MINIREVIEW

Intestinal Microvascular Endothelium and Innate Immunity in Inflammatory Bowel Disease: a Second Line of Defense?

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Impairment of the intestinal epithelial barrier function is presently understood to be a key pathogenic step in the initiation and development of chronic human inflammatory bowel diseases (IBD), which include Crohn’s disease (CD) and ulcerative colitis (UC). Invasion by microbial pathogens has been proven to be a crucial event in the perpetuation of the disease process (21, 49). Limited data are available on how mucosal cellular compartments—which include local leukocyte populations as well as nonimmune cells such as fibroblasts and endothelial cells (EC)—might respond to invading enteric pathogens. In IBD, these pathogens might include adherent-invasive pathovars of Escherichia coli, which have been associated with ileal Crohn’s disease involvement for some patients (20). However, studies assessing mucosal invasion should be interpreted with caution in view of the fact that enteric microbes potentially contaminate deeper mucosal layers during sampling and sectioning of tissue specimens (79). Several mechanisms of innate and adaptive immunity are thought to orchestrate the immune response (10, 57), serving a sentinel function by communicating with other local immune cell populations. In the setting of inflammation, primary cultures of human intestinal microvascular endothelial cells (HIMEC) have been shown to express antigen presentation functions in vitro (34). In addition, local immune cell populations normally residing in the bowel lumen are able to translocate to the mucosal and submucosal layers (72). Having reached this subepithelial compartment, microbes will normally be recognized by pathogen-associated molecular pattern (PAMP) receptors, including Toll-like receptors, which are expressed by mucosal immune and nonimmune cells. These cell populations include macrophages, neutrophils, and dendritic cells, as well as intestinal myofibroblasts (63) and EC (52) (Fig. 1). In addition to their function as a second mechanism of defense, EC are able to rapidly mount an innate immune response (10, 57), serving a sentinel function by communicating with other local immune cell populations. In the setting of inflammation, primary cultures of human intestinal microvascular endothelial cells (HIMEC) have been shown to possess antigen presentation functions in vitro (34). In addition, human EC are believed to promote proinflammatory signaling to distant immune cells by release of EC-derived soluble mediators, including chemokines such as interleukin 8 (IL-8; also known as CXCL8) (8) and RANTES (regulated on activation, normal T-cell expressed and secreted; also known as CCL5) (74), upon stimulation with bacterial antigens, including LPS. If all of these mechanisms and cellular interactions fail, due to either overwhelming bacterial contamination or a delayed and attenuated immune response, pathogens will be able to enter the intestinal microcirculation, resulting in systemic infection and septic illness.

ENDOTHELIAL HETEROGENEITY AND HIMEC CULTURE

Most of the knowledge of how microvessels respond to inflammatory challenges is obtained from in vivo animal studies. However, rodent models are known to have significant differences from human vascular biology. For example, gut-specific adhesion molecules, including the lymphocyte homing factor...
TOLL-LIKE RECEPTORS AND BACTERIAL SENSING IN EC

TLRs expressed on EC contribute to early stages of the immune response against various microbial agents and represent an essential component of the innate immune system. Toll-like receptors are highly conserved members of a large family of PAMP recognition receptors, which are activated upon binding of their respective ligands (4). Independently of antigen-processing functions, TLR signaling occurs rapidly as a mechanism of innate immunity. TLRs are therefore critically involved in the primary response of spatially organized mucosal cells in the setting of microbial encounters (1). The human TLR family belongs to the family of IL-1 receptors and comprises 10 type I transmembrane receptor molecules (TLR1 to TLR10), which are expressed by antigen-presenting cells such as activated macrophages and dendritic cells (1). In addition, TLRs are expressed by a multitude of mucosal cell populations, including intestinal myofibroblasts (63) and intestinal epithelial cells (17). Known microbial TLR agonists include PAMPs such as polyacylated lipopeptides (recognized by TLR1–TLR2 [TLR1/2] and TLR2/6 heterodimers), viral double-stranded RNA (TLR3), bacterial LPS (TLR4), and bacterial flagellin (TLR5), as well as nonmethylated bacterial CpG DNA (TLR9), which have been reported to have beneficial effects as immunostimulatory agents in experimental models of inflammatory bowel disease (67, 68). Binding of a PAMP to a TLR evokes a rapid proinflammatory response, which is transduced by TLR-associated adaptor molecules, including myeloid differentiation factor 88 (MyD88) and IL-1 receptor-associated kinase (IRAK), resulting in activation of intracellular protein kinases and nuclear translocation of the activated transcription factor nuclear factor κB (NF-κB) (for reviews, see references 17 and 80). Previous studies published by our group have indicated that HIMEC respond rapidly to stimulation with the TLR agonists LPS (TLR4) from E. coli (57) and flagellin from Salmonella spp. (TLR5) (52) by expression of proinflammatory effector molecules, including endothelial adhesion factors (intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1]) and secreted cytokines, such as IL-6 and IL-8 (Fig. 2).

