Epithelial Toll-Like Receptor 5 Is Constitutively Localized in the Mouse Cecum and Exhibits Distinctive Down-Regulation during Experimental Colitis

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Received 25 June 2005/Returned for modification 10 August 2005/Accepted 18 October 2005

We recently demonstrated that the pattern recognition receptors (PRRs) toll-like receptor 2 (TLR2), TLR4, and CD14 are expressed in mouse colonic epithelium in a compartmentalized manner. Here we report the localization of TLR5, the receptor for bacterial flagellin, and its distinctive down-regulation during experimental colitis. Guts from normal BALB/c mice and those with dextran sodium sulfate (DSS)-induced colitis were compared. Each gut was divided into seven segments (stomach, small intestine [three parts], and colon [three parts]), and epithelial cells and crypt units were collected by scraping and EDTA treatment, respectively. Northern blotting showed that TLR5 mRNA was preferentially expressed in the epithelium of the proximal colon in normal mice. Laser capture microdissection coupled to reverse transcriptase PCR confirmed this localization. TLR5 protein expression reflected mRNA expression, as evidenced by Western blotting. In mice with acute colitis, inflammation occurred mainly in the distal colon. Interestingly, while TLR2, TLR4, and CD14 were up-regulated in the inflamed colon, TLR5 was down-regulated at both the mRNA and protein levels. Decreased TLR5 expression was more evident during chronic colitis. Additional in vitro studies using a mouse cell line, Colon-26, showed that gamma interferon (IFN-γ) time- and dose-dependently down-regulates TLR5. In conclusion, epithelial cells, mainly in the proximal colon, constitutively express TLR5. TLR5 expression is down-regulated in vivo during acute and chronic DSS-induced colitis, in contrast to the expression of TLR2, TLR4, and CD14. The mechanism governing TLR5 regulation may therefore differ from that controlling other PRRs. Finally, IFN-γ may be involved in down-regulating TLR5 expression.

The concept of a highly efficient innate immune system that recognizes potential pathogens by detecting lipopolysaccharides (LPSs), peptidoglycans, lipopeptides, flagellin, or many other highly preserved and unvarying structural molecules (27) is now widely accepted, and innate immunity acts as the first-line defense against pathogenic microorganisms in mammals. Although the innate immune system is basically activated by pathogen-related molecules, this activation seems to be driven by several kinds of strategically regulated pattern recognition receptors (PRRs). Because of the variety of PRRs and their sophisticated intracellular signal transduction systems, the innate immune system is increasingly regarded as highly complex.

Since the discovery of human toll (22), several new receptors and ligands have been recognized, and more are constantly being added to the arena. Toll-like receptors (TLRs) are transmembrane proteins that share a leucine-rich repeat ectodomain, which confers high specificity for particular ligands (4). TLRs also share an intracellular domain which contains a toll-interleukin-1 (IL-1) receptor homology domain common to the IL-1 receptor family and the IL-18 receptor (36). Ligand binding to TLRs triggers activation of the IL-1 receptor-associated kinase via the adaptor protein MyD88, formation of a complex with tumor necrosis factor receptor-associated factor 6, and activation of downstream cascades leading to the activation of nuclear factor κB and other factors required for the transcription of proinflammatory cytokine genes (2, 18).

The goal of innate immunity is early discrimination between pathogens and nonpathogens; this task is of extreme importance in anatomic regions in which the epithelial cells are constantly exposed to microbes, such as the gut mucosa-luminal interface. We have recently demonstrated differential expression of the PRRs TLR4, TLR2, and CD14 in the gastrointestinal mucosae of normal and colitic mice (32). We have also found that TLRs are compartmentalized not only at the cellular level, but also within different colonic segments in mice. These findings may help to explain the segmental distribution of colonic inflammation observed in murine experimental models (19, 20).

