Toll-like receptor (TLR) signaling represents one of the best studied pathways to implement defense mechanisms against invading microbes in human being as well as in animals. TLRs respond to specific microbial ligands and to danger signals produced by the host during infection, and initiate downstream cascades that activate both innate and adaptive immunity. TLRs are expressed by professional immune cells and by the large majority of non-hematopoietic cells, including epithelial cells. In epithelial tissues, TLR functions are particularly important because these sites are constantly exposed to microorganisms, due to their location at the host interface with the environment. While at these sites specific defense mechanisms and inflammatory responses are initiated via TLR signaling against pathogens, suppression or lack of TLR activation is also observed in response to the commensal microbiota. The mechanisms by which TLR signaling is regulated in mucosal epithelial cells include differential expression and levels of TLRs (and their signaling partners), their cellular localization and positioning within the tissue in a fashion that favors responses to pathogens while dampening responses to commensals and maintaining tissue homeostasis in physiologic conditions. In this review, the expression and activation of TLRs in mucosal epithelial cells of several sites of the human body are examined. Specifically, the oral cavity, the ear canal and eye, the airways, the gut, and the reproductive tract are discussed, along with how site-specific host defense mechanisms are implemented via TLR signaling.

**Keywords:** epithelial cells, mucosal tissues, pattern recognition receptors, immunity, bacteria

INTRODUCTION

All organisms have some form of protective mechanisms against pathogens. In many instances, innate immunity functions are the first, and sometimes, the only barrier to infection by invading organisms. In human beings, innate immunity is not only mediated by professional immune cells, but also by non-professional cell types that contribute to defense responses by secreting substances with anti-microbial activity and inflammatory mediators that favor rapid and direct involvement of professional immune cells. Many, if not all, of these responses are dependent on detection of invading microorganisms. The toll-like receptors (TLRs) family is one of the best characterized among several cellular effectors for pathogen detection (1). TLRs are a family of trans-membrane proteins widely expressed by eukaryotic cells and recognize ligands that are present in virtually all types of microorganisms. Once binding takes place, activation of signaling pathways downstream of TLRs plays a major role in directing both innate and adaptive host immune responses. Thus, TLRs represent one of the first and most important lines of defense against bacterial, viral and fungal pathogens and parasites that may interact with and harm the human host. However, two major aspects relative to TLR-dependent pathogen recognition and subsequent responses need to be carefully considered: most microorganisms that colonize the human host are not pathogens, and, depending on the site of colonization/infection, different defense responses may be necessary or appropriate to counteract such infections. This review explores how TLR signaling is regulated in mucosal epithelial cells to mediate specific host responses to commensal or pathogenic microbial infections. Such control is exerted via a number of mechanisms, including regulation of receptor expression levels, cellular localization (i.e. cytosolic or surface expression) and positioning within the tissue (apical or basolateral expression), and also depending on the tissue body site.

TOLL-LIKE RECEPTORS: OVERVIEW OF STRUCTURE AND SIGNALING PATHWAYS

Toll-like receptors were discovered almost two decades ago and their importance in regulation of immune responses was immediately recognized, enhancing our understanding of many phenomena that define host innate and adaptive immunity. TLRs recognize microbial and viral products with specific structural features. Such products are classified as pathogen-associated molecular patterns (PAMPs) (2). As many microorganisms colonize the human host without causing disease, the term CAMPs has been introduced for commensal-associated molecular patterns (3). As many microorganisms colonize the host without causing disease, the term CAMPs has been introduced for commensal-associated molecular patterns (or the more generic term MAMPs, for microbial-associated molecular patterns) that are also recognized by TLRs (3). In addition, endogenous ligands that induce inflammation in the absence of infection can also activate TLR-dependent signaling and are defined as danger-associated molecular patterns (DAMPs) (4).
Toll-like receptors are trans-membrane proteins that contain a horseshoe-shaped extracellular or cytoplasmic leucine-rich repeat (LRR) domain and an intra-cytoplasmic toll/IL-1R (TIR) domain [homologous to the corresponding intracellular domain of the IL-1 receptor (IL-1R)], which are connected by a single trans-membrane domain. The LRR domain is responsible for ligand recognition and the TIR domain for intracellular signal transfer.

In humans 10 TLRs have been identified to date and comprise both extracellular and intracellular receptors (1). TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 are surface-expressed and recognize extracellular microorganisms and ligands. TLR3, TLR7, TLR8, and TLR9 are intracellular, localizing into cytosolic endosomal compartments via a UNC-93B-assisted translocation mechanism (5), and are engaged by microorganisms and ligands that have already crossed the cell membrane barrier. In some instances, intracellular TLRs, such as TLR3 and TLR9, can be expressed on the cell surface, and extracellular TLRs, such as TLR4, can also have an intracellular localization, depending on the cell type (6, 7). For all TLRs, ligand binding to the LRR domain induces formation of receptor homodimers or, in some cases, heterodimers (1). A resulting TIR domain conformational change allows interactions between TIR domains of adjacent TLRs and binding of additional adaptor proteins essential for triggering intracellular signaling cascades. Adaptor proteins identified to date include the myeloid differentiation factor 88 (MyD88) (8), the MyD88 adaptor-like (Mal) (9) [also called TIR domain-containing adaptor protein, TIRAP (10)], the TIR domain-containing adaptor protein inducing interferon-β (TRIF) (11) [also called TIR-containing adaptor molecule, TICAM (12)], and the TRIF-related adaptor molecule (TRAM) (13). TLR signaling is also subject to negative regulation by a variety of inhibitory factors, including the Toll-interacting protein (Tollip), IRAK-M, the sterile α- and HEAT-Armadillo-motif-containing protein (SARM), and the B cell adaptor for PI3K (BCAP) (14), which inhibit downstream steps in the TLR-dependent signaling cascades. The crystal structure of several TLRs has been solved, either alone or in complex with ligands (15–19), expanding our understanding of the molecular mechanisms of TLR activation and of the co-factors that are required for signaling.

With the exception of TLR3, all TLRs require MyD88 recruitment to the TIR domain. TLR2 and TLR4 signaling require not only MyD88 but also the cooperation of Mal/TIRAP (Figure 1). Through MyD88, members of the IL-1R-associated protein kinases (IRAKs) IRAK4, IRAK1, and IRAK2 are activated (20). This is directly followed by activation of the tumor necrosis factor receptor-associated factor-6 (TRAF6) (21) and RIP (22), which proceed to activate a complex made of TGF-β-activated kinase 1 (TAK1) and TAK1-binding proteins (TAB1, TAB2, and TAB3). Lastly, gene expression regulatory factors of the MAPK family (ERK, JNK, p38) and NF-κB are activated (Figure 1), inducing cell survival and proliferation, immune cell activation, production of pro-/anti-inflammatory mediators (cytokines and chemokines), interferons, and anti-microbial products. Activation of intracellular TLR7, TLR8, and TLR9 also proceeds via MyD88, but can trigger TRAF6, IRAK4, and TRAF3-dependent activation of IRF7, which translocates to the nucleus and induces production of type-I interferon (13, 23) (Figure 1).

A MyD88-independent pathway is triggered by TLR3 and TLR4 (in addition to the TLR4/MyD88-dependent signaling pathway) and, potentially, by TLR2 (24, 25). The TLR3 MyD88-independent pathway is mediated by TRIF and the TNF receptor associated factor protein TRAF3, inducing non-canonical IκKs, TBK1, and IκKs pathways, activation of IRF3, and secretion of type-I IFNs (26, 27) (Figure 1). TLR3-TRIF signaling can also drive activation of MyD88-dependent downstream components TRAF6 and RIP1, thus converging on activation of NF-κB (Figure 1). The TLR4/MyD88-independent pathway leads to recruitment of TRIF via activation of TRAM and downstream segregation of cell activation via both TRAF6/RIP1 and TRAF3/IRF3 pathways (27) (Figure 1).

**TLR LIGANDS: OVERVIEW OF INTERACTIONS**

Toll-like receptors recognize a large variety of structurally defined, but not necessarily structurally related ligands. TLR3, TLR5, TLR7,
TLR8, and TLR9 recognize “unique type” of ligands. TLR3 recognizes viral double-strand RNA (dsRNA) and synthetic analogs of dsRNA, while TLR7 and TLR8 recognize viral single-strand RNA, miRNA and the anti-viral compounds, imidazoquinolines (31–34), and TLR9 recognizes unmethylated CpG DNA of bacterial and viral origin (35) as well as the malaria pigment, hemozoin (likely due to its being coated with malarial DNA (36)). A unique ligand for TLR10 is currently unknown but this receptor is thought to heterodimerize with TLR2 and share recognition of ligands that are in common with those that are recognized by TLR1. Recently, a role for TLR10 has been suggested in responses to pathogen infections (37, 38), Crohn’s disease (39), and even cancer (40).

