteri, however, can inhibit L. casei-induced proinflammatory cytokines whereas the antiinflammatory cytokine IL-10 remains unaltered (90). In contrast, Borruel et al. reported that 2 L. casei strains reduced the release of TNF-α in human intestinal mucosa cells and prevented the inflammatory reaction induced by a nonpathogenic E. coli strain (156). LGG was shown to inhibit LPS- or LTA-induced TNF-α secretion by macrophages. When macrophages were exposed to both LPS and LTA, the inhibition was only partial, suggesting that simultaneous TLR2 and TLR4 engagement partially overcomes the TNF-α-inhibitory activity of LGG. Based on these observations, it was proposed that probiotics produce soluble immunomodulatory substances. Because TNF-α was the only cytokine inhibited in this study, inhibition does not seem to involve the NF-κB pathway (160). Lan et al. revealed that LGG suppressed E. coli- and B. ovatus-induced proinflammatory cytokine mRNA accumulation and protein secretion in a murine colonic epithelial cell–bacteria coculture system (165).

In a mouse allergy model, mice fed with L. casei strain Shirota had a significantly lower titer of ovalbumin-specific Ig E compared with nonprobiotic controls. In addition, the levels of T111-associated cytokines, such as IFN-γ and IL-2, produced by splenic cells were higher than those in the control group, in contrast to T12-associated cytokines (IL-4, IL-5, and IL-6) (150). B. adolescentis and B. longum, which are commonly found to be the main residents of the adult bifidobacterial flora, triggered more proinflammatory TNF-α, IL-6, and IL-12 secretion but induced less IL-10 production, compared with infant-type bifidobacteria (153). Moreover, 4 different strains of LAB have been shown to reduce the T12 cytokine production by PBMCs from allergic patients in vitro (154). These differences in cytokine induction are explained by variations of PGN in the cell wall of the different bifidobacteria. Furthermore, unmethylated CpG-DNA motifs are present in varying amounts in different bacterial species and strains (153). A lactobacillus strain, as well as a bifidobacterium, attenuated colitis in IL-10–deficient mice, most likely through the observed reduction of T111 cytokines and the induction of the TGF-β (157).

More recently, the probiotic bacterium L. plantarum was shown not to induce IL-1β, TNF-α, and IFN-γ production in healthy or inflamed gut mucosal cells, but significantly higher IL-10 titers indicate an antiinflammatory effect (161).
### TABLE 3: Studies on the induction of immune modulating molecules and effector substances by lactic acid bacteria

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</tbody>
</table>

$^1$↑ induction or increase, ↓ suppression or reduction, ↔ no effect, ( ) small effect. Abbreviations: CD, cluster of differentiation; IFN, interferon; iNOS, inducible nitric oxide synthase; LAB, lactic acid bacteria; LGG, Lactobacillus rhamnosus Gorbach and Goldin; LTA, lipoteichoic acid; MHC, major histocompatibility complex; NF-κB, nuclear factor κB; NK cell, natural killer cell; PBMC, peripheral blood mononuclear cell; TGF, transforming growth factor; T$_b$ cell, T helper cell.
Bacterial DNA recovered from feces, collected after probiotic administration, induced IL-10 and reduced IL-1β in PBMCs, whereas the DNA recovered from untreated individuals induced higher IL-1β than IL-10 secretion. The high CpG content of bifidobacterium species suggests that the DNA composition may account for the enhanced IL-10 secretion (158). DNA from probiotic bacteria was shown to protect mice from experimental colitis in a TLR9-dependent manner (168).

Probiotic bacteria regulate the delicate balance between necessary and excessive immune responses. So probiotics support an adequate immune response in case of infection by enhancing Th1 responses, associated with the release of proinflammatory cytokines (IL-10 and TGF-β) (Table 3), probably through the induction of regulatory Tr1 cells and Th3 cells. IL-10 and TGF-β suppress Th2 cells, which are associated with allergic reactions. This suppression is enhanced by the Th1 polarization that further suppresses Th2 reactions (Fig. 5). Therefore, probiotics can be useful in preventing and treating allergic disorders, switching a Th2 toward a Th1 response. This reflects the higher incidence of allergy reaction in children with low levels of LAB or an “adult-like” Bifidobacterium flora (169–171). Supplementation of the diet with LGG used as a single strain or in combination with B. lactis Bb-12 or L. reuteri reduces the frequency and/or severity of atopic eczema in children (172–176). Not all probiotic strains, however, can induce a Th1-to-Th3 switch.

Probiotic bacteria may also protect the intestine by competing with pathogens for attachment, by strengthening tight junctions between enterocytes, or by enhancing the mucosal immune response to pathogens (133). A preparation containing lactobacilli and bifidobacteria was found to enhance the epithelial barrier function, thus reducing Salmonella invasiveness (177), probably by competing for epithelial binding sites or receptors. Along the same line, cow’s milk-induced gut permeability impairment in suckling rats was reduced by LGG (178). Changes in the gut tightness may indirectly influence immune responses that control the amount and quality of Ags that reach gut lymphoid cells and organs.

Probiotics have been shown to be beneficial in inflammatory bowel diseases and antibiotic-associated diarrhea (155). The duration of acute diarrhea in children and the amount of shed rotaviruses in the stools have been reduced following the administration of LGG as a single strain or in combination with L. reuteri (179–181). The frequency of airways infections was also reduced in children who received LGG-enriched milk

Figure 5 Hypothesis of immunomodulation by probiotics. Abbreviations: B, B cell; DC, dendritic cell; Eo, eosinophil; IFN, interferon; Mø, macrophage; MC, mast cell; PAMP, pathogen-associated microbial pattern; TGF, transforming growth factor; Th cell, T helper cell; −, inhibition; −−, induction. In case of exposure to high doses of PAMPs following infection, probiotics can support an adequate immune response by enhancing Th1 responses. Th1 response is associated with the release of proinflammatory cytokines, resulting in enhanced phagocytic activity and activation of NK cells. In case of exposure to low doses of PAMPs, probiotics suppress the Th2 response by promoting Tr1/Th3-mediated secretion of the antiinflammatory cytokines IL-10 and TGF-β. This suppression is enhanced by the probiotics-mediated stimulation of Th1 cells, which counteract Th2 reactions by IFN-γ release.
for 7 mo in day-care centers (182). In adults, the intake of a mixture of 3 probiotic strains for at least 3 mo significantly shortened common cold episodes by almost 2 d and reduced the severity of symptoms (183).

These results suggest that probiotics may also have extra-intestinal effects. But the beneficial effect of probiotics on healthy people is likely to be limited to risk reduction, in terms of intestinal effects. But the beneficial effect of probiotics on severity of symptoms (183).

shortened common cold episodes by almost 2 d and reduced the mixture of 3 probiotic strains for at least 3 mo significantly for 7 mo in day-care centers (182). In adults, the intake of a probiotic. A better understanding of the intestinal innate immune response and the molecular interaction between the gut microbiota and the host will help to design better prophylactic or therapeutic strategies to prevent inflammatory bowel diseases and allergic or autoimmune reactions.

**Literature Cited**


143. Miettinen M, Lehtonen A, Julkunen I, Matikainen S. Lactobacilli and microbiota basis of microflora-host interactions 771S