The present study focuses on TLR5, the receptor for flagellin from gram-positive and -negative bacteria (18). Flagellin is the main structural protein of bacterial flagella. MyD88 seems to be an essential signal transducer for TLR5, since mice deficient in this adaptor are completely unresponsive to flagellin (11). Epithelial cell lines derived from a variety of tissues, including the gut, have been demonstrated to express TLR5 (8, 34, 44), suggesting that this receptor plays an important role in epithelial function. Intestinal epithelial cells express TLR5 on their basolateral sides when forming monolayers (8, 12). Since

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there is no information concerning the in vivo expression of TLR5 in the mouse gut, we aimed to characterize TLR5 localization in the BALB/c mouse gut and to determine how it is modulated during dextran sulfate sodium (DSS)-induced colitis.

MATERIALS AND METHODS

Animal model, experimental design, and sampling. Seven-week-old male specific-pathogen-free BALB/c mice (Nihon Clea, Tokyo, Japan) were studied with the approval of the Ethics Committee for Animal Experimentation of Shimane University, DSS solution (2.5%, wt/vol) was administered as drinking water for 7 days to induce acute colitis. To produce chronic colitis, DSS solution was administered for 7 days, followed by plain drinking water for 10 days, and this cycle was repeated six times. Matched control and experimental animals were sacrificed, starting 24 h after the end of each DSS administration period, by an overdose of diethyl ether. The gut was dissected, opened on an ice-cold surface, rinsed, and divided into seven segments: the glandular stomach, proximal, medial, and distal small intestine; and proximal, medial, and distal colon, as previously published (32). The proximal colon segment corresponds anatomically to the cecum. In general, three methods of epithelial cell sampling were used: scraping of cells from the mucosal surface, detachment of crypts using EDTA the cecum. In general, three methods of epithelial cell sampling were used: scraping of cells from the mucosal surface, detachment of crypts using EDTA, and laser capture microdissection of cells from frozen gut. In a previous study (32), it was shown that scraping is a valid method of obtaining representative samples of gut epithelial cells; the samples contained 94.2% ± 2.1% epithelial cells, 0.56% ± 0.1% CD1c-cells, 1.21% ± 0.1% CD45R cells, and 2.48% ± 0.4% F4/80+ cells, as shown by immunostaining of cyt centrifuged preparations. Although it is likely that nonepithelial cell contaminants may contribute to the findings, the quantity of leukocytes present in the scraped samples is not high enough to be detected by Northern blotting using a CD45 probe as a leukocyte marker (32). Detached colonic crypt samples were taken from the proximal and distal colonic segments. To detach the crypts, the segments were everted and treated with EDTA, resulting in the collection of epithelial samples with <5% contaminating nonepithelial cells (23). For laser microdissection, gut samples were embedded in Tissue-Tek O.C.T. compound (Sakura Finetechanical, Tokyo, Japan), frozen on dry ice, and stored at −70°C. A total of 61 mice were used in the experiments.

Cells and reagents. The BALB/c mouse-derived cancer cell line Colon-26 (13, 39) was obtained from the Cell Resource Center for Biomedical Research of Tohoku University, Colon-26 is an undifferentiated carcinoma induced by the carcinogen N-nitroso-N-methylurethane (13). The human colon cancer cell line HT29 was obtained from the American Type Culture Collection (Manassas, VA). The cells were maintained in RPMI 1640 (ICN Biomedicals, Aurora, OH) supplemented with 10% fetal bovine serum (ICN Biomedicals) and penicillin-streptomycin-ampicillin (GIBCO BRL, Grand Island, NY) and kept at 37°C in an incubator under a 5% CO2 atmosphere and constant humidity. When needed, cell growth was arrested by culture in fetal bovine serum-free media for 16 h. Colon-26 and HT29 membranes interfered from Pierce Endogen (Rockford, IL). Other proteins tested were mouse IL-1β, IL-10, mouse IL-6 (in vitro), and human hepatocyte growth factor (Sigma, and human hepatocyte growth factor (Sigma).