Ligand discrimination by TLR2 and TLR4 is a complex process that is not only dependent on ligand compatibility but also requires the presence of specific co-receptors and accessory molecules. In the case of TLR4, recognition of its best characterized ligand, bacterial lipopolysaccharide (LPS) occurs when this is in complex with the accessory molecules lipid binding protein (LBP) and the lipid A binding protein (CD14), and is presented to TLR4 in the presence of specific co-receptors and accessory molecules. In the presence of CD14, all LPS types can induce TLR4 activation via both pathways. In the absence of CD14, vs. rough or lipid A). In the presence of CD14, all LPS types can induce TLR4 activation via both pathways. In the absence of CD14, smooth LPS fails to induce TLR4 activation, while lipid A induces signaling via Mal/MyD88 (42). The details of the molecular interactions of TLR4 with its accessory molecules and ligands and have been elucidated in elegant crystal structure studies (17). A novel factor involved in TLR4-mediated signaling has been described, the TLR4-interactor with LRRs, TRIL, which is highly expressed in the brain and enhances TLR4-dependent signaling by LPS (43). Some types of bacterial LPS signal via TLR2, for example Porphyromonas gingivalis LPS, although some components of TLR4-dependent signaling are also involved (44). In addition to LPS, TLR4 also recognizes viral components and endogenous ligands, such as β-defensin 2 (45), high mobility group box 1 protein (HMGB1) (46), fibronectin extra domain A (F-EDA), heat shock proteins and other molecules (47), although the contribution of contaminating LPS to the effect of some of these molecules is still unclear.

An even more complex picture characterizes signaling via TLR2, which can form heterodimers with either TLR1 or TLR6. TLR2 recognizes a broad range of ligands with very different structural features. The first described TLR2 ligands are lipopeptides and lipoproteins, shown to engage TLR2/TLR1, TLR2/TLR6 heterodimers depending on different acyl group patterns. The synthetic triacylated lipoprotein Pam3CSK4 is a specific ligand for the TLR2/TLR1 dimer (48) while the diacylated lipoprotein Pam2CSK4 binds to and signals via the TLR2/TLR6 dimer (but can also function via TLR2/TLR1) (49, 50). The molecular and structural details of the TLR2/TLR1- and TLR2/TLR6-ligand complexes have been elucidated by co-crystallization studies that have paved the way in defining these sophisticated interactions and the role of the accessory molecules CD14, LBP, and CD36 in ligand-driven complex formation (16, 51, 52). Other bacterial ligands for TLR2 include cell wall components such as lipoteichoic acid (LTA) (53), glycolipids, lipoarabinomannan (54), β-glucans (55) and zymosan (56). TLR2 activation by peptidoglycan (PG) is controversial and this molecule is also known to signal via another intracellular pattern recognition receptor, Nod2, a member of the nucleotide oligomerization domain (NOD)-like receptors (NLRs) family (57–59). In addition, bacterial ligands of diverse origin with no structural similarities and no lipid components have also been shown to activate cells via TLR2 signaling, for example porins and toxins. Porins from Neisseriae species, Fusobacterium nucleatum and Chlamydia induce TLR2/TLR1-dependent signaling (60–64), while Shigella and Salmonella porins induce signaling via TLR2/TLR6 (65, 66).

Haemophilus porin is also considered to be a TLR2 ligand (67). Other well-described TLR2 protein ligands are the pentameric B subunit of the Escherichia coli type II heat-labile enterotoxin [LT-Ile-B (5)] (68), bacterial fimbraea (69) and the PEP18 protein from Mycobacterium tuberculosis (70).

Furthermore, endogenous ligands and DAMPs are also associated with TLR2-dependent signaling, including HSPs, HMGB1, uric acid, fibropectin and other extracellular matrix proteins, and some types of LPS, as discussed in the previous section.

**TRLS IN HUMAN EPITHELIAL CELLS: OVERVIEW OF EXPRESSION AND FUNCTIONS**

In humans TLR expression is nearly ubiquitous in immune cells, where it drives innate and adaptive immune mechanisms such as activation of antigen-presenting cells (APCs), secretion of inflammatory mediators, T cell differentiation and antibody production. By contrast, TLR expression is less widespread in cells of non-hematopoietic origin, such as epithelial cells (Table 1). Since TLRs are specialized in recognition of microbial products, it appears reasonable that they have evolved to be localized at the best potential host/microbe interface for a rapid initial response. For example, depending on the cell type and the body location, TLR protein expression may not be detected despite the presence of TLR mRNA, extracellular TLRs may present an intracellular localization in endosomal compartments (i.e., TLR4) while intracellular TLRs can be found on the cell surface (i.e., TLR3 or TLR9), and selected TLRs can be expressed in a tissue-specific manner. TLR-dependent activation of immune responses by a pathogen is indiscriminately triggered in APCs, but a similar modality of activation of epithelial cells may lead to unnecessary responses to the large number of commensal organisms found throughout the majority of non-sterile body surfaces that are in constant contact with the environment (Figure 2). The best and most studied examples include the selective expression of TLR2 and TLR4 by cells of mucosal epithelial sites such as the oral cavity, the upper and lower airways (including the nasal passage), the ear and the eye, the gut and the reproductive tract, as well as the skin (even if the majority of the skin tissue comprises cells of non-mucosal nature). Although the first function of these cells is that of offering a mechanical barrier against pathogens, they also have an intimate relationship with both circulating and local immune cells [i.e., resident neutrophils, dendritic cells (DCs) and macrophages].
### Table 1 | TLR mRNA and protein expression in mucosal epithelial cells.

| Tissue                  | mRNA                                | TLR       |
|-------------------------|-------------------------------------|-----------|
| **ORAL EPITHELIA**      |                                     |           |
| Gingival                | TLR1 (71, 72), TLR2 (73–75), TLR3 (73), TLR4 (73, 75, 76), TLR5 (71, 72), TLR6 (71, 72), TLR7 (73), TLR8 (71–73), TLR9 (71, 72) | TLR1 (71), TLR2 (71–73, 75–78), TLR3 (71, 73, 78), TLR4 (71–76), TLR5 (71, 77, 78), TLR6 (71, 72, 78), TLR7 (71, 73), TLR8 (71), TLR9 (71, 74, 75, 79) |
| Salivary                | TLR1–TLR10 (80, 81)                 | TLR1–TLR4, TLR7 (80) |
| Tonsillar               | TLR1–TLR6, TLR9, TLR10 (80, 82)     | TLR2, TLR3 (82) |
| Ear epithelia           | TLR2–TLR4, TLR9 (83–86)             | TLR2–TLR4, TLR9 (83–86) |
| **OCULAR EPITHELIA**    |                                     |           |
| Corneal                 | TLR1 (67), TLR2 (87–90), TLR3 (87–89), TLR4 (87, 87–90), TLR5 (87, 91), TLR6 (87), TLR7 (87, 88), TLR9 (87–89), TLR10 (87) | TLR1 (92), TLR2 (87, 90, 92–98), TLR3 (87, 87–90, 92, 93), TLR4 (87, 92, 90, 93, 96–98), TLR5 (87, 91–95, 97), TLR6 (92, 92), TLR9 (87, 89) |
| Conjunctival            | TLR1 (67), TLR2, TLR3 (87, 88), TLR4 (87, 88, 99), TLR7 (87, 88), TLR9 (87, 88, 99), TLR10 (87) | TLR3 (88), TLR4 (88, 99), TLR9 (99) |
| Retinal                 | TLR1–TLR2 TLR9 (100)                | TLR2–TLR4 (100) |
| Iris                    | TLR4 (98)                           | TLR4 (98) |
| **AIRWAY EPITHELIA**    |                                     |           |
| Nasal                   | TLR1–TLR10 (101, 102)               | TLR2 (102, 103), TLR3 (102), TLR4 (103) |
| Tracheal/bronchial      | TLR1 (7, 81, 104), TLR2 (7, 81, 104, 105), TLR3 (7, 81, 104), TLR4 (7, 81, 104, 106), TLR5–TLR10 (7, 81, 104) | TLR1, TLR2 (7, 104, 105, 107), TLR3 (7, 104, 107), TLR4 (7, 104, 106, 107), TLR5, TLR6 (7, 104, 107), TLR7, TLR9, TLR10 (7) |
| Lung                    | TLR1 (81, 108), TLR2 (81, 108–111), TLR4 (81, 101, 103, 109, 111, 112), TLR5, TLR6 (81, 108) | TLR2 (105, 109, 110), TLR4 (103, 109–112), TLR5 (108) |
| **GUT EPITHELIA**       |                                     |           |
| Esophageal              | TLR1–TLR6 (113)                     | TLR1–TLR3 (113), TLR4 (113, 114), TLR5 (113) |
| Gastric                 | TLR2, TLR4, TLR5 (115–117)          | TLR2, TLR4, TLR5 (115–117) |
| Intestinal              | TLR1 (81), TLR2 (81, 118, 119), TLR3 (81), TLR4 (81, 118, 120–122), TLR5–TLR10 (81) | TLR2 (81, 118, 119) TLR3 (123), TLR4 (118, 120–122), TLR5 (119, 123), TLR9 (123) |
| M cells/Paneth cells    |                                     | TLR2, TLR4, TLR5 (124) |
| **GENITO-URINARY EPITHELIA** |                                     |           |
| Male                    | TLR1, TLR2 (81), TLR3 (81, 125–127), TLR4–TLR7 (81), TLR8 (81, 125–127), TLR9, TLR10 (81) | TLR1 (129, 130), TLR2 (129–131), TLR3, TLR4, TLR6 (129–131) |
| Female                  | TLR1–TLR6, TLR9, TLR10 (129, 130)   | TLR1 (129, 130), TLR2 (129–131), TLR3, TLR4, TLR6 (129–131) |
| Vagina                  | TLR1–TLR6, TLR9, TLR10 (130, 130)   | TLR1–TLR3, TLR5, TLR6, TLR9 (130, 132) |
| Endocervix/decidua      | TLR1–TLR3, TLR5–TLR9 (130, 132)     | TLR1–TLR3, TLR5, TLR6, TLR9 (130, 132) |
| Endometrium, uterus/fallopian tubes | TLR1–TLR6 (130), TLR7–TLR9 (130, 133, 134) | TLR1, TLR2 (130), TLR3 (130, 134), TLR4–TLR6 (130), TLR7–TLR9 (130, 132–134) |
| Urinary tract/renal     | TLR1–TLR6, TLR9 (135, 136)          | TLR2–TLR4 (135, 137–140), TLR5, TLR9 (137) |
Direct defense functions mediated by TLR signaling in mucosal epithelial tissues include induction of anti-microbial substances and other soluble mediators for local and systemic control of infections. Production of anti-microbial substances is beneficial for controlling mucosal epithelial tissue colonization/infection but production of inflammatory mediators is a double-edged sword. In fact, while pro-inflammatory cytokines trigger recruitment and activation of APCs at the site of infection, they may also favor inflammatory tissue damage. One example is the damaging effect of TNF-α on oral bone integrity. Following infection by oral pathogens such as *P. gingivalis*, gingival epithelial cells, and macrophages that are recruited to this site secrete high levels of TNF-α, which then causes enhanced inflammatory oral bone loss. In addition, uncontrolled secretion of inflammatory cytokines is not desirable for maintaining local tissue homeostasis in the presence of the commensal microbiota. On the other hand, lack of inflammatory responses is similarly dangerous in the instances when pathogen infections or commensal imbalance may occur.

