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ORIGINAL ARTICLE

Increase in the Tight Junction Protein Claudin-1 in Intestinal Inflammation

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Abstract
Background and Aims Studies have shown a decrease in key tight junction (TJ) proteins such as ZO-1 and occludin in both inflammatory bowel disease (IBD) and experimental models of inflammation. Our group has also shown an increase in claudin-1 in experimental colitis.
Methods IEC-18 cells were treated with increasing doses of tumor necrosis factor alpha (TNFα). The TJ was assessed by transepithelial resistance (TER), permeability, Western blot, PCR, and immunofluorescence. Mucosal samples from patients with ulcerative colitis (UC), Crohn's disease (CD), and without IBD (normal) were assayed for TJ proteins occludin and claudin-1 by Western blot and a ratio of claudin-1 to occludin (C:O) was calculated.
Results IEC-18 cells had increased permeability, decreased TER and an increase in claudin-1 with TNFα treatment. In human specimens, there was a decrease in occludin and an increase in claudin-1 leading to a significant increase in the C:O ratio in diseased UC colon compared to non-diseased UC colon ($P < 0.001$) and normal colon ($P < 0.01$). In CD, the C:O ratio was similar in all CD tissue irrespective of disease status.
Conclusions Treatment of IEC-18 cells with TNFα, a key inflammatory cytokine in IBD, led to a significant increase in claudin-1 expression. There was a significant increase in the C:O ratio in diseased colon in UC compared to the healthy appearing UC colon and normal controls. The C:O ratio was unchanged in CD despite presence or abscence of gross disease. This suggests that there may be an underlying difference in the TJ between UC and CD.

Keywords TNFα · Crohn's disease · Ulcerative colitis · Claudin-1 · Occludin

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Introduction

Inflammatory bowel disease (IBD) is a disorder of the gastrointestinal tract (GI) that results from uncontrolled inflammation. The etiology is unknown but is thought to be triggered by an environmental antigen in a genetically susceptible person. IBD consists of 2 subtypes, ulcerative colitis (UC), which is a mucosal disease that only involves the colon and rectum, and Crohn's disease (CD), which can involve the full thickness of the intestinal wall and can also occur anywhere within the GI tract. UC and CD are felt to be two distinct diseases although in many patients with isolated colonic disease it can be difficult to differentiate between the two.

An imbalance between the pro- and anti-inflammatory cytokines can lead to immune-mediated diseases such as IBD. Tumor necrosis factor-alpha (TNFα), a pro-inflammatory cytokine, has been shown to be increased in the serum and tissue of patients with IBD [1–3]. TNFα plays such an important role in the disease process that Infliximab®, a genetically constructed IgG1 murine–human chimeric molecule that binds both soluble and membrane bound TNFα, is currently the most effective medical treatment for CD and is being increasingly used in the treatment of UC.

There are several clusters of proteins that reside between epithelial cells and form a physiologically active barrier to

the intracellular space. The tight junction complex (TJ) is the most apical of these complexes. The organization of the TJ is similar to other intercellular junctions and consists of transmembrane proteins that mediate adhesive function linked to underlying plaque proteins, such as ZO-1, that in turn associate with the cytoskeleton [4, 5]. Occludin and the family of claudins make up the transmembrane proteins. Occludin is a 65-kda transmembrane protein thought to play both a functional and structural role defining the paracellular barrier [6, 7]. The claudins comprise a multigene family, ranging in size from 20 to 26 kda, in which at least 24 members have been identified so far [6, 8]. Like occludin, the claudins have four transmembrane domains but they do not show any sequence homology to occludin, and only 13–70% homology to each other [8, 9]. The different claudins have diverse functions depending on cell type and the host organism [6].

Alterations in TJ structure and function have been seen in both human IBD and experimental models of intestinal inflammation [10–19]. Studies have shown a decrease in key TJ proteins such as ZO-1 and occludin which correspond to an increase in intestinal permeability and decrease in transepithelial resistance (TER) [13, 19]. Using the dextran sulfate sodium (DSS) model of colonic inflammation, our group has shown not only a decrease in ZO-1 but also a seemingly counterintuitive increase in claudin-1, suggesting a possible compensatory role for claudin-1 in the damaged TJ [13]. TNFα signaling via NF-κB has been shown to modify the TJ and alter the expression of its component proteins [20, 21]. Addition of TNFα to cultured monolayers of colonic epithelial cells results in an increase in paracellular permeability, a decrease in TER and a displacement of ZO-1 from its usual position on the cell membrane [22, 23]. Here, we have studied the effect of TNFα on claudin-1 in an in vitro epithelial cell culture model and also the changes in occludin and claudin-1 in human CD and UC tissue specimens.