RELEVANCE OF ENDOTHELIAL TLRs IN MUCOSAL IMMUNOLOGY

A list of TLRs and a summary of their characteristics are given in Table 1.

**TLR1.** TLR1 and TLR2 are crucial receptors for the recognition of bacterial triacylated lipoproteins/lipopeptides, which include 19-kDa mycobacterial lipoprotein (54). Little is known about the expression of TLR1 in EC so far. TLR1 represents the first TLR homolog described for mammals and is expressed in leukocytes and HUVEC (40). Conflicting results regarding the expression of TLR1 in HIMEC-1 cells, an immortalized human microvascular endothelial cell line, have been published by Fichorova et al. and Spitzer et al. (26, 78). The mechanisms transduced by TLR1 activation are poorly defined but are believed to exhibit modulating functions in the setting of TLR2- and TLR6-mediated responses.

**TLR1 overexpression by transfection of human HeLa cells**
has been found to alter the PAMP detection specificity of TLR2. In addition, coexpression of TLR1 leads to diminished recognition of bacterial modulin by TLR2 in a murine macrophage cell line (86). Surprisingly, a report by Spitzer et al. indicates that stable transfection of HMEC-1 cells with TLR1 leads to abrogation of LPS-induced TLR4 signaling, which is considered a major innate response mechanism in the defense arsenal of EC (78).

The relevance of this mechanism remains unclear but leaves room for speculation that TLRs expressed on human EC might have interdigitating functions, influencing each other’s detection specificity patterns. Data on the expression and relevance of TLR1 in human IBD are lacking so far.

**TLR2.** Compared to other TLRs, TLR2 possesses a less specific role as a PAMP receptor and is associated with innate inflammatory responses to lipoteichoic acid (LTA) and bacterial, mycobacterial, and spirochetal lipoproteins (16). TLR2 has been shown to form heterodimers with TLR1 or TLR6. TLR1/2 heterodimers are specific receptors for triacylated lipopeptides, whereas TLR2/6 heterodimers show reactivity in response to the binding of diacylated lipopeptides and peptidoglycan (PGN) (4). In contrast to other TLRs, recognition of microbial components by TLR2 is dependent on association with an additional TLR, such as TLR1 or TLR6 (54). The first evidence showing human endothelial expression of TLR2 comes from Zhang and colleagues, who used HMEC-1 cells to characterize the signaling pathways utilized by human endothelial cells stimulated with bacterial LPS (87). Work conducted by Faure et al. revealed a proinflammatory up-regulation of endothelial TLR2 expression after stimulation with gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), and bacterial LPS in an NF-κB-dependent manner.

HMEC-1 cells are rendered responsive to TLR2 agonists by overexpression of TLR2, indicating that human EC are capable of mounting innate and adaptive immune responses to TLR2 agonists in vitro (25).