Laser capture microdissection. Fresh frozen tissues were cut into 5-µm-thick sections in a cryostat, mounted on glass slides, fixed with 70% ethanol for 30 s, and washed with deionized water for 20 s. The sections were stained with hematoxylin for 30 s, dehydrated in an ethanol gradient, counter stained with eosin Y for 1 min, dehydrated again, cleared with xylene, and air dried. Microdissection was performed using the Arcturus LM200 laser microdissection system (Olympus, Tokyo, Japan), as reported previously (45). Briefly, the selected area of tissue was covered with laser capture transfer film and pulsed with a laser beam, diameter (30 µm; duration, 5 µs; power, 30 mW). The size of the spot produced by these conditions was just large enough to match the size of the epithelial cells. Each sample consisted of 3,000 shots.

RT-PCR. For reverse transcriptase (RT) PCR, the cells were homogenized and their total RNA was isolated by the guanidine thiocyanate-phenol-chloroform method. The details of the protocol have already been published (32). Ten micrograms of RNA from each scraped gut segment, or the total amount of RNA obtained from 3,000 laser shots, was reverse transcribed. Two microliters of cDNA was then amplified using DNA polymerase (AmpliTag Gold; PE Applied Biosystems, Foster City, CA). The minimum number of PCR cycles required to visualize the target gene signals was 30 standard cycles. The TLR5 primers were 5'-GGA CAT TGA AGG ATT TGA AGA TG-3' and 5'-GGAGCCA CCT TCT GTA TGC TTG GAA TA-3'. The CD45 and β-actin primers have been published previously (32). The PCR products were electrophoresed on an agarose gel with ethidium bromide. β-Actin was amplified as an internal control, and in the case of laser capture samples, all gene signals were semiquantitated by densitometry and standardized to the β-actin signals. To exclude amplification from contaminating genomic DNA, the primers were designed to span different exons when possible, and a sample of nontranscribed RNA was used as a negative control during amplification. To check for carryover contamination, a non-template control reaction was also included.

Northern blotting. Scraped epithelial cells, colonic crypt extracts, or cultured Colon-26 cells were processed to extract RNA as described above for RT-PCR. Twenty micrograms of RNA from the epithelial cells or crypt extracts, or 40 µg of RNA from the Colon-26 cells, were electrophoresed in a denaturing gel, transferred and UV-linked onto a nitrocellulose membrane. Probe DNA sequences were amplified from normal mouse gut cDNA. The materials and methods used to produce the probes have previously been published, as have the primers sequences used for the amplification of TLR2, TLR4, CD14, and IL-1β (32). The TLR5 probe (300 bp) was constructed by RT-PCR using the primers described above for RT-PCR. The primers used for construction of the keratin 19 (KRT19) probe (436 bp) were 5'-CTG TGCTG ATG GCC TGC TG-3' and 5'-TTC AGG TAG GCC AGC TCC T-3'. For construction of the probes, the amplified cDNA was cloned by ligation into a plasmid vector (pCRII, TA Cloning Kit Dual Promoter; Invitrogen, Carlsbad, CA), which was subsequently transfected into competent cells and cultured. The extracted plasmid was restricted and purified. The probe sequences were confirmed using a Big Dye Terminator v1.1 Cycle Sequencing Kit on an ABI PRISM 310 genetic analyzer (PE Applied Biosystems). The probes were labeled with γ-32P-ATP and hybridized at 56°C for 24 h. When possible, hybridization of the studied genes was performed sequentially on the same membrane. The membranes were washed with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.2% sodium dodecyl sulfate for 5 min, first at room temperature and then at 50°C. The signals were detected and measured with a bioimage analyzer (BAS 2000 Il; Fujix, Tokyo, Japan) and were standardized to β-actin.