Methods

Cell Culture and TNFα Treatment

IEC-18 cells (rat ileal intestinal epithelial cells) [American type culture Collection (ATCC), Manassas, VA, USA] were maintained in media (DMEM; ATCC), 0.1 Unit/ml Bovine insulin (Sigma, St. Louis, MO, USA), and 5% fetal bovine serum (Gemini Bioproducts, West Sacramento, CA, USA) at 37°C and 5% CO_2. 1×10^5 cells were plated into 0.4-μ 12-well transwell plates (Corning, Lowell, MA, USA) and TER measured daily (see below). Three days after the cells were plated, when TER was stable, the media was removed from the transwell chambers and replaced with fresh media and TNFα (R + D Systems, Minneapolis, MN, USA) at concentrations of 0, 5, 50, 100, or 200 ng/ml. The TNFα was added to the basolateral chamber and the treatment day was designated as day 0. Plates were treated with TNFα for 48 h and then used for permeability, immunofluorescence, or Western blot as described below.

TER

TER was measured daily with an EVOM (World Precision Instruments, Sarasota, FL, USA) starting 24 h after the cells were plated (day −3). Each well was measured three times and the mean calculated. The background of the control wells (wells with media only, no cells) was subtracted from the wells containing cells. The change in TER between day 0 (untreated) and day 2 (48 h post-treatment) was calculated for each well ($n = 13$).

Permeability

On treatment day 2, 48 h after the addition of TNFα, the media were removed from both the apical and basolateral chambers of the transwell plate. Two hundred microlitre of RITC dextran 70,000 (Sigma) was added to the apical chamber and 1.5 ml of media (see above) supplemented with HEPES to a pH of 6.8 was added to the basolateral chamber. Then, 50 μl of media were harvested from the basolateral chamber at 30 min, 2, 5, and 8 h. Fluorescence was measured using the Typhoon System 9400 (Amersham Bioscience, Pittsburgh, PA, USA) according to the manufacturer's instructions. Permeability was then calculated using the following formula:

$$\text{Permeability } (P_o) = [(F_A \Delta t) V_A](F_L A)$$

where F_A = basolateral fluorescence, F_L = apical fluorescence, Δt = change in time, A = surface area of the filter, and V_A = volume of the basolateral chamber [24] $N = 4$.

Preparation of Fresh Tissue Samples

Fresh operative specimens from patients undergoing ileocolectomy or colon resection for CD, UC or non-IBD diseases such as constipation or cancer (ND) were obtained. Segments of the terminal ileum (TI) and colon were collected. CD and UC tissues were designated as healthy or diseased by gross appearance of the mucosa at the time of tissue collection. The mucosa was scraped from the segments of tissue, sonicated in an antiprotease buffer [6 M Urea, 0.1% Triton X-100, 10 mM Tris (pH 8.0), 1 mM DTT, 5 mM MgCl2, 5 mM EGTA, 150 mM NaCl, 0.2 mM PMSF] to release membrane bound proteins, and

stored at −70°C until use. Use of the human tissue samples was approved by the Milton S. Hershey Medical Center Institutional Review Board.

Western Blot

On day 2 (48 h of TNFα treatment), IEC-18 cell monolayers were lysed in antiprotease buffer (see above for fresh tissue preparation). The protein concentration in the IEC-18 cell lysates and human mucosal samples were measured with BCA protein assay Kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Then, 20 μg of IEC-18 cell lysate or 40 μg of mucosal sample was loaded on a gradient SDS minigel and run against high molecular weight standards for 1 h at a constant voltage of 100 V. A standard control was run on all gels to allow for intergel comparison. The proteins were then transferred to nitrocellulose over 1 h at 100 V. The blots were blocked in 5% non-fat milk for 1–2 h to prevent non-specific binding.

For IEC-18 cells, the blot was incubated with polyclonal rabbit-anti-claudin-1 at 1:1,000 or mouse monoclonal anti-occludin-1 at 1:1,000 overnight at 4°C. The blots were then washed and incubated with the rabbit or mouse HRP conjugated secondary antibody (1:1,000), respectively, for 1 h. Immunoreactive bands were detected using a chemiluminescence kit (Perkin Elmer Life Sciences, Boston, MA, USA) and autoradiographic film. IEC-18 Blots were stripped and reprobed for actin to confirm lane loading. Band densities were determined using Quantiscan densitometer software (Biosoft, Ferguson, MO, USA). A ratio of TJ protein of interest to actin was calculated and then a ratio to the standard control was reported for all doses of TNFα. ($n = 4$.)