Specific oxidized phospholipids, which accumulate at sites of extensive inflammatory endothelial cell activation, have been shown to be potent desensitizers of TLR2 agonistic activity, pointing to a possible fine-tuning mechanism in limiting endothelial cell activation in inflamed tissues (85). Also, physical factors, including laminar fluid flow, have been shown to reduce TLR2 expression and PAMP responsiveness in human EC (23), suggesting the possibility that impairment of microvascular flow, as observed in IBD-associated microvascular dysfunction (36), confers a high susceptibility to TLR2 ago-

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**TABLE 1. Pathogen recognition receptors expressed by human endothelial cells**

<table>
<thead>
<tr>
<th>Pathogen recognition receptor</th>
<th>Known ligand(s)</th>
<th>Expression in human EC</th>
<th>Response to ligand in human EC</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1/2</td>
<td>Triacylated lipopeptides</td>
<td>+</td>
<td>+</td>
<td>26, 40</td>
</tr>
<tr>
<td>TLR2</td>
<td>PGN, LTA, lipoarabinomannan</td>
<td>+</td>
<td>+</td>
<td>25, 87</td>
</tr>
<tr>
<td>TLR2/6</td>
<td>Diacylated lipopeptides</td>
<td>+</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>TLR3</td>
<td>Viral dsRNA, poly(I:C)</td>
<td>+</td>
<td>+</td>
<td>40, 53, 83</td>
</tr>
<tr>
<td>TLR4</td>
<td>LPS, LTA</td>
<td>+</td>
<td>+</td>
<td>57</td>
</tr>
<tr>
<td>TLR5</td>
<td>Flagellin</td>
<td>+</td>
<td>+</td>
<td>52</td>
</tr>
<tr>
<td>TLR7/8</td>
<td>Antiviral imidazoquinolines (Imiquimod, R-848), viral ssDNA</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>TLR9</td>
<td>Unmethylated bacterial Cpg DNA</td>
<td>+ (PCR)</td>
<td>Unknown</td>
<td>Unpublished observation</td>
</tr>
<tr>
<td>TLR10</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>NOD1</td>
<td>Intracellular bacteria, gram-negative PGN</td>
<td>+</td>
<td>+</td>
<td>60-62</td>
</tr>
<tr>
<td>NOD2</td>
<td>Intracellular bacteria, muramyl dipeptide</td>
<td>+</td>
<td>+</td>
<td>59</td>
</tr>
</tbody>
</table>

*Human endothelial cells have been shown to constitutively express an arsenal of pathogen recognition receptors, including Toll-like receptors and NOD proteins 1 and 2. Activation of these receptors by their cognate ligands leads to a ligand-specific innate immune response, characterized by expression of leukocyte adhesion molecules, induction of angiogenesis, and secretion of soluble immune mediators, including cytokines and chemokines.

*dsRNA, double-stranded RNA; ssRNA, single-stranded RNA.

**+**, expression or response.
nists. Similar mechanisms might be responsible for the enhanced expression of TLRs in atherosclerotic plaques (24), further supporting the idea of a possible link between cardiovascular disease and innate immunity (19).

To date, no specific findings that these receptor combinations are expressed on human EC have been reported, and so far their roles in human IBD remain undefined.

**TLR3.** In 2001, Miettinen et al. were able to detect up-regulation of TLR3 expression in human EC by IFN-α and IFN-γ (53). These findings were corroborated by Hijiya et al., who found weak constitutive expression of TLR3 in primary HUVEC (40). A very recent article by Tissari and coworkers has further substantiated the proinflammatory response elicited by poly(I:C) in HUVEC. Stimulation with IFN-α and IFN-β was effective in enhancing TLR3 expression in HUVEC, increasing cellular responsiveness to poly(I:C), resulting in enhanced expression of the recently identified type III interleukins IL-28 and -29. Moreover, expression of STAT1, a signal-transducing molecule important for interferon-induced signals, was markedly enhanced after stimulation with poly(I:C) in HUVEC (83). Our own experiments have indicated that HIMEC constitutively express TLR3 (J. Heidemann, unpublished data). Using an array of classical proinflammatory stimuli, we were able to enhance the expression of TLR3 in HIMEC by IFN-γ and TNF-α but not by LPS or IL-1β treatment. In addition to its well-characterized functions in the regulation of adaptive immunity, IFN-γ is considered a pivotal cytokine in the defense against viral infections (76). In this context, it appears to function as a sensitizer for viral antigens in HIMEC. Interestingly, simultaneous stimulation with TNF-α and LPS led to attenuation of the TNF-α-induced response (J. Heidemann, unpublished data). Viral double-stranded RNA was initially identified as the specific ligand for TLR3, signaling through the proinflammatory transcription factor NF-κB (5). In our hands, poly(I:C), an experimental ligand for TLR3, was effective in up-regulating mRNAs for IL-12-related molecules, including IL-12p35 and Epstein-Barr virus (EBV)-induced gene 3 (EBI3) (Heide- mann, unpublished), which have been identified as important immune mediators in the setting of Th1-driven immune responses (28, 47, 65). These findings further support the concept that human intestinal EC might be able to mount initial steps of an antiviral response when challenged by viral antigens.