The inflammatory cytokines and chemokines most frequently produced by epithelial cells via TLR stimulation include those directly involved in inflammatory and immune regulation (i.e., IL-1α and IL-1β, IL-6, IL-10, IL-13, TNF-α, and TGF-β), those with chemotactic effects (such as IL-8, MCP-1, MIP-1, and RANTES) and growth and differentiation factors (i.e., IL-3, IL-7, G-CSF, and GM-CSF). The activity of some of these mediators encompasses several of the categories mentioned above. However, some promote inflammation and amplify immune responses, for example IL-1β, IL-8, RANTES, or TNF-α, while others dampen such responses, such as IL-10, IL-37, and TGF-β (141). Besides secreting inflammatory mediators, epithelial cells influence mucosal innate and adaptive immunity by also producing factors that directly affect DC, B and T cell functions, such as the B cell-activating factor of the TNF family (BAFF), a proliferation-inducing ligand (APRIL) (142, 143) and type-I interferons.

**TLR EXPRESSION AND RESPONSES IN HUMAN MUCOSAL EPITHELIAL TISSUES**

**ORAL EPITHELIUM**

The gingival epithelium is composed of a variety of cell types including keratinized and non-keratinized, stratified and flat squamous cells, which are exposed to an enormous number of microorganisms of both commensal and pathogenic nature. It is thought that up to 10^{10} bacteria can be found in the oral cavity. In physiologic conditions, a relatively small number of resident immune cells, including neutrophils, lymphocytes and monocytes/macrophages, are located within the oral cavity epithelial tissue. Upon oral pathogen infection, TLR-dependent gingival inflammation causes an influx of neutrophils, monocytes and lymphocytes to facilitate bacterial clearance (144).

Toll-like receptor expression and functions in the oral cavity are very important for the maintenance of oral tissue homeostasis because of the constant presence of commensal microbes (Table 1). Expression of mRNA for TLR1 to TLR9 has been detected in oral epithelial cells (including the tongue), although actual TLR protein expression and cellular localization can be variable and inducible. TLR2 is highly expressed in cells of the gingival basal layer but lower levels are observed in cells of the superficial layers, more exposed to the environment and to microorganisms. While an opposite spatial relationship may be expected to insures recognition of colonizing microorganisms, this is a strategy that facilitates TLR-dependent inflammatory response only when invading pathogens are detected in the basal cell layer. A similar pattern is observed for TLR1, TLR3, TLR4, TLR5, and TLR9 expression, also depending on the state of the tissue (inflamed vs. non-inflamed) (71, 72). TLR7 and TLR8 expression is comparable in both healthy and infected tissues. In addition, acute and persistent gingival inflammation also enhances the expression of TLR2 and TLR4, favoring downstream local innate immune responses (73). In chronic oral inflammatory conditions (i.e. bacterial periodontitis or other pathologies), TLR4 expression in the gingival epithelium decreases, likely to dampen inflammatory responses that may exacerbate damage to oral tissue and bone (76). Variable levels of TLR expression have also been observed as a consequence of other oral chronic inflammatory conditions, for example caused by lichen planus. In this case, increased TLR4 and TLR9 protein expression and decreased TLR2 mRNA have been detected (74, 75). High constitutive expression of TLR1, TLR2, TLR3, TLR4, and TLR7 mRNA has also been shown in vitro in salivary gland epithelial cells, with TLR3 protein levels being the highest (80). mRNA for all TLRs except TLR7 and TLR8 has been observed in tonsillar epithelial cells at the junction between the oral cavity and the airways. A strong expression of TLR2 and TLR3 mRNA is observed in both tonsillar cell lines and primary cells, and detection of actual TLR2 and TLR4 proteins and their activity appears variable (82).
The likely most relevant defense mechanism that is mediated by TLR signaling in the oral cavity is induction of anti-microbial substances such as defensins (α-, β-, and θ-type). Human β-defensin (hBD)-1 to hBD-4 mRNA and proteins are expressed in the oral epithelium (145). hBD-1 is constitutively expressed, hBD-2 and hBD-3 are inducible in the basal layer epithelial cells via TLR2, TLR3, TLR4, TLR5 and TLR9 signaling, and by general inflammatory conditions of the gingival epithelium (i.e., in the presence of IL-1β and TNF-α). hBD-4 is only induced by bacterial infections (146). In a feedback mechanism, β-defensins also induce TLR signaling and recruitment/activation of immature DCs, monocytes and memory T cells in the oral epithelium, which thus places these substances at a cross-road between an immune effector and an immune inducer produced by epithelial cells (147). Overall, activation of TLR2 signaling is generally more frequent than that of TLR4 in gingival epithelial cells, inducing a strong activation of MAPKs and NF-κB pathways than controls production of antibacterial substances (148).

Oral epithelial cells do not generally secrete high levels of inflammatory mediators, likely to avoid excessive local innate immune responses resulting in tissue destruction. Secretion of IL-8 in response to TLR9, TLR2 and TLR5 stimulation and, to a lesser extent, to TLR4 stimulation has been shown in gingival epithelial cells, which is enhanced by a prior cell exposure to IFN-γ (77, 79, 149). Besides IL-8, secretion of other inflammatory cytokines directly involved in innate immunity, such as IL-1β and TNF-α, as well as that of APCS chemo-attractants has been shown. Thymic stromal lymphopoietin (TSLP) is also expressed by oral epithelial cells following TLR3, TLR5 and TLR2/TLR6 activation (78).

**EAR EPITHELIA**

Toll-like receptor expression has been detected in the ear epithelium (Table 1), indicating that this tissue is suited to respond to pathogens and initiate immune and defense responses accordingly. Primary epithelial cells of the human ear and middle ear/inner ear epithelial cell lines express functional TLR2 but not TLR4, shown by their responsiveness to Haemophilus influenzae whole cell lysates stimulation in vitro and up-regulation of defensins mRNA expression, but not to purified LPS (83). TLR2-dependent stimulation is inhibited by anti-TLR2 blocking antibodies (84). From immunohistochemistry studies of biopsies from the normal ear canal and acquired cholesteatoma (an abnormal growth of keratinized squamous epithelium), it appears that expression of TLR2, TLR3 and TLR4 is detected and regulated as a function of cholesteatoma (85). Studies on TLR expression and function in animal models support expression of TLR2, TLR4, and TLR9 in auditory cells (86).

**OCULAR EPITHELIA**

A larger number of studies exist regarding TLR expression in the human eye (93) as compared to the ear (Table 1). Expression of mRNA for TLR1, TLR2, TLR3, TLR4, TLR5, TLR6 and TLR9 has been shown in human corneal epithelial cells (88), where extracellular TLR3 and intracellular TLR2 and TLR4 expression can be detected (6). TLR2 and TLR5 functionality in these cells has been assessed by loss-of-activity using anti-TLR2 and -TLR5 antibodies. It is not clear whether lack of TLR4 or MD-2 protein expression is involved in un-responsiveness to TLR4 ligands (97). Corneal epithelial cells respond to TLR stimulation mostly by secreting defensins and by further regulating expression of TLR mRNA (87, 92, 94, 95). In retinal epithelial cells, TLR2, TLR3, and TLR4 mRNA expression has been detected and is regulated by signaling via TLR3, which induces high levels of TLR3 and TLR9 protein expression (89, 100). Induction of interferons, IL-8 and MCP-1 can be induced by anti-TLR3 antibodies, but stimulation via TLR9 only induces IL-8 secretion. mRNA for TLR1, TLR6, TLR7 and TLR9 and TLR4 protein expression has also been detected in section of whole human eyes and iris epithelial cells (98). Lastly, TLR9 is expressed in conjunctival epithelial cells and its expression is regulated by nerve growth factors (99). In the human eye, the major function of TLR-dependent signaling is induction of anti-microbial substances. While hBD-1 to hBD-4 are expressed constitutively, hBD-2 expression is increased by TLR2 and TLR5 stimulation for example, in a Pseudomonas aeruginosa infection model of corneal epithelial cells (90, 95). In the ocular epithelial tissue, expression of hBD-9 is also induced by TLR2, TLR3, TLR4 and TLR5 signaling (150, 151). In immortalized human eye tissue, mRNA expression and secreted IL-1β, IL-6, IL-8, MCP-1 and sICAM-1, IL-32, IL-33 and TNF-α are induced by TLR2, TLR3, TLR4 and TLR5 signaling in response to viral and bacterial stimulation (91, 94, 96, 152).

