Expression of TLR-4 in colon of children with Crohn’s disease

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Abstract

Toll-like receptor (TLR)-4 plays a key role in microbial recognition, induction of antimicrobial responses and control of tolerance in the gut. The aim of the study was to analyze TLR-4 expression in colon of children with Crohn’s disease (CD) in comparison with histological changes. The tissue specimens were obtained from colon of 18 children with recognized CD. Histological stage of inflammation was determined in all specimens. TLR-4 protein was determined by immunohistochemistry. In non inflamed mucosa TLR-4 was found only in lamina propria, whereas in inflamed tissue it was expressed both in epithelium and in lamina propria. TLR-positive cells infiltrating lamina propria were located below basal membrane, and their number increased in specimens with moderate inflammation compared to tissues with slight inflammation. TLR-4 positive cells located in lamina propria created conglomerates in mucosa with moderate inflammation whereas they were dispersed in specimens with slight inflammatory changes. The most intense epithelial expression of TLR-4 was found in mucosa with moderate inflammation. Neither TLR-4 positive cells in lamina propria, nor TLR-4 expression in epithelium were found in sections with severe inflammation and granuloma. Our results show that TLR-4 expression in CD mucosa is dependent on severity of inflammation. The increased intensity in mucosa with moderate inflammation, but not with grade injury could suggest protective role of TLR-4 in CD pathogenesis.

Key words: Crohn’s disease, TLR-4, inflammatory bowel disease, epithelial cells

Introduction

Inflammatory bowel disease (IBD) comprising Crohn’s disease (CD) and ulcerative colitis (UC), is a chronic inflammatory disorder caused by multifactorial conditions in genetically predisposed host. There is increasing evidence that disturbed homeostasis between intestinal microbiota and mucosal immunity is a critical determinant of chronic intestinal inflammatory processes in IBD. Toll-like receptors (TLRs) expressed on intestinal epithelial cells play a key role in microbial recognition, induction of antimicrobial responses and control of tolerance in the gut [2, 3, 7]. TLRs comprise a class of 13 mammalian type I transmembrane glycoproteins which contain multiple leucine-rich repeat motifs (LRR) and a conserved region called the Toll-interleukin-1 receptor (RIR) domain [1]. The TIR domain consists of sites...
essential for interaction between TLR subunits as well as a recruitment of cytoplasmic adaptor proteins to initiate the downstream signaling cascades. TLRs recognize microbiota/viral-associated both commensal and pathogen molecular patterns. TLRs are expressed throughout the whole gastrointestinal tract by a wide variety of cells including enterocytes/colonocytes, Paneth cells, goblet cells, subepithelial myofibroblasts and specialized immune cell subsets within the intestinal lamina propria, such as macrophages, dendritic cells, and lymphocytes. In the healthy intestine TLR-4, recognizing bacterial lipopolysaccharides (LPS) are present only in small amounts on both epithelial and lamina propria cells [5, 7-10]. Our studies in germ-free mice presented that TLR-4 expression in intestine avoided of microbes is expressed in apical part of colon epithelium and cytoplasm of immature crypt cells [11]. In contrast, mice reared in conventional conditions, colonized with non-pathogenic intestine microbiota expressed TLR-4 in lower amounts and in different pattern – this receptor was only localized in crypt cells. Thus, TLR-4 activation in healthy conditions is minimized to maintain mucosal and commensal balance. It is supposed that TLR hyperactivation may provoke chronic inflammation. TLR-4 expression is significantly increased in epithelium and lamina propria cells throughout the lower gastrointestinal tract in active CD and UC [5, 7, 8]. Thus, the upregulation of TLR-4 could be responsible for sustained activation of the immune system in IBD patients. On the other hand, experimental induction of IBD with dextran sodium sulphate (DSS) in mice showed that TLR-4 signaling may exert cytoprotective characteristics [6, 10].

The aim of present study was to analyze TLR-4 expression in colon of children with CD in comparison with a histological stage of inflammation.

Materials and methods

In the prospective study the specimens were obtained from 18 children with recognized CD being patients of the Children Memorial Health Institute, Department of Gastroenterology, Hepatology and Immunology. The diagnosis of CD was based on clinical, radiographic, endoscopic and histopathological findings. The study was approved by local ethical committee and was performed with informed written consents of parents.

Histological analysis

Colonic biopsy specimens were taken from the endoscopically active lesions (inflamed mucosa) as well as from mucosa which did not present pathological changes (normal mucosa). The biopsy was fixed in 10% buffered formalin solution for 24h, dehydrated in graded alcohol and embedded in paraffin. Subsequently, paraffin was removed from 4 μm thick sections and they were stained with hematoxylin and eosin.

Immunohistological determination of TLR-4

Colon biopsy were used to obtain 4 μm-thick sections. Sections were de-paraffined and rehydrated using a graded alcohol series. Antigen retrieval was done using 0,01 M citrate buffer pH 6,0. Slides were boiling for 30 minutes, washed in phosphate buffer solution (PBS) pH 7,4, and incubated for 10 minutes at room temperature in 3% H2O2 to block the activity of endogenous peroxidases. After further incubation for 30 minutes with 10% normal goat serum, primary polyclonal rabbit anti-human TLR-4 (Santa Cruz Biotechnology) diluted 1:5 in PBS was applied for 2 hours in room temperature. Then slides were washed with and incubated with secondary antibody goat anti-rabbit (Jackson Immunoresearch) diluted 1:200 in PBS for 1 hour in room temperature. Reactions were developed using AEC-chromogen (Dakocyton). Finally, slides were counterstained with hematoxylin. Negative controls were prepared by replacing the primary antibody with PBS.