Single studies have addressed the hypothesis that mucosal (super)infections with EBV occur as possible pathogenetic events in the development of human IBD and Th1-driven immune responses, including granuloma formation. Although a large number of EBV-infected mucosal cells were detectable in actively inflamed bowel mucosae of both CD and UC patients in a study published by Gehlert and colleagues, the authors were unable to prove a significant correlation between expression of EBI3 and EBV-infected cells (28).

**TLR4.** TLR4 has been widely accepted as the best-characterized endothelial pathogen receptor. Bacterial LPS, the identified TLR4 ligand, has been understood as a classical proinflammatory activating factor in human EC for many years (10). The proinflammatory activation of human EC elicited by LPS has been extensively studied, and the resulting endothelial response mechanisms, e.g., secretion of cytokines, up-regulation of leukocyte adhesion molecules, and major histocompatibility complex class II-associated antigen presentation, have been meticulously characterized. LPS bound to LPS-binding protein is readily recognized by the LPS receptor complex expressed on the outer endothelial cell membrane, consisting of TLR4 and CD14, which functions as a costimulatory molecule. Binding of LPS results in activation of intracellular signaling cascades, which involve TLR-associated adaptor molecules such as MyD88, IRAK-1, and TNF receptor-associated factor 6 (TRAF6), the last of which has been identified as a signaling molecule required for LPS-induced microvascular angiogenesis (66). Activation of these signaling molecules eventually leads to activation and nuclear translocation of the proinflammatory transcription factor NF-κB (4, 66). In HIMEC, LPS stimulation leads to up-regulation of the adhesion molecules E-selectin, ICAM-1, and VCAM-1 as well as of IL-6 and IL-8, among others, fostering an acute-phase proinflammatory response. Accordingly, experimental endothelial leukocyte adhesion, as measured by static and dynamic leukocyte adhesion assays, was dramatically enhanced as well (57). Given that large amounts of enteric flora-derived LPS reside in the bowel lumen and owing to the marked human intestinal endothelial reactivity shown in these experimental series, there is room for speculation that hyperresponsiveness to enteric LPS may occur as a central step in the pathophysiology of human IBD.

**TLR5.** Recently it has been shown that human EC constitutively express significant levels of the receptor for bacterial flagellins, TLR5. Upon challenge with flagellins derived from clinical *Escherichia* and *Salmonella* isolates, HIMEC were shown to up-regulate their surface levels of ICAM-1, which is considered a classical leukocyte adhesion factor in the setting of inflammation. This was accompanied by enhanced endothelial leukocyte transmigration rates, as assessed by in vitro assays. Immunohistochemistry of normal human colonic mucosa revealed substantial immunoreactivity for TLR5 in the mucosal microvasculature, providing evidence for a second line of defense in the immune reaction to mucosally invading flagellated bacteria (52). This hypothesis is supported by previous data published by Gewirtz and coworkers, who were able to show constitutive and functional expression of TLR5 on the basolateral but not the apical side of human colonic epithelial cells in situ (29).

Once the epithelial barrier keeping bacterial flagellins away from the subepithelial compartment is torn down, TLR5 expressed on EC might serve a sentinel function in detecting flagellated microbes before they enter the circulation, thereby potentially saving the organism from septic illness. Although recently there is a growing body of evidence hinting at flagellins as dominant bacterial antigens involved in the pathogenesis of CD (50, 75, 82), the role of TLR5 expressed in human IBD is still uncertain.