**AIRWAY EPITHELIUM**

Crucial regulation of TLR expression is well-documented in the airways (Table 1), where it critically influences airway defense mechanisms and local immune responses. While expression of TLR mRNAs is generally detected in respiratory tract epithelial cells, protein expression often varies, depending on tissue site and on host physiologic vs. disease condition (i.e., normal vs. inflammation or allergy) (7, 104). The most relevant TLRs in the airways epithelia are TLR2 and TLR4. Their expression is maintained at low levels and preferentially on the basal cell layers. Following infection by pathogens and in inflamed tissues, increased expression of TLR2 and TLR4 has been reported (101, 109, 110). TLR4 is often intracellular, initially located in the Golgi complex, but is readily transferred to the cell surface for pathogen recognition (112). TLR2 and TLR4 responses in the airway epithelium are also influenced by the constitutively low or absent expression of MD-2 and CD36 (106, 153) and by negative TLR regulatory factors such as IRAK-M and Tollip, which dampen cell activation in physiologic conditions. Exposure to TLR4 ligands, such as killed H. influenzae or its purified P6 outer membrane protein, to TNF-α and IFN-γ can induce MD-2 expression and cell responsiveness. TLR3 and TLR5 also play important roles in the human airway epithelia by recognizing viral and bacterial ligands (107, 108).

Toll-like receptor-dependent activation of airway epithelial cells can induce different responses, depending on the tissue location along the respiratory tract. The upper respiratory epithelium, which includes the nasal cavity, pharynx, and the larynx, is generally in contact with a high number of (mostly commensals) organisms [up to 10⁶ organisms/nasal cavity and up to 10⁴ organisms in the nasopharynx (154)]. The first and most common defense response in these epithelia is production of mucus. Expression of mucins, the major protein components of mucus, is induced directly, by
TLR signaling and indirectly, by high levels of IL-8 and TNF-α induced via TLRs (155). In turn, mucins can also further regulate TLR signaling (156). In epithelial cells of the nasal mucosa, TLR signaling induces production of anti-microbial substances including hBD-1 to hBD-4, relevant for defense mechanism in both disease conditions and allergy (103). In the lower respiratory tract, which includes the trachea, primary bronchi, and the lungs, TLR-dependent responses are also regulated through expression levels and localization mechanisms. These epithelial tissues are relatively sterile but can become exposed to microorganisms descending from the upper respiratory tract. Studies of human lung epithelial tissues have shown TLR4 expression and signaling in response to bacterial LPS (112) and in disease conditions, such as chronic obstructive pulmonary disorder (COPD), asthma and allergy (103, 111). In tracheal epithelial cells, TLR3 expression on the luminal and basal side, and TLR2, TLR6 and TLR1 basolateral expression, have been reported. Low levels of TLR2, TLR4, TLR5, TLR7, TLR9 and TLR10, and high levels of TLR6 are shown, and TLR3, TLR7, and TLR9 are present in both intracellular compartments and on the cell surface (7). Also in lower respiratory tract, TLR signaling induces production of anti-microbial substances. HBD-1 is constitutively expressed in these epithelia, hBD-5 and hBD-6 are not expressed and increased expression of hBD-2 to hBD-4 is observed in a TLR-dependent manner (105, 106, 157). Besides defensins, other antibacterial molecules are induced by TLR signaling, such as lysozyme, nitric oxide (NO), and LL-37 (158). TLR signaling also leads to production of cytokines. Soluble inflammatory mediators in the upper and lower respiratory epithelia are desirable for promoting local recruitment of professional phagocytic cells that participate in pathogen clearance. Accordingly, TLR-dependent secretion of TNF-α, IL-8, MIP-1α, MIP-1β, RANTES, GRO-α, -β, and -γ, IL-6, IL-5, and TGF-β promotes an influx of neutrophils, eosinophils, monocytes, NK cells, macrophages and DCs at these sites (102, 159). Secretion of type-I and type-III IFNs (including IL-28 and IL-29) is also reported, predominantly via TLR3 signaling (7, 160), suggesting an active protective process against viral infections. Other TLR-dependent mediators of immune responses that are induced in the airways include BAFF and APRIL, which favor interaction of airway epithelial cells with B cells and DCs (161).

**GUT EPITHELIUM**

The mucosal epithelial tissue of the digestive system, or gut, is tightly connected to both the oral cavity and the respiratory tract. This epithelium is also colonized by an enormous number of commensal microorganisms (approximately 10^{13}–10^{14}) and occasionally, a small number of pathogens from similar species to those found in the oral and respiratory tracts. Differential TLR expression and functions are observed in epithelial cells of these tissues, depending on the specific location (i.e., the esophagus, the stomach, the small intestine or the large intestine) (Table 1), and depending on the local commensal flora in each of these tissues. Generally, mRNA for TLR1 to TLR9 has been reported, but only a low, constitutive expression of TLR2, TLR4 and TLR5 (and of MD-2) is observed on the cell basolateral side (120).

Few studies have shown TLR expression in the esophagus. TLR4 mRNA expression and functional activity in response to LPS have been shown in biopsies of cancerous and normal esophageal epithelial tissues in vitro and ex vivo (114). Esophageal epithelial cells also express high levels of TLR2 and TLR3 mRNA and, to a lower extent, TLR1 and TLR5 mRNA. Following stimulation of TLR3 with Poly I:C, TLR2 mRNA expression is up-regulated, although these cells are unresponsive to PG and Pam3CSK4. In addition, cell incubation with flagellin also fails to induce esophageal epithelial cell activation, suggesting that both TLR2 and TLR5 proteins may not be expressed. Secretion of high levels of IL-8 via TLR3 activation is consistent with responses triggered by infection with intracellular pathogens (113). Only a few studies exist on stomach epithelial cells. In normal gastric epithelial cells, constitutive and inducible expression of TLR2, TLR4 and TLR5 mRNA and protein has been shown in response to bacterial components and whole organisms (115, 116). Inducible expression of TLR4 and TLR2-dependent secretion of cytokines in vitro can be inhibited by blocking anti-TLR2 antibodies (117).

The small and large intestine areas present the highest concentration of commensal microorganisms as compared to all the other mucosal epithelial sites. Here, TLR expression and control of signaling pathways is extremely important. Intestinal epithelial cells (IECs) may either lack expression of TLR4, MD-2 and CD14 or, if expressed, TLR4 may be located in intracellular compartments to avoid hyper-responsive ness to LPS from commensal organisms (118, 121, 122). Up-regulation of MD-2 and TLR4 can be induced by high local levels of IFN-γ or TNF-α, which is thought to contribute to chronic colitis associated with Crohn’s disease (120). TLR2 mRNA and low levels of TLR2 protein are expressed in these cells at a sub-apical location and high levels of TLR5 are detected on the cell’s basolateral side, thus sensing flagellin only when microorganisms cross the intestinal epithelial barrier during active invasion (119). TLR9 activation at the apical or basolateral side of IECs may induce secretion of different cytokines, depending on whether NF-κB pathways are triggered (123). Polar expression of TLRs is a mechanism common to the oral, airway and gut epithelia for preventing unnecessary and potentially detrimental inflammatory response to commensal colonizers. Additional control mechanisms for TLR-mediated intestinal cell activation include negative regulation of TLR signaling via Tollip and the single Ig IL-1 receptor-related molecule (SIGIRR) (162). Toll-like receptor-dependent production of α-defensins (hD-5 and hD-6), β-defensins (hBD-1, hBD-2, and hBD-3) and other bactericidal substances has been shown in the gut (163). In contrast to the predominant induction of TLR-dependent anti-microbial products in the oral and airway epithelium, secretion of inflammatory mediators is an important outcome in the gut epithelium. Pro-inflammatory cytokines including IL-1β, IL-7, IL-8, IL-15, and IL-18 drive local recruitment of PMNs and other leukocytes (164, 165), and IL-18 secretion further amplifies IL-2 and INF-γ production, influencing production of mucus and its composition. However, considering that an overly robust inflammatory response is detrimental to the host, gut epithelial cells also produce IL-10 and TGF-β, which play a role in tissue repair processes and re-establish the barrier function of the gut epithelia (166).

The significance of TLR-dependent cytokine production by IECs is also related to the presence of M cells, Paneth cells, and -
and mucus-producing goblet cells within the epithelial tissue. M cells, or membranous epithelial cells, are located above Peyer’s patches and other lymphoid areas and provide an important bridge between epithelial cells and professional immune cells. These cells facilitate antigen sampling and transport through the epithelial layer into lymphoid areas and accelerate both innate and adaptive immune responses (167). M cells express higher levels of TLR4 on the apical surface, in contrast to enterocytes, and provide signals for further activation of immune cells to produce secretory IgA for control of both pathogens and commensals and other mediators of immunity such as BAFF and APRIL (168). Paneth cells, epithelial cells of the small intestine, express TLRs for recognition of pathogens and produce anti-microbial substances (124).