Western blotting. Fresh frozen tissues were washed four times with phosphate-buffered saline containing 100 µg/ml of the protease inhibitor phenylmethysulfonyl fluoride, and then 2 µl of protease inhibitor cocktail (Sigma, St. Louis, MO) was added. Adherent Colon-26 cells were washed once with phosphate-buffered saline before lysis. The composition of the lysis buffer has been published previously (32). The lysates were centrifuged at 20,000 × g for 20 min at 4°C to separate the supernatant. One hundred micrograms of protein (estimated by the Bradford method) was loaded into each lane for fractionation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was washed three times in Tris-buffered saline (TBS) (10 mM Tris, 150 mM NaCl, pH 7.6), blocked in 5% skim milk in Tween-TBS for 2 h, and then incubated for 24 h with a rabbit anti-human TLR5 polyclonal antibody (Ab) that cross-reacts with mouse TLR5 (H-127; Santa Cruz Biotechnology) at a dilution of 1/500 and a temperature of 16°C. The membrane was washed three times with washing buffer, and then incubated with a 1:1000 dilution of goat anti-rabbit horseradish peroxidase-conjugated secondary antibody in PBS-C at room temperature for 1 h. The blots were developed using a chemiluminescent substrate (Amersham Pharmacia Biotech). The blot was washed and reprobed with anti-β-actin Ab as an internal control.

Statistical analysis. Numerical analyses were performed using a statistical software package (SPSS for Windows, release 10.0.1; SPSS Inc., Chicago, IL). The results were compared using the Mann-Whitney U test. A P value of <0.05 was considered statistically significant. Unless stated otherwise, all results are expressed as means ± standard errors.

RESULTS

TLR5 mRNA is expressed mainly in the proximal colonic (cecal) epithelium in normal mouse gut. Northern blotting revealed abundant TLR5 mRNA transcripts in the epithelial scrapings from the proximal colonic segments of normal mice. In contrast, TLR2 and TLR4 were mainly found in the medial and distal colonic segments, respectively (Fig. 1A and B). CD45 mRNA was undetectable by Northern blotting (data not shown), confirming that the Northern blot signals could not be

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attributed to contaminant leukocytes. To confirm the expression of TLR5 by epithelial cells, we used laser capture microdissection to dissect individual colonic epithelial cells. Using RT-PCR to assess TLR5 mRNA expression, we observed results similar to those obtained by blotting the total RNA from the epithelial scrapings. RT-PCR detected the highest level of TLR5 in the proximal colonic epithelium by laser capture microdissection and a low level of the lymphocyte marker CD45 in all the colonic segments (Fig. 1C).

The gut epithelial expression pattern of TLR5 protein agrees with that of its mRNA. To determine whether the expression pattern of TLR5 protein agrees with that of its mRNA, Western blots of protein extracted from the epithelial scrapings were probed with a polyclonal Ab that recognizes human and mouse TLR5 (8, 16, 34). The blots showed reactive signals at approximately 97 kDa, the molecular size of TLR5 (8), in both the mouse gut samples and the mouse-derived cancer cell line Colon-26. Mouse spleen and the human cancer cell line HT29 were used as controls (44). In concordance with the mRNA results, TLR5 protein was strongly expressed in the mouse proximal colon (Fig. 1D).

TLR5 exhibits distinctive down-regulation during acute DSS-induced colitis. Because epithelial PRRs have been implicated in the initiation or perpetuation of colitis in a mouse model of colitis (23), the behavior of TLRs was determined in the DSS-induced model of colitis. Administration of DSS for 7 days produced acute colitis that affected mainly the distal colon, as evidenced by intense expression of IL-1β mRNA (Fig. 2A). No signal for CD45 mRNA was demonstrated by Northern blotting in the scrapings from colitic animals (data not shown). Although its expression was weak, TLR5 mRNA was still detectable in the proximal and medial segments of the colon, as in normal animals. In contrast, TLR2, which is also expressed mainly in the medial and proximal colonic segments in normal mice, showed increased expression in all segments, including the distal colon (Fig. 2B).