**TLR6.** Information on TLR6 expression in human EC is very limited to date. In unstimulated HMEC-1 EC, TLR6 has been shown to be expressed constitutively at substantial levels. Bulut and coworkers have reported that endothelially expressed TLR6 functionally cooperates with TLR2 in transducing pathogen-specific signals evoked by bacterial antigens, including soluble tuberculosis factor, *Borrelia burgdorferi* outer surface protein A lipoprotein (OspA-L), and phenol-soluble modulin, which is a complex composed of three *Staphylococcus*
epidermidis-derived antigens (16). Expression of TLR6 in human IBD has not been determined so far, and the pathogenetic role of TLR6 expressed in human EC remains unclear.

**TLR7 and TLR8.** R-848, a low-molecular-weight antiviral compound of the imidazoquinoline family, has been reported to be the ligand for TLR7 and, to a lesser extent, TLR8 (39, 45). The specificity of R-848 for human TLR7 and TLR8 has been confirmed by genetic complementation studies. R-848 is able to stimulate activation of NF-κB in human HEK 293 embryonic kidney cells stably transfected with human TLR7 or TLR8 but not with human TLR2, TLR3, or TLR9. Although R-848 has been shown to activate both TLR7 and TLR8, TLR7 shows 10-fold-higher sensitivity to R-848 (45). These data suggest a possible redundancy between these two TLRs. Recent reports have indicated that TLR7 and TLR8 are receptors sensing viral single-stranded RNA (22, 38). According to a recent study published by Tissari et al., TLR7 and TLR8 are not expressed in HUVEC, and HUVEC are unresponsive to treatment with R-848 as assessed by Northern blot analysis, electrophoretic mobility shift assays for NF-κB activation, and phospho-STAT1 immunoblotting (83). In contrast, Gunzer et al. have detected constitutive expression of TLR7 in murine endothelial cells in vitro and in vivo (31). Interestingly, systemic challenge of mice with the TLR7/TLR8 agonist R-848 leads to a dramatic but transient generalized enhancement of endothelial adhesiveness, accompanied by a rapid and almost complete depletion of leukocytes from the circulation. In addition, administration of R-848 leads to up-regulation of endothelial adhesion molecules in vitro and in vivo, accompanied by enhanced leukocyte rolling on endothelial surfaces in treated animals in vivo (31). Corresponding data on the endothelial expression of these TLRs and their possible relevance in the setting of mucosal defense are lacking so far.

**TLR9.** TLR9, the receptor for unmethylated bacterial CpG DNA, has evoked lively interest among basic scientists and clinical IBD researchers (55). A growing body of evidence suggests that CpG DNA motifs exert marked immunomodulatory effects on murine and human lymphocytes in vitro and murine lymphocytes in vivo (7, 67). Downstream signaling pathways of TLR9 appear to be broadly similar to those of TLR4, including activation of the mitogen-activated protein kinases (MAPks) JNK and p38 as well as of the IκB kinase complex. Effects elicited by TLR9 stimulation include support of B-cell proliferation as well as resistance to apoptosis, release of the acute-phase interleukins IL-6 and IL-12, and activation of monocytes/macrophages and NK cells (81). Interestingly, human colonic epithelial cells have been shown to respond to bacterial CpG DNA stimulation by up-regulation of IL-8, supporting the concept of an active intestinal epithelial barrier capable of initiating rapid innate immune responses (3, 64). The group of Rachmilewitz and coworkers has reported on the beneficial effects of immunostimulatory DNA (ISS-ODN) derived from bacterial CpG DNA sequences when administered in various murine colitis models, including dextran sodium sulfate (DSS)-induced colitis, dinitrobenzene sulfonic-acid-induced colitis, and the murine colitis arising spontaneously in IL-10−/− knockout mice (67). In all models of experimental and spontaneous colitis, treatment of diseased mice with bacterial CpG DNA was effective in ameliorating clinical, biochemical, and histologic scores of colonic inflammation. Further, ISS-ODN administration inhibited the induction of proinflammatory cytokines and chemokines and suppressed colonic matrix metalloproteinases (67). More recent data published by this group have provided evidence that intragastric and subcutaneous administration of unmethylated bacterial DNA (the ligand of TLR9) was effective in reducing the severity of DSS-induced colitis. The severity of DSS-induced colitis in TLR2- and TLR4-deficient mice was significantly decreased by intragastric administration of gamma-irradiated probiotics, whereas for TLR9-deficient mice, gamma-irradiated probiotics had no effect. The authors conclude that the protective effects of probiotics are conferred by bacterial DNA rather than by their metabolites or their ability to colonize the intestine. In these reports, the expression of TLR9 in intestinal EC was not assessed (68).