**GENITO-URINARY TRACT EPITHELIUM**

Toll-like receptor expression by epithelial cells of the genito-urinary tract is also fine-tuned to specifically respond only to pathogens, because of the potential large number of commensal organisms in the different compartments of this mucosal site (Table 1). In this epithelium, additional variables need consideration, such as the diversity of cell composition of the reproductive tract epithelium in females vs. males, the nature of the commensal flora and the likelihood of exposure to pathogens for each anatomical section, thus suggesting a tissue-specific immune surveillance. Few studies have been carried out on the human male genital tract (MGT), while more abundant data exist on mouse and rat models (169). TLR expression in the human MGT is generally low. In different sections of the MGT, for example in the epididymis, vas deferens, seminal vesicles and testes, epithelial cells do not appear to express TLR mRNA. In the prostate, intracellular expression of TLR3 and TLR8 is detected and that of TLR9 in the penile urethra, although it is not widespread to all individual cells. In vitro studies of primary urethral and prostate cells, epididymal-vas deferens have indicated a potential cell susceptibility to activation by TLR2 ligands. Similar observations have been made in seminal vesicles (although overall TLR expression is not well-studied in these cells) (81, 125–127). One of the reasons for such low TLR-dependent signaling in the MGT also correlates with protection of sperm cells development, which would not benefit from occurring in a pro-inflammatory environment where cells are highly responsive to stimulation via TLRs (despite protective functions against infections) (170). In addition, the commensal microflora burden of the MGT is rather low.

Toll-like receptor expression is better described in epithelial cells of the female genital tract (FGT). In vaginal epithelial cells, TLR1 to TLR6 and TLR9 are expressed, with high levels of TLR2 and TLR4 proteins (129, 130). In epithelial cells of upper FGT regions, TLR4 expression is not fully ascertained (133). It is likely low in the endocervical and ectocervical epithelial tissues (171) but these cells express TLR1, TLR2, and TLR6 and are responsive to TLR2 and TLR5 activation. Other reports indicate expression of mRNA for TLR7, TLR8 (weak), TLR9 and detectable protein levels of TLR3 (extracellular) and TLR9 in these cells (130, 132). In the sterile regions of the FGT, the fallopian tubes and uterus, TLR1, TLR7, TLR8 and TLR9 mRNA is detected, likely due to the sensitivity of these sites to viral infections (130). Uterine epithelial cells express TLR1 to TLR9 but are only susceptible to activation by TLR2, TLR3, TLR5, and to some extent, TLR4 agonists (134). Endometrial epithelial cells express TLR1 to TLR6 and TLR9, but low TLR5 and TLR6 levels are observed in isolated endometrial epithelial cells as compared to the whole tissue (130). In most of the FGT epithelia, TLR10 mRNA is also detected. Clearly, such variable expression of TLRs throughout the FGT supports different responses to potential pathogens and controls local homeostasis in the absence of infection.

Although in part physically distinct from the genital tract epithelium, cells of the urinary tract epithelia (comprising the urethra, bladder, ureters, and the kidneys) have also been shown to express TLRs. TLR2, TLR3, TLR4, TLR5, and TLR9 are expressed in various sites of the urinary tract epithelium (137), but renal epithelial cell lines and primary human proximal tubule cells do not express TLR4 and are unresponsive to LPS (138, 139). Expression of soluble MD-2 and CD14 also correlates with responsiveness of these cells to LPS. In the bladder and in the kidney epithelia, expression of TLR4 is an important surveillance strategy against Gram negative bacteria infections, particularly uropathogenic *E. coli*, controlling inflammatory responses to such infections (140). Heightened susceptibility to urinary tract infections (UTIs), asymptomatic and persistent bacteriuria have been associated to low levels of TLR4 expression and TLR4 polymorphisms in human being (135).

Although there are tissue-specific differences in TLR expression in the MGT and the FGT, production of anti-microbial substances is observed in both tissues. TLR-dependent secretion of hBD-1, DEFB118, DEFB126, and SPAG11 (172) is elicited in the epididymis, testis, and prostate (173) and that of HD-5 and hBD-1 in the vagina, the ectocervix and at high levels particularly in the endocervix, uterus and fallopian tubes. By contrast, different patterns of cytokine and chemokine secretion characterize TLR-dependent responses in the MGT and the FGT, where the delicate balance between homeostasis and inflammation can influence fertility and reproduction processes. In the FGT, mild inflammatory responses are observed, possibly due to an intrinsic bias of this tissue to exposure to large numbers of commensals. TLR-dependent stimulation of FGT epithelial cells in *vitro* induces secretion of IL-1α, IL-1β, IL-6, IL-8 and TNF-α (64, 174), and cyclooxygenase 2 (COX-2), an inducible enzyme associated with mucosal inflammation (131), but few studies exist to support these findings in *vivo*. During infection by sexually transmitted pathogens, such as *Chlamydia or Neisseria gonorrhoeae*, secretion of IFN-γ, IL-10, IL-12, IL-1β, IL-6, and IL-8 has been reported in the cervix, fallopian tubes, and cervical secretions (174, 175). In the fallopian tubes and the uterus, the presence of endometrial epithelial cells, with similar functions than the intestinal M cells, favors secretion of inflammatory cytokines that can influence local immune responses (176). In epithelial cells of the MGT, data gathered mostly from *in vitro* studies have identified secretion of IL-6, IL-8, TNF-α, and IL-1β following TLR stimulation (64, 128, 177). A consequence of genito-urinary tract epithelia inflammation, recruitment of professional APCs and PMNs to the site of infection leads to symptoms such as purulent discharge and local tissue inflammation. These symptoms are observed at varying extent in both the MGT and FGT.
CONCLUSION

It is well established that cell activation and signaling via TLRs is crucial for induction of host immune and defense responses against microorganisms. During the course of life, human beings encounter a large number of commensal bacteria. The majority of commensals do not alter local homeostasis and integrity of the tissues that they colonize, and some have even beneficial effects at mucosal sites, for example the gut or the reproductive tract. Naturally, host mucosal epithelia are also exposed to potential pathogens. In many cases, these microorganisms only cause disease when they succeed in colonizing the appropriate infection site(s) or in crossing the mucosal epithelial barriers that separate the host from the environment. Potential pathogenicity and diseases may also arise in the event of cross-colonization of mucosal epithelial tissues by commensal organisms that are not specific for that given body site. Thus, epithelial cells of mucosal sites have evolved to implement specific control mechanisms for bacterial recognition and for initiating or suppressing local tissue-specific immune responses against disease-causing organisms or commensals, respectively. Regulation of TLR expression, cellular localization, and functions in mucosal epithelial cells provides one of the mechanisms by which bacteria/host cell interactions are directed to avoid onset of persistent local inflammation. For example, in mucosal epithelia, expression of TLRs on the cell surface is strongly regulated between the apical and basolateral sides of cells, ensuring that immune response only takes place if pathogens cross these tissues and preventing tissue damage in the absence of benefits for the host. Similarly, expression of endosomal TLRs and other cytosolic PRRs including NLRs and RIG-like receptors (RLRs) ensures recognition of intracellular pathogens. Thus, induction of specific responses is targeted to pathogen organisms’ clearance and resolution of infection. The importance of TLR regulation is also apparent in the control of host diseases and conditions that can result from abnormal TLR expression and inflammation.

ACKNOWLEDGMENTS

The authors thank John F. Love, M.D., Ph.D. (Boston University) for critical reading of the manuscript. The authors thank NIH/NIAID grants U19 AI084048-01 and R01 AI40944-01.

REFERENCES

1. Sasaki M, Yamamoto M. Pathogen recognition receptors: ligands and signaling pathways by toll-like receptors. Int Rev Immunol (2013) 32:116–33. doi:10.3109/08830185.2013.774391

2. Akira S, Hemmi H. Recognition of pathogen-associated molecular patterns by TLR family. Immunit Lett (2003) 85:85–95. doi:10.1016/S0165-2478(02)00228-6

3. Cario E, Brown D, McKee M, Lynch-Devaney K, Gerken G, Podolsky DK. Commensal-associated molecular patterns induce selective toll-like receptor-3 trafficking from apical membrane to cytoplasmic compartments in polarized intestinal epithelium. Am J Pathol (2002) 160:165–73. doi:10.1016/S0002-9440(01)63460-X

4. Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol (1994) 12:991–1045. doi:10.1146/annurev.immunol.12.1.991

5. Brinkmann MM, Spooner E, Hooe K, Beutler B, Ploegh HL, Kim YM. The interaction between the ER membrane protein UNC93B and TLR3, 7, and 9 is crucial for TLR signaling. J Cell Biol (2007) 177:265–75. doi:10.1083/jcb.200612056

6. Utz M, Nochi T, Iang MH, Park EI, Igarashi O, Hino A, et al. Intracellularly expressed TLR2s and TLR4s contribute to an immunosilent environment at the ocular mucosal epithelium. J Immunol (2004) 173:3337–47. doi:10.4049/jimmunol.173.5.3337