Results

Histology

All sections obtained from macroscopically “affected” mucosa presented histological features of inflammation determined as slight, moderate or severe inflammation. In most sections obtained from non-affected mucosa chronic nonspecific inflammation was observed. Typical granulomas were found in two sections with severe inflammation.

TLR-4 expression

TLR-4 was found in cytoplasm of single mononuclear cells in lamina propria, but not in epithelial cells of non-inflamed mucosa. TLR-4 positive cells were located below basal membrane. The number of cells expressing TLR-4 in lamina propria increased in sections with slight and moderate features of inflammation. TLR-4 – positive cells were mainly located near the epithelium layer (Fig. 1), and some of them crossed through the basal membrane and placed intra epithelial cells. In contrast to tissues with slight inflammation where TLR-4 positive cells were dispersed, in tissue sections with moderate inflammation those cells created conglomerates (Fig. 2). TLR-4 was also found in epithelial cells, but only in mucosa with moderate inflammation. Both cytoplasmic and apical patterns of TLR-4 expression were observed in epithelium. Cytoplasmic expression was mainly detected in colonocytes covering conglomerates of TLR-4 positive cells, whereas a apical expression was typical for colonocytes located under lamina propria deprived of TLR-4 positive cells. Interestingly, neither TLR-4 positive cells in lamina propria, nor TLR-4 expression in colonocytes were found in sections with severe inflammation and granuloma (Fig. 3).

Discussion

The first evidence that TLR-4 expression was increased in the intestine of IBD patients was given by Cario and Podolsky more than 10 years ago [5]. They presented that TLR-4 was abundantly expressed by epithelial cells and cells infiltrating lamina propria of UC and CD patients. In-
Interestingly, enhanced expression of TLR-4 was found in inflamed and non-inflamed mucosa. In contrast to this study, we found that expression of TLR-4 varied in tissues with different histological stages. The highest expression intensity was found in mucosa with moderate inflammation both in epithelium and in lamina propria. TLR-4 was not present either in epithelial cells of non-inflamed mucosa and of tissue with slight inflammation or in tissue with severe inflammatory features and granuloma. Similarly, the highest amounts of TLR-4 positive cells were demonstrated in tissues with moderate features of inflammation. Hausman et al. also presented a significant increase in TLR-4 expression in inflamed intestinal mucosa compared with non-inflamed mucosa in patients with CD, but they found TLR-4 only in cells infiltrating lamina propria [8]. However, neither Hausmann et al. [8] nor Cario and Podolsky [5] characterized the inflammatory changes in examined tissues. Our results give the first evidence that the TLR-4 expression depends on severity of inflammation, and could suggest a protective role of TLR-4 in CD mucosa. Recently, the importance of TLR-4 activation in maintaining the epithelial barrier has been described in DSS-induced experimental colitis [6, 10]. Fukata et al. showed that DSS treatment of TLR-4 knockout mice was associated with striking reduction in acute inflammatory cells compared with wild-type mice despite similar degrees of epithelial injury [6]. TLR4-/- mice experienced earlier and more severe bleeding than control mice. Similar results were seen with MyD88-/- mice, suggesting that this is the dominant downstream pathway. Mesenteric lymph nodes from TLR4-/- and MyD88-/- mice more frequently grew gram-negative bacteria [6]. Thus, TLR-4 signaling through the adapter molecule MyD88 is important in intestinal response to injury and in limiting bacterial translocation. We presented that intestinal mucosa which did not express TLR-4 presented the grave injury with granuloma. Upregulation of TLR-4 in CD mucosa with moderate inflammation could be a result of response on epithelial damage, and not a reason of activation of pro-inflammatory immune response.

In our study we observed both apical and cytoplasmic localization of TLR-4 in epithelial cells. Cario and Podolsky showed that the epithelial expression pattern differed between CD and UC [5]. Intense staining was mostly present at basolateral surfaces in mucosal sections of UC patients, while in most CD samples TLR-4 was found at the apical pole of intestinal epithelial cells. We found that expression pattern depends on the presence of TLR-4 positive cells in lamina propria. Cytoplasmic expression was limited to epithelium covering conglomerates of TLR-4 positive cells. Cario et al. presented on epithelial cell lines that TLR-4 expression pattern is dependent on the state of cell differentiation as well as on activation by specific bacterial ligands e.g. LPS [4]. Intense staining at the apical surface was found in differentiated epithelial cells, whereas in nondifferentiated cells TLR4 was mostly present in the cytoplasmic compartment. TLR-4 activation with LPS induced redistribution of the receptor from apical part to cytoplasmic compartment near basolat-

![Fig. 1 TLR-4 positive cells (arrow) in colon with slight inflammation](image1)

![Fig. 2 Colon specimen with moderate inflammation. 1. Conglomerates of TLR-4 positive cells in lamina propria, 2. Positive expression of TLR-4 in cytoplasm of epithelial cells, 3. Positive expression of TLR-4 in apical part of epithelium](image2)

![Fig. 3 Colon specimen with severe inflammation and granuloma. Single TLR-4 positive cells in lamina propria (arrow)](image3)
eral membrane. Thus, our result suggest that cytoplasmic expression pattern in epithelial cells could be a result of TLR-4 activation or immaturity of rebuilt epithelium. However, no presence of TLR-4 in tissues with severe inflammation indicates that this receptor could be necessary for rebuilding of injured epithelial cells.

Concluding, we suggest that increased intensity in mucosa with moderate inflammation, but not with grade injury could suggest protective role of TLR-4 in CD pathogenesis.

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References