Regarding endothelial expression of TLR9, Li et al. (48) have described constitutive and regulated expression of TLR9 in pulmonary EC isolated from mice and rats. Stimulation of EC with CpG DNA induced a potent proinflammatory response as indicated by enhanced NF-κB- and p38 MAPK-dependent expression of IL-8 and ICAM-1 in mouse pulmonary EC. Additionally, a synergistic effect of CpG DNA and LPS on the endothelial proinflammatory response was observed (48). In our experimental series, we have observed weak constitutive expression of TLR9 mRNA in HIMEC; expression increased slightly after stimulation with bacterial LPS (Heidemann, unpublished). However, we have not yet been able to reproduce the proinflammatory effects of bacterial CpG DNA on HIMEC.

**TLR10.** At present, TLR10 remains the only orphan member among the family of human TLRs. TLR10 has no rodent homolog, and no natural or synthetic ligand to TLR10 has been discovered. TLR1, TLR6, and TLR10 genes have been localized on chromosome 4p14 and display striking structural similarities (35). As with TLR1–TLR2 and TLR2–TLR6 heterodimers, TLR10 appears to form functional heterodimers with TLR1 and TLR2 (35). Like other TLRs, TLR10 has been shown to interact with the signaling adaptor protein MyD88. The expression of TLR10 has been shown to be restricted to B cells and plasmacytoid dendritic cells (18, 35, 43), and no evidence of human endothelial TLR10 expression has been presented so far.

**SENSING OF INTRACELLULAR PATHOGENS BY EC**

Nucleotide oligomerization domain (NOD)-encoded proteins represent a family of recently identified intracellular pathogen recognition receptors. One of the most important findings in IBD research was the identification of NOD2/CARD15 mutations as potential risk factors for the development of Crohn’s disease in a fraction of patients (32, 44, 58). In later publications, mutations in NOD2/CARD15, a putative intracellular pathogen recognition receptor, were associated with specific Crohn’s disease manifestations, including stenosing disease and complicated clinical courses (2, 6). Subsequent publications have described the expression of NOD2/CARD15 in various intestinal epithelial cell lines (43, 70). Data published by Hisamatsu et al. have indicated that NOD2/CARD15 might serve as an intracellular antibacterial factor in these cells, and loss of NOD2/CARD15 gene function in these cells was hypothesized to be a potential predisposing factor for the...
development of IBD (41). In 2005, HUVEC were shown to display regulated cytosolic expression of NOD2 upon proinflammatory stimulation with bacterial LPS, TNF-α, and IL-1β. The authors of this study concluded that expression of NOD2 in endothelial cells may be relevant for the detection of intracellular pathogen-associated molecular antigens (59).

Furthermore, recent evidence has indicated that human EC express the intracellular pathogen recognition receptor NOD1, which recognizes a muramopeptide from gram-negative peptidoglycan (30). In an elegantly designed study, Opitz and colleagues have characterized the proinflammatory effects elicited by the strictly intracellular bacterium Chlamydia pneumoniae in HUVEC cultures. Viable but not heat-inactivated chlamydiae were required in order to induce proinflammatory activation, as measured by endothelial IL-8 secretion, indicating that cell permeation by the bacteria is required to induce proinflammatory responses. However, heat-inactivated bacteria were able to induce an NF-κB-mediated response when bacteria were transfected intracellularly, whereas extracellular stimulation did not elicit this response. Moreover, stimulation of the proinflammatory response was independent of chlamydial LPS, as shown by preincubation with the LPS-binding antibacterial polyoxymyxin B (60, 62). Signaling pathways involved in endothelial intracellular bacterial sensing are p38 MAPK and activation of NF-κB, as shown by inhibition experiments on pathogen-induced IL-8 secretion (61).