7. Joaonnida I, Ye F, McNally B, Willette M, Flano E. Toll-like receptor expression and induction of type I and type III interferons in primary airway epithelial cells. J Virol (2013) 87:3261–70. doi:10.1128/JVI.01956-12

8. Medzhutov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, et al. MyD88 is an adaptor protein in the hTLR-1 receptor family signaling pathways. Mol Cell (1998) 2:253–8. doi:10.1016/S1097-2765(00)80136-7

9. Fitzgerald KA, Palsson-McDermott EM, Bowie AG, Jefferies CA, Mansell AS, Brady G, et al. Mal (MyD88-adaptor-like) is required for toll-like receptor-4 signal transduction. Nature (2001) 413:78–83. doi:10.1038/35092578

10. Horng T, Barton GM, Medzhutov R. TIRAP: an adaptor molecule in the toll-like signaling pathway. Nat Immunol (2001) 2:835–41. doi:10.1038/nn901-835

11. Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, et al. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science (2003) 301:663–4. doi:10.1126/science.1087262

12. Oshiumi H, Matsumoto M, Funami K, Akazawa T, Seya T, TICAM-1, an adaptor molecule that participates in toll-like receptor-3-mediated interferon-beta induction. Nat Immunol (2003) 4:161–7. doi:10.1038/nm886

13. Fitzgerald KA, Rowe DC, Barnes JS, Caffrey DR, Visintin A, Letz E, et al. LPS-TLR4 signaling to IRF-3 and NF-kappaB involves the toll adapters TRAM and TRIF. J Exp Med (2003) 198:1043–55. doi:10.1083/jem.20031023

14. Ihle JS, Kadowaki S, Kato S, Tanaka Y, Matsuda T, Shima H, et al. Role of TRAF6 in the TLR2 signaling pathway that induces the transcription of IL-12. J Immunol (2001) 167:1342–50. doi:10.4049/jimmunol.167.5.1342

15. Bell JK, Botos I, Hall PR, Askins J, Shiloach J, Davies DR, et al. The molecular basis for the differential effects of LPS on TLR2 and TLR4 signaling. J Immunol (2004) 172:2913–7. doi:10.4049/jimmunol.172.5.2913

16. Poole E, Liu W, Zhao H, Nusrin M, Fan T, Xu L, et al. Structural basis of TLR5-flagellin recognition and signaling. Science (2008) 321:654–6. doi:10.1126/science.1161312

17. Kim HM, Park BS, Kim JI, Kim SE, Lee J, Oh SC, et al. Crystal structure of the TLR4 extracellular domain. J Endotoxin Res (2006) 12:375–8. doi:10.1177/0960918X06031870

18. Joo MS, Kim SE, Heo YJ, Lee MB, Kim HM, Paik SG, et al. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell (2007) 130:1071–82. doi:10.1016/j.cell.2007.09.008

19. Kim HM, Park BS, Kim JI, Kim SE, Lee J, Oh SC, et al. Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist eritoran. Cell (2007) 130:906–17. doi:10.1016/j.cell.2007.08.002

20. Yoon SI, Kurnasov O, Nataraajan V, Hong M, Gudkov AV, Osterman AL, et al. Structural basis of TLR5-flagellin recognition and signaling. Science (2012) 335:859–64. doi:10.1126/science.1215584

21. Tanji H, Ohto U, Shibata T, Miyake K, Shimizu T. Structural reorganization of the toll-like receptor 8 dimer induced by agonistic ligands. Science (2013) 339:1426–9. doi:10.1126/science.1242919

22. Akira S, Yamamoto M, Takeda K. Role of adaptors in toll-like receptor signaling. J Immunol (2004) 173:2913–7. doi:10.4049/jimmunol.173.5.2913

23. Meylan E, Burns K, Hofmann K, Blancheteau V, Martinon F, Kelliher M, et al. Mal (MyD88-adaptor-like) is required for toll-like receptor-4 signal transduction. Nature (2001) 413:78–83. doi:10.1038/35092578

24. Burns E, Eliyahu T, Uematsu S, Akira S, Nussbaum G. TLR2-dependent inflammatory response to Porphyromonas gingivalis in MyD88 independent,
porins are potent protective immunogens with adjuvant properties. Immunol- 
ology (2013) 139:459–71. doi:10.1111/imn.12093

76. Goldiero M, Goldiero M, Finamore E, Rossano F, Gambuzza M, Catania MB, et al. Haemophilus influenzae porin induces toll-like receptor 2-mediated cytokine production in human monocytes and mouse macrophages. Infect Immun (2004) 72:1204–9. doi: 10.1128/IAI.72.2.1204-1209.2004

77. Beklen A, Hukkanen M, Richardson R, Konttinen YT. Immunohistochemical activity correlations of variant forms of the B pentamer of Escherichia coli type II heat-labile enterotoxin LT-Ib with toll-like receptor 2 binding. Acta Crystallogr D Biol Crystallogr (2012) 68:1604–12. doi:10.1107/S0907444912038917

78. Davey M, Liu X, Ukai T, Jain V, Gudino C, Gibson FC III, et al. Bacterial fim- 

ogy

79. Takai T, Chen X, Xie Y, Vu AT, Le TA, Kinoshita H, et al. TSLP expression 

80. Beklen A, Sorsa T, Konttinen YT. Toll-like receptors 2 and 5 in human gingival 

81. Uehara A, Fujimoto Y, Fukase K, Takada H. Various human epithelial cells 

82. Lange MJ, Lasiter JC, Misfeldt ML. Toll-like receptors in tonsillar epithelial cells. Mol Cell Biol (2005) 25:8866–92. doi:10.1128/MCB.25.22.8866

83. Lim DJ, Lasiter JC, Miscfeldt ML. Toll-like receptors in tonsillar epithelial cells. Int J Pediatr Otorhinolaryngol (2009) 73:631–20. doi:10.1016/j.ijporl.2008.12.013

84. Lee HY, Takeshita T, Shimada J, Akopyan A, Woo JJ, Han H, et al. Induction of beta-defensins by NTL5 requires TLR2-mediated MyD88 and IRAK. TRAF6- p38MAPK signaling pathway in human middle ear epithelial cells. BMC Infect Dis (2008) 8:87. doi:10.1186/1471-2334-8-87

85. Szczepanski M, Szyfter W, Jenek R, Wrobel M, Lisew ska IM, Zemor ski J. Toll-like recep- tors 2, 3 and 4 (TLR-2, TLR-3 and TLR-4) are expressed in the microenvi- ronment of human acquired cholesteatoma. Eur Arch Otorhinolaryngol (2006) 263:605–7. doi:10.1007/s00405-006-0030-1

86. Tanigawa T, Oikuh h E, Morikawa A, Hayashi K, Sato T, Shihata R, et al. Immunological role of prostaglandin E2 production in mouse auditory cells in response to LPS. Innate Immun (2013) doi:10.1177/1775349513505378

87. Redfern RL, Reim RV, Mc Dermott AM. Toll-like receptor activation modulates antimicrobial peptide expression by ocular surface cells. Exp Eye Res (2011) 92:209–20. doi:10.1016/j.exer.2010.12.005

88. Erdinest N, Aviel V, Mallem D, Antony E, Yahalom C, Mechoulam H, et al. Expression and activation of toll-like receptor 3 and toll-like receptor 4 on human corneal epithelial and conjunctival fibroblasts. J Inflamm (Lond) (2014) 11:3. doi:10.1186/1476-9255-11-3

89. Eishara N, Yamagami S, Chen L, Tokura T, Iwatsuo M, Ushio H, et al. Expression and function of toll-like receptor-3 and -9 in human corneal myofibroblasts. Invest Ophthalmol Vis Sci (2007) 48:3069–76. doi:10.1167/iovs.06-0968

90. Kumar A, Zhang J, Yu FS. Toll-like receptor 2-mediated expression of beta- defensin-2 in human corneal epithelial cells. Microbes Infect (2006) 8:380–9. doi:10.1016/j.micinf.2005.07.006

91. Du JW, Zhang F, Capo-Aponte JE, Tachado SD, Zhang J, Yu FS, et al. AsialoGM1-mediated IL-8 release by human corneal epithelial cells requires coexpression of TLR5. Invest Ophthalmol Vis Sci (2006) 47:4810–8. doi:10.1167/ iovs.06-0250

92. Ma P, Wang Z, Pliigelnder SC, Li DQ. Toll-like receptors mediate induction of peptidoglycan recognition proteins in human corneal epithelial cells. Exp Eye Res (2010) 90:130–6. doi:10.1016/j.exer.2009.09.021

93. Pearlman E, Sun Y, Roy S, Kar marmar M, Hiss AG, Szczotka-Flynn L, et al. Host defense at the ocular surface. Int Rev Immunol (2013) 32:4–18. doi:10.1080/ 
94. Zhang J, Xu K, Ambari B, Yu FS. Toll-like receptor 5-mediated corneal epithelial inflammatory responses to Pseudomonas aeruginosa flagellin. Invest Ophthal- mol Vis Sci (2003) 44:4247–54. doi:10.1167/iovs.03-0219

95. Evans DJ, Fleissig SM. Why does the healthy cornea resist Pseudomonas aerugi- nosa infection? Am J Ophthalmol (2013) 155:961–70. doi:10.1016/j.ajo.2013.01.001