Next, we studied the expression of TLR5 during the recovery phase of DSS-induced colitis. TLR5 mRNA levels were decreased during colitis, and remained down-regulated during the first day after the end of DSS administration. Interestingly, a transient peak in TLR5 mRNA expression was observed on day 4. In contrast, TLR2 was strongly increased on days 1 and 4 after the end of the DSS administration period (Fig. 2C). The expression of TLR5 protein was similar to that of its mRNA. Compared with normal mice, TLR5 levels were diminished during DSS-induced colitis, although the normal localization in the proximal colon was preserved (Fig. 2D). Northern blotting of the colonic crypt samples detached by EDTA treatment yielded results in agreement with those obtained from mucosal scrapings (Fig. 3A).

Polarized responses of PRRs: TLR5 expression is abolished during chronic colitis, while TLR2, TLR4, and CD14 are up-regulated. Chronic colitis was induced in order to observe the modulation of PRRs during recurrent inflammation. Cyclic administration of DSS produced episodes of colitis manifested by diarrhea, gross intestinal bleeding, marked loss of body weight, and shortening of the colon, as described by others (30). During chronic colitis, the constitutive expression of TLR5 mRNA in the proximal colon was diminished during the first DSS cycle and was completely abolished thereafter. The
opposite reaction was observed with the other PRRs studied; TLR2, TLR4, and CD14 were up-regulated in the proximal colon from the fourth DSS cycle onward. Since we have already shown that acute DSS-induced inflammation is localized in the distal colon of BALB/c mice, the up-regulation of TLR2, TLR4, and CD14 in the proximal colons of mice with chronic colitis is likely to reflect the extension of the inflammatory process from the distal to the whole colon. This conclusion was supported by increased expression of IL-1\(\beta\) in the proximal colon from cycle 6. An interesting observation was that higher levels of TLR2, TLR4, and CD14 appeared in the proximal colon prior to IL-1\(\beta\). The expression of KRT19 was deter-
mock treated. (C) Inhibition of TLR5 protein expression by IFN-γ regulated in Colon-26 cells as the exposure time to IFN-γ showed a time-dependent effect. TLR5 expression was more down-regulated in H9R25 cells than in the latter. In contrast to TLR5, TLR2 was up-regulated by IFN-γ in both arrested and nonarrested cells, although the effect was stronger in the former. The down-regulatory effect was reproduced in both cell cycle-arrested and nonarrested cells. Typical results with nonarrested cells are shown. β-Actin was used as an internal control.

![IFN-γ down-regulates TLR5 in vitro](image)

**FIG. 4.** IFN-γ down-regulates TLR5 in vitro. (A) Like the in vivo results, the opposite modulation of TLR5 and TLR2 expression was also observed in the mouse carcinoma cell line Colon-26. Treatment with IFN-γ during 24 h decreased TLR5 mRNA expression but increased that of TLR2. RNA expression was evaluated by Northern blotting. (B) IFN-γ down-regulated the TLR5 protein level in a dose-dependent manner. Colon-26 cells were treated with IFN-γ or were mock treated. (C) Inhibition of TLR5 protein expression by IFN-γ also showed a time-dependent effect. TLR5 expression was more down-regulated in Colon-26 cells as the exposure time to IFN-γ (40 ng/ml) was increased. Experiments were done in triplicate with arrested and nonarrested cells. Typical results with nonarrested cells are shown. β-Actin was used as an internal control.

mimed to test whether all the RNA samples contained similar amounts of total mRNA of epithelial origin despite the development of recurrent inflammation, and this was found to be the case (Fig. 3B).

**IFN-γ down-regulates TLR5 in vitro.** As IFN-γ has been shown to regulate the expression of some TLRs in vitro (38, 40), we tested its effect on cultured murine Colon-26 adenocarcinoma cells. This cell line expresses TLR5 mRNA and protein at levels detectable by both Northern (Fig. 4A) and Western (Fig. 1D, 4B) blotting. IFN-γ was shown to be a down-regulator of TLR5 mRNA expression in Colon-26 cells. The down-regulatory effect was reproduced in both cell cycle-arrested and nonarrested cells, although the effect was stronger in the latter. In contrast to TLR5, TLR2 was up-regulated by IFN-γ in Colon-26 cells (Fig. 4A). The effect of IFN-γ on TLR5 expression was also reproduced at the protein level. IFN-γ produced a time- and dose-dependent effect on TLR5 protein expression in Colon-26 cells (Fig. 4B and C). Other cytokines (IL-1β, IL-4, IL-6, and macrophage inflammatory protein 2) and growth factors (epidermal growth factor and hepatocyte growth factor) failed to modulate TLR5 expression in this cell line (data not shown).