In addition to expression of TLRs on EC surfaces, evidence suggests that human EC are able to detect intracellular bacteria through NOD1, generating an innate immune response. The relevance of this finding for intestinal inflammation and IBD remains to be elucidated.

HYPOTHESIS: POTENTIAL ROLE OF PATHOGEN TOLERANCE IN CHRONIC GUT INFLAMMATION

In the pathogenetic sequence of IBD, a breakdown of the intestinal epithelial barrier is considered to be a central and initial step of mucosal inflammation. Once the mucosal epithelium is disrupted, luminal pathogens and antigens are able to traverse the former barrier, infiltrating the underlying mucosal layers. Microbial antigens recognized by immune cells residing in the mucosa, as well as by EC, trigger an innate immune response, which in turn results in further steps of an adaptive immune response, leading to antigen presentation by antigen-presenting cells, antibody production, and generation of a specific cellular antibacterial defense. In human IBD, sustained inflammatory activation in response to LPS is believed to support damaging effects also, thus playing a central pathogenic role in endotoxic shock and human IBD. Endotoxin tolerance, the LPS-induced transiently impaired inflammatory response to subsequent LPS challenge, has been described at the cellular and molecular level most extensively for monocytes and macrophages. A previous study published by Ogawa and coworkers has indicated that HIMEC possess features characteristic of endotoxin tolerance (57). More specifically, HIMEC responded to repeated stimulations with bacterial LPS by decreased leukocyte binding capacities in static and dynamic adhesion assays, underlining the physiological relevance of this phenomenon in the setting of prolonged enteric antigen exposure, as in chronic IBD. Endotoxin tolerance appears to represent an important down-regulatory mechanism to prevent excessive inflammation and subsequent tissue damage in response to repeated LPS exposure. Endotoxin tolerance in HIMEC was accompanied by altered HIMEC gene activation patterns, which included down-regulation of the endothelial adhesion factors E-selectin and VCAM-1 as well as the acute-phase interleukin IL-6. Assessment of signaling pathways in HIMEC rendered tolerant to endotoxin suggests that modulation of NF-κB as well as p44/42 MAPK, p38 MAPK, and JNK activation might be central events associated with LPS tolerance in HIMEC. Repeated LPS stimulation also resulted in enhanced manganese superoxide dismutase expression in HIMEC, which was correlated with a significant reduction in superoxide anion generation after LPS challenge. Superoxide radicals are known to increase leukocyte-EC adhesion, and superoxide dismutase is known to inhibit increased adhesion mediated by superoxide through degradation of superoxide anions. Interestingly, TLR4 expression was not altered during the LPS tolerance experiments, suggesting that this phenomenon is dependent on the above mechanisms rather than on modulation of TLR4 expression. The authors hypothesize that LPS tolerance in HIMEC may represent an important mechanism in controlling gut inflammation during intestinal immune homeostasis in physiology and pathophysiology (57). Whether additional mechanisms of pathogen tolerance in HIMEC might be involved in controlling mucosal inflammation, e.g., by flagellin or bacterial CpG DNA motifs, has not been determined and leaves room for further speculation.

CONCLUSION

There is accumulating evidence that human endothelial cells residing in mucosal barriers are equipped with an array of microbial pattern recognition receptors that serve cardinal functions in the generation of innate immune responses. Upon microbial challenge, endothelial cells armed with such receptors are able to elicit rapid response mechanisms, which include up-regulation of leukocyte adhesion molecules and induction of angiogenesis, as well as paracrine signaling to local immune cells and systemic secretion of immune mediators to the circulating bloodstream. Human endothelial cells are capable of detecting both extracellular and intracellular microbial invaders by antigen-specific receptor combinations (e.g., TLR heterodimers) and activation of NOD family proteins. As demonstrated for various immune cell populations, human endothelial cells physiologically display mechanisms of endotoxin tolerance, and the dysregulation of this mechanism might play a central role in immune-mediated diseases where microbial antigens perpetuate the inflammatory process, including human IBD.

ACKNOWLEDGMENT

The authors do not declare any competing interests.

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evokes epithelial IL-8 production by a MAPK-dependent, NF-κB-independent pathway. FASEB J. 17:1319–1321.