96. Zhao J, Wu X. Aggregilus fungitius antigens activate immortalized human corneal epithelial cells via toll-like receptors 2 and 4. Curr Eye Res (2008) 33:447–54. doi:10.1016/j.jao.2008.10.013

97. Zhang J, Kumar A, Wheeler M, Yu FS. Lack of TLR-2 expression in human corneal epithelial cells is an underlying mechanism of lipopolysaccharide (LPS) unresponsiveness. Immunol Cell Biol (2007) 85:141–8. doi:10.1080/0888881070124847

98. Brito BE, Zamora DO, Bonnah RA, Pan Y, Planck SR, Rosenbaum JT. Toll-like recep- tors mediate induction of p38MAPK signaling pathway in human middle ear epithelial cells. Oral Microbiol Immunol (2004) 19:261–7. doi:10.1111/j.1399-302X.2004.00473.x

99. Takai T, Chen X, Xie Y, Vu AT, Le TA, Kinoshita H, et al. TSLP expression induced via toll-like receptor pathways in human keratinocytes. Methods Enzym- 

100. Kumar MV, Nagineni CN, Chin MS, Hooks JJ, Detrick B. Innate immunity to AsialoGM1-mediated IL-8 release by human corneal epithelial cells. Immunol Lett (2008) 118:65–6. doi:10.1016/j.imlet.2008.03.012

101. Misera A, Stampachiacchere B, Normando EM, Lambiase A, Bonini S, Bonini S. Nerve growth factor modulates toll-like receptor (TLR) 4 and 9 expression in cultured primary VRC conjunctival epithelial cells. Mol Vis (2009) 15:3207–44.

102. Kumar MV, Nagineni CN, Chin MS, Hooks JJ, Detrick B. Innate immunity to the retina: toll-like receptor (TLR) signaling in human retinal pigment epithelial cells. J Neuroimmunol (2004) 153:7–15. doi:10.1016/j.jneuroim.2004. 04.018

103. Dong Z, Yang Z, Wang C. Expression of TLR2 and TLR4 messenger RNA in the epithelial cells of the nasal airway. Am J Rhinol (2005) 19(3):236–9,

104. Oikuh E, Sato T, Shihata R, Hayashi K, Sato T, Shihata R, et al. Induction of beta-defensins by NTL5 requires TLR2-mediated MyD88 and IRAK. TRAF6- p38MAPK signaling pathway in human middle ear epithelial cells. BMC Infect Dis (2008) 8:87. doi:10.1186/1471-2334-8-87

105. Wang X, Zhang Z, Louboutin JP, Moser C, Weiner DJ, Wilson JM. Airway TLR-mediated pathways regulate house dust mite-induced allergic disease in the upper and lower airways. J Allergy Clin Immunol (2013) 131:549–61. doi:10.1016/j.jaci.2012.07.050

106. Sha Q, Truong-Tran AQ, Plitt JR, Beck LA, Schleimer RP. Activation of airway toll-like receptor agonists. FASEB J (2003) 17:358–64. doi:10.1096/fj.03-3808OC

107. Shang X, Zeng H, Louboutin JP, Moser C, Weiner DJ, Wilson JM. Airway TLR-mediated regulation of human beta-defensin-2 through toll-like recep- tors 2. FASEB J (2003) 17(12):1727–9. doi:10.1096/fasebj.2002-0616fe

108. Isa HP, Kline JN, Penisten A, Apicella MA, Gioannini TL, Weiss J, et al. Endo- toxin responsiveness of human airway epithelial cells is limited by low expres- sion of MD-2. Am J Physiol Lung Cell Mol Physiol (2004) 287:L428–37. doi:10.1152/ajplung.00377.2003
107. Berube J, Bourdon C, Yao Y, Rousseau S. Distinct intracellular signaling pathways control the synthesis of IL-8 and RANTES in TL1R/TL2, TL3R or NO3I activated human airway epithelial cells. *Cell Signal* (2009) 21:448–56. doi:10.1016/j.cellsig.2008.12.001

108. Zhang Z, Louboutin JP, Weiner DJ, Goldberg JB, Wilson JM. Human airway epithelial cells sense *Pseudomonas aeruginosa* infection via recognition of flagellin by toll-like receptor 5. *Infect Immun* (2005) 73:7151–60. doi:10.1128/IAI.73.11.7151-7160.2005

109. Armstrong L, Medford AR, Uppington KM, Robertson J, Witherden IR, Tetley TD, et al. Expression of functional toll-like receptor-2 and -4 on airway epithelial cells. *Am J Respir Cell Mol Biol* (2004) 31:241–5. doi:10.1165/rcmb.20040078OC

110. Regueiro V, Morena D, Campos MA, Margareto J, Garmendia J, Benitez R, et al. Human airway epithelial cells express the toll-like receptor-7 and -8 and respond to the bacterial flagellin protein. *FEMS Immunol Med Microbiol* (2005) 45:221–6. doi:10.1016/j.femsim.2005.06.007

111. MacRedmond RE, Greene CM, Dorscheid DR, McElvaney NG, O'Neill SJ, et al. Toll-like receptors and decreased toll-interacting protein in gastric mucosa in patients with *Helicobacter pylori* infection increases the expression of inflammatory tumorogenic cytokines and chemokines as well as components of the toll-like receptor and NF-kappaB pathways in human prostate epithelial cells. *Mol Cell Probes* (2014) 28(4):147–54. doi:10.1016/j.mcp.2014.01.006

112. Guillot L, Medjane S, Le Barillec K, Balloy V, Danel C, Chignard M, et al. Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human beta-defensin-2 in vaginal epithelial cells. *Microbes Infect* (2005) 7:1117–27. doi:10.1016/j.micinf.2005.03.016

113. Pivarcsi A, Nagy I, Kereck A, Kis K, Kenderessy-Szabo A, Szell M, et al. Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human beta-defensin-2 in vaginal epithelial cells. *Microbes Infect* (2005) 7(11):1177–27. doi:10.1016/j.micinf.2005.03.016

114. Svanborg C, Godaly G, Hedlund M. Cytokine responses during mucosal infection. *J Pediatr Gastroenterol Nutr* (2001) 32:297–12. doi:10.1097/000057902000

115. Lim DM, Narasimhan S, Michaylina CZ, Wang ML. TLK3-mediated NF-κB signaling in human esophageal epithelial cells. *Am J Pathol* (2009) 175:425–33. doi:10.1016/j.ajpath.2009.06.2029

116. MacRedmond RE, Greene CM, Dorscheid DR, McElvaney NG, O'Neill SJ, et al. Toll-like receptors and decreased toll-interacting protein in gastric mucosa in patients with *Helicobacter pylori* infection increases the expression of inflammatory tumorogenic cytokines and chemokines as well as components of the toll-like receptor and NF-kappaB pathways in human prostate epithelial cells. *Mol Cell Probes* (2014) 28(4):147–54. doi:10.1016/j.mcp.2014.01.006

117. Miyazaki J, Kawai K, Oikawa T, Johraku A, Hattori K, Shimazui T, et al. Uroepithelial expression of TLR4 associated MD-2 in intestinal epithelial cells: a toll-like receptor that prevents infection by uropathogenic bacteria. *Science* (2006) 313:3913–22. doi:10.1126/science.1133270

118. Takenaka R, Yokota K, Ayada K, Mizuno M, Zhao Y, Fujinami Y, et al. Toll-like receptor 4-associated MD-2 in intestinal epithelial cells: an essential role in host defense pathway. *Helicobacter* (2006) 11:224–8. doi:10.1111/j.1103-2284.2006.00111.x

119. Takeda K, Oka S, Kiyono H, Kawai K, Sato S, Noguchi T, et al. Toll-like receptor 4 in alveolar macrophages and in human lung tissues: expression and modulation by inflammatory stimuli. *Am J Respir Cell Mol Biol* (2003) 28:439–49. doi:10.1165/ajrccm.84.0.12010386

120. Sato S, Takeda K, Oka S, Kiyono H, Kitamura Y, Noguchi T, et al. Toll-like receptor 4 in alveolar macrophages and in human lung tissues: expression and modulation by inflammatory stimuli. *Am J Respir Cell Mol Biol* (2003) 28:439–49. doi:10.1165/ajrccm.84.0.12010386

121. Biswas A, Wilmanski J, Forsman H, Hrncir T, Hao L, Tlaskalova-Hogenova H, et al. Toll-like receptor 4 in alveolar macrophages and in human lung tissues: expression and modulation by inflammatory stimuli. *Am J Respir Cell Mol Biol* (2003) 28:439–49. doi:10.1165/ajrccm.84.0.12010386

122. Song J, Abraham SN, TLK3-mediated immune responses in the urinary tract. *Curr Opin Microbiol* (2008) 11:66–73. doi:10.1016/j.mib.2007.12.001

123. Schaefer TM, Fahey JV, Wright JA, Wira CR. Innate immunity in the human female reproductive tract: antiviral response of uterine epithelial cells to the TLR3 agonist poly(I:C). *J Immunol* (2005) 174:992–1002. doi:10.4049/jimmunol.174.2.992

124. Pivarcsi A, Nagy I, Kereck A, Kis K, Kenderessy-Szabo A, Szell M, et al. Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human beta-defensin-2 in vaginal epithelial cells. *Microbes Infect* (2005) 7:1117–27. doi:10.1016/j.micinf.2005.03.016