**DISCUSSION**

As demonstrated at both the mRNA and protein levels, TLR5 expression is localized mainly in the epithelial cells of the proximal colonic segment in BALB/c mice. Other TLRs are also preferentially expressed in other segments of the colon (32). TLR4, the main LPS receptor, is present mainly in the distal colon, while TLR2, which detects peptidoglycans and lipopeptides, is expressed in the medial colonic segment. CD14, another LPS receptor, is expressed in the same areas as TLR2 and TLR4. Consistent with its high bacterial load (6), the mouse colon shows strong expression of these TLRs. The constitutive localization of TLR5 in the proximal colon, which corresponds anatomically to the cecum, implies that this organ may have special functions related to innate immunity against flagellated bacteria. Consistent with this, cecectomy has been reported to increase gut coliform counts and to make mice more susceptible to peroral challenge with the intestinal pathogen *Salmonella enterica serovar Enteritidis* (42).

Collection of a relatively pure epithelial cell population from the mouse gut mucosa in a reproducible manner is a technical challenge. Our approach consisted of three different methods: mucosal scrapings, EDTA crypt detachment, and laser capture microdissection. We have described and validated the first method previously (32). The scraping technique extracts mostly intestinal villi and crypts, and the disaggregated cell samples contain more than 92% epithelial cells, as shown by immunocytochemistry. On the other hand, the EDTA technique provides crypt unit samples appropriately characterized by others (23). Although it is unavoidable when using these techniques to get nonepithelial cells, it is also known that Northern blotting, the method we have used to assess mRNA expression, is not as sensitive in detecting genes represented in low copy numbers in proportion to predominant epithelial cell genes. Thus, we assessed CD45 as a panleukocyte marker in the Northern blots, but no signal was detected. Therefore, we suggest that Northern blot signals most likely represent epithelial gene expression. Finally, using RT-PCR coupled to laser capture permitted us to selectively extract the front line of crypt cells, and we were able to demonstrate TLR5 expression, which is in agreement with results from scrapings and EDTA-extracted crypts. Laser capture also showed that CD45-positive nonepithelial cells were localized mostly in the small intestine. Thus, the differences in expression shown here most likely reflect the expression in epithelial cells.

We used the DSS-induced model of experimental colitis to determine whether the expression patterns of the genes for TLRs change during DSS-induced inflammation. The pathogenic mechanisms responsible for the induction of colitis by DSS are not completely known. Defects in the epithelial barrier (15); impaired macrophage function (17, 30); direct cytotoxicity to epithelial cells by DSS; interference with the normal
interaction between epithelial cells, intestinal lymphocytes, and the extracellular matrix (28); and disruption of the interaction between the epithelium and gut flora (10, 31) have all been reported as possible mechanisms. In our experiments, a low concentration of DSS induced distal colitis, as indicated by increased IL-1β expression. As reported previously, expression of TLR4, TLR2, and CD14 is increased in the inflamed colon (32). TLR5, on the other hand, was down-regulated at both the mRNA and protein levels in the inflamed proximal colon, whereas strong TLR5 expression was found in normal mice. These findings suggest that the regulatory mechanisms governing TLR5 expression during colonic inflammation are different from those controlling TLR2, TLR4, and CD14. The results from the DSS-induced chronic colitis model further supported those obtained from the acute colitis model. In chronic colitis, abolition of TLR5 expression in the epithelium of the proximal colon was observed after the second cycle of DSS. Unlike with TLR5, expression of the mRNAs for the other PRRs (TLR2, TLR4, and CD14) was progressively increased in the proximal colon during chronic DSS-induced colitis, reflecting expansion of the inflammatory process from the distal colon to the proximal colon, as corroborated by an increase in IL-1β. These data implicate alterations in the genes for PRRs not only in the process of acute DSS-induced inflammation but also in the chronic condition.