125. Pivarcsi A, Nagy I, Kereck A, Kis K, Kenderessy-Szabo A, Szell M, et al. Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human beta-defensin-2 in vaginal epithelial cells. *Microbes Infect* (2005) 7:1117–27. doi:10.1016/j.micinf.2005.03.016

126. Pivarcsi A, Nagy I, Kereck A, Kis K, Kenderessy-Szabo A, Szell M, et al. Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human beta-defensin-2 in vaginal epithelial cells. *Microbes Infect* (2005) 7:1117–27. doi:10.1016/j.micinf.2005.03.016

127. Sellami H, Said-Sadier N, Znazen A, Gdoura R, Ojcius DM, Hammami A. Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human beta-defensin-2 in vaginal epithelial cells. *Microbes Infect* (2005) 7:1117–27. doi:10.1016/j.micinf.2005.03.016

128. Zhang D, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA, et al. Toll-like receptor 4 explains endotoxin hyporesponsiveness in human intestinal epithelial cells. *Microbiol* (2005) 2:784–92. doi:10.1128/IAI.00930-04

129. Pivarcsi A, Nagy I, Kereck A, Kis K, Kenderessy-Szabo A, Szell M, et al. Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human beta-defensin-2 in vaginal epithelial cells. *Microbes Infect* (2005) 7:1117–27. doi:10.1016/j.micinf.2005.03.016

130. Zhang D, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA, et al. Toll-like receptor 4 explains endotoxin hyporesponsiveness in human intestinal epithelial cells. *Microbiol* (2005) 2:784–92. doi:10.1128/IAI.00930-04
144. Scott DA, Krauss J. Neutrophils in periodontal inflammation. *Front Oral Biol* (2012) **156**:56–83. doi:10.1515/00329672

145. Liu J, Chen J, Xu X, He, L, Chen L. The expression of hBDs in the gingival tissue and keratinocytes from healthy subjects and periodontitis patients. *Arch Oral Biol* (2014) **59**:193–8. doi:10.1016/j.archoralbio.2013.11.007

146. Lu Q, Darveau RP, Samarayake WP, Wang CY, Jin L. Differential modulation of human (beta)-defensins expression in human gingival epithelia by *Porphyromonas gingivalis* lipopolysaccharides with tetra- and penta-acylated lipid A structures. *Innate Immun* (2009) **5**:325–35. doi:10.1080/1530992090140899

147. Hazlett L, Wu M. Defensins in innate immunity. *Cell Tissue Res* (2011) **349**:175–88. doi:10.1007/s00441-010-1022-4

148. Chung WO, Dale BA. Differential activation of nuclear factor-kappaB signaling pathways for gingival epithelial cell responses to oral commensal and pathogenic bacteria. *Oral Microbiol Immunol* (2008) **23**:119–26. doi:10.1111/j.1399-302X.2007.00398.x

149. Uchra A, Sugawara S, Takada H. Priming of human oral epithelial cells by interferon-gamma to secrete cytokines in response to lipopolysaccharides, lipoteichoic acids and peptidoglycans. *J Med Microbiol* (2002) **51**:626–34.

150. Mohammed I, Suleman H, Otri AM, Kulkarni BB, Chen P, Hopkinson A, et al. Localization and gene expression of human beta-defensin 9 at the human ocular surface epithelium. *Invest Ophthalmol Vis Sci* (2010) **51**:4677–82. doi:10.1167/iovs.09-4674

151. Otter AM, Mohammed I, Al-Aqaba MA, Fares U, Peng C, Hopkinson A, et al. Variable expression of human beta defensins 3 and 9 at the human ocular surface in infectious keratitis. *Invest Ophthalmol Vis Sci* (2012) **53**:757–61. doi:10.1167/iovs.11-8467

152. Zhang L, Che C, Lin J, Liu K, Li DQ, Zhao G. TLR-mediated induction of proinflammatory cytokine IL-32 in corneal epithelium. *Curr Eye Res* (2013) **38**:630–8. doi:10.1007/s00428-2012-763102

153. Ohnisih T, Muroi M, Tanamoto K. The lipopolysaccharide-recognition mechanism in cells expressingTLR4 and CD14 but lacking MD-2. *FEMS Immunol Med Microbiol* (2007) **51**:84–91. doi:10.1111/j.1365-2672.2007.00281.x

154. Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, Pace NR. The epithelial cells produce B cell-activating factor of TNF family by an IFN-beta-dependent mechanism. *J Immunol* (2006) **177**:2424–32. doi:10.4049/jimmunol.168.5.2424

155. Tenagu S, Hamil KG, Birse CE, Ruben SM, French FS, Hall SH. Antibacterial properties of the sperm-binding proteins and peptides of human epididymal 2 (H2E) family: salt sensitivity, structural dependence and their interaction with outer and cytoplasmic membranes of *Escherichia coli*. *Biochem J* (2003) **372**:473–83. doi:10.1042/BJ20030225

156. Com E, Bourgeon F, Evrard B, Ganz T, Collou D, Jegou B, et al. Expression of antimicrobial defensins in the male reproductive tract of rats, mice, and humans. *Biol Reprod* (2003) **68**:95–104. doi:10.1095/biolreprod.102.005389

157. Fichorova RN, Desai PJ, Gibson FC III, Genco CA. Distinct proinflammatory cytokine II expression and antigen presentation by human endometrial cells. *Infect Immun* (2009) **78**:1523–32. doi:10.1128/IAI.69.9.5840-5848.2010

158. Agrawal T, Vats V, Salhan S, Mittal A. The mucosal immune response to *Chlamydia trachomatis* infection of the reproductive tract in women. *J Reprod Immunol* (2009) **83**:180–90. doi:10.1016/j.jromi.2009.07.013

159. Wallace PK, Yeaman GR, Johnson K, Collins JE, Guyre PM, Wira CR. MHC class II expression and antigen presentation by human endometrial cells. *J Steroid Biochem Mol Biol* (2001) **76**:203–11. doi:10.1006/jsbi.2000.0419

160. Al- Moulay N, Eley A. Interaction of Chlamydia trachomatis serovar E with male genital tract epithelium results in secretion of proinflammatory cytokines. *J Med Microbiol* (2007) **56**:1025–32. doi:10.1099/jmm.0.47241-0

161. Dixit E, Kagan JC. Intracellular pathogen detection by RIG-I-like receptors. *Innate Immun* (2009) **13**:476–86. doi:10.1016/j.innimp.2009.07.013

162. Rahman AH, Eisenberg RA. The role of toll-like receptor family of innate immunity pattern-recognition receptors are abundant in the male rat reproductive tract. *Biol Reprod* (2012) **87**:56–64. doi:10.1095/biolreprod.106.059410

163. Fraczek M, Kurpisz M. Inflammatory mediators exert toxic effects of oxidative stress on human spermatozoa. *Anatol J* (2007) **28**:325–33. doi:10.1246/anaj.106.001149

164. Agrawal T, Vats V, Salhan S, Mittal A. The mucosal immune response to *Chlamydia trachomatis* infection of the reproductive tract in women. *J Reprod Immunol* (2009) **83**:180–90. doi:10.1016/j.jromi.2009.07.013

165. Wallace PK, Yeaman GR, Johnson K, Collins JE, Guyre PM, Wira CR. MHC class II expression and antigen presentation by human endometrial cells. *J Steroid Biochem Mol Biol* (2001) **76**:203–11. doi:10.1006/jsbi.2000.0419

166. Al- Moulay N, Eley A. Interaction of Chlamydia trachomatis serovar E with male genital tract epithelium results in secretion of proinflammatory cytokines. *J Med Microbiol* (2007) **56**:1025–32. doi:10.1099/jmm.0.47241-0

167. Dixit E, Kagan JC. Intracellular pathogen detection by RIG-I-like receptors. *Innate Immun* (2009) **13**:476–86. doi:10.1016/j.innimp.2009.07.013

168. Rahman AH, Eisenberg RA. The role of toll-like receptor family of innate immunity pattern-recognition receptors are abundant in the male rat reproductive tract. *Biol Reprod* (2012) **87**:56–64. doi:10.1095/biolreprod.106.059410

169. Fraczek M, Kurpisz M. Inflammatory mediators exert toxic effects of oxidative stress on human spermatozoa. *Anatol J* (2007) **28**:325–33. doi:10.1246/anaj.106.001149

170. Agrawal T, Vats V, Salhan S, Mittal A. The mucosal immune response to *Chlamydia trachomatis* infection of the reproductive tract in women. *J Reprod Immunol* (2009) **83**:180–90. doi:10.1016/j.jromi.2009.07.013

171. Wallace PK, Yeaman GR, Johnson K, Collins JE, Guyre PM, Wira CR. MHC class II expression and antigen presentation by human endometrial cells. *J Steroid Biochem Mol Biol* (2001) **76**:203–11. doi:10.1006/jsbi.2000.0419

172. Al- Moulay N, Eley A. Interaction of Chlamydia trachomatis serovar E with male genital tract epithelium results in secretion of proinflammatory cytokines. *J Med Microbiol* (2007) **56**:1025–32. doi:10.1099/jmm.0.47241-0

173. Dixit E, Kagan JC. Intracellular pathogen detection by RIG-I-like receptors. *Innate Immun* (2009) **13**:476–86. doi:10.1016/j.innimp.2009.07.013