We found that the expression of TLR5 mRNA showed dynamic changes during the period of recovery from DSS-induced colitis. A transient increase was seen on day 4, after continued down-regulation on day 1. The fourth day of recovery is the main day on which epithelial regeneration occurs (31). These data therefore suggest a possible effect of certain growth factors on the expression of TLR5. Unfortunately, our in vitro studies failed to demonstrate any stimulatory effect of hepatic or epidermal growth factors on TLR5 expression in colonic epithelial cells. Like growth factors, IFN-γ has been reported to be increased during the development of murine DSS-induced colitis (7, 21, 24, 29, 35, 41). IFN-γ up-regulates the expression of TLR4 in human colonic epithelial cell lines (1). Therefore, we investigated the effect of this Th1 cytokine on TLR5 expression in Colon-26 mouse cells. In this cell line, IFN-γ down-regulated TLR5 but up-regulated TLR2. Although there are no published studies concerning the expression of TLR2 and TLR5 in mouse epithelial cell lines, TLR2 expression has been shown to be stimulated by LPS in alveolar macrophages and lung tissue, whereas TLR5 was down-regulated in alveolar macrophages following the application of inflammatory stimuli (33). These observations agree with the results of our study. The expression of TLR2 and TLR4 in mouse renal epithelial cells has also been shown to be stimulated by IFN-γ (42). Interestingly, it has been shown that the increase in IFN-γ is accelerated late in the chronic phase of DSS-induced colitis (5, 24), which is in good correlation with our observation of transcriptional down-regulation of TLR5 and up-regulation of TLR2 and TLR4 in the proximal colon during chronic DSS administration. IFN-γ in the DSS-induced inflamed intestine is produced mainly by the mononuclear cells located at the base of the lamina propria and by intraepithelial lymphocytes (5, 14). Therefore, this cytokine may act as a paracrine down-regulator of TLR5 and an up-regulator of TLR2 in the colonic epithelium.

IFN-γ has been shown to up-regulate the expression of TLR4 in human monocytes and polymorphonuclear cells (41) and of CD14 in human gingival fibroblasts (42). The mechanism by which IFN-γ up-regulates the transcription of TLR genes includes IFN-γ receptor-mediated binding and oligomerization, with further sequential activation of the Janus tyrosine kinase and STAT1 (signal transducer and activator of transcription) pathways. Under certain conditions, IFN-γ can also activate STAT3 instead of STAT1; this switch leads to opposite effects on inflammation and cell survival (43). We speculate that this inhibitory pathway may be the mechanism for the down-regulation of TLR5 expression.

It was observed that IL-12-induced IFN-γ production increased inflammation in acute DSS-induced colitis (44). Thus, it may be expected that intestinal inflammation would be lower in IFN-γ knockout mice. In a recent study, colonic inflammation during DSS-induced colitis was found to be lower in IFN-γ knockout mice than in wild-type mice, but the values did not reach statistical significance (45).

The present data demonstrate that, like other PRRs, TLR5 is expressed in normal mouse intestinal epithelial cells in a segmental manner, with special localization in the proximal colonic segment. TLR5 expression is down-regulated in vivo during DSS-induced colitis, probably as a consequence of increased levels of IFN-γ. The modulation of TLR5 levels during inflammation contrasts with the responses of TLR2, TLR4, and CD14 in both acute and chronic models of DSS-induced colitis. These results have important implications for the understanding of the functions of PRRs in the normal mouse gut and in the pathogenesis of DSS-induced colitis.

ACKNOWLEDGMENT

This work was supported in part by grants-in-aid for scientific research from the Ministry of Education, Science, Sports, and Culture of Japan.

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