For fresh tissue blots, the blot was cut in half horizontally at about 50 kda to allow simultaneous probing for both occludin and claudin-1 without stripping the blot. The top half of the Western blot was incubated with monoclonal mouse anti-occludin and the bottom half was incubated with polyclonal rabbit-anti-claudin-1, both at 1:1,000 overnight at 4°C. In doing this, the same lane on the nitrocellulose was used to measure occludin and claudin-1. A positive control for both proteins was used on each Western blot. The blots were then washed and incubated with the appropriate HRP conjugated secondary antibody (1:1,000) for 1 h. Immunoreactive bands were detected using a chemiluminescence kit (Perkin Elmer Life Sciences) and autoradiographic film. Band densities were determined using Quantiscan densitometer software. A ratio of claudin-1 to occludin (C:O) was calculated for each sample and corrected for the ratio of claudin-1 to occludin in the control lane. ($n \geq 11$ for ND, $n \geq 9$ for CD, $n \geq 16$ for UC.)

PCR

Confluent IEC-18 monolayers were treated with 0, 5, or 50 ng/ml of TNFα for 4, 8, 12, or 24 h. Total RNA was then isolated using RNEasy Mini (Qiagen, Germany). The total RNA concentration was measured using a spectrophotometer at 260 nm. The purity of the RNA was determined using the A260/A280 ratio and was approximately 2.1. One microgram of total RNA was reverse transcribed into cDNA using Invitrogen Superscript III (Invitrogen, Carlsbad, CA, USA). Primers for claudin-1 were as follows: forward: 5′-TCTGGCAAGTCTAGCAGTTTGTG-3′ and reverse: 5′-GCTAAGCTGCTAACCCTGTGGT-3′. PCR amplification for claudin-1 was performed at 95°C for 5 min, (94°C for 30 s, 57°C for 45 s, 72°C for 30 s) for 35 cycles, then 72°C for 4 min. Primers for GAPDH were as follows: 5′-AACGGATACATTGGGGGTAG-3′ and reverse: 5′-CA TGGAGAAGGCTGGGGCTC-3′. PCR amplification for GAPDH was performed at 94°C for 5 min, (94°C for 30 s, 55°C for 45 s, 72°C for 45 s) for 30 cycles, then 72°C for 7 min. For both, 10 μl of the PCR products were run on 2% agarose gels and stained with ethidium bromide. Gels were photographed with Gel Doc-it Imaging System (UVP, Upland, CA, USA). Claudin-1 was normalized to GAPDH.

Statistics

Permeability, TER, and Western blot calculations were compared using ANOVA with Newman-Keuls multiple comparison test, using Graph Pad Prism (San Diego, CA, USA). Statistical significance was considered achieved when $P < 0.05$.

Results

TNFα Induces Expression of Claudin-1

Western blot was performed on IEC-18 TNFα-treated and -untreated cell monolayers. In the untreated monolayer, there was a small but measurable amount of claudin-1 detected. When 5 ng/ml of TNFα was added there appeared to be an increase in claudin-1 expression but it was not statistically significant. However, with the addition of 50 and 100 ng/ml of TNFα, there was a significant increase in the amount of claudin-1 (Fig. 1a, b). This suggests that pro-inflammatory cytokines in intestinal epithelial cells can lead to the induction of claudin-1.

PCR for claudin-1 was performed on IEC-18 cell monolayers treated with and without TNFα for 4–24 h and normalized to GAPDH. There was minimal detected claudin-1 in the untreated cells at all time points. With

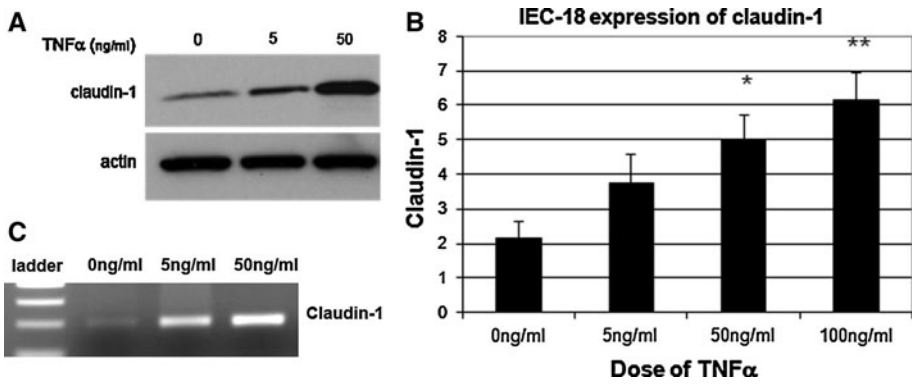

Fig. 1 Induction of Claudin-1 by TNFα. IEC-18 monolayers were treated on the basolateral side of the monolayer with increasing doses of TNFα. **a** Sample Western blot showing increase in claudin-1 with TNFα treatment. Actin confirms equivalent lane loading. **b** Graphical summary of IEC-18 Western blots showing mean claudin-1 expression ± standard error. There was a significant increase in claudin-1 by Western blot starting at 50 ng/ml of TNFα, $n = 4$. $^{*}P < 0.05$ versus 0 ng/ml, $^{**}P < 0.01$ versus 0 ng/ml. Claudin-1 is expressed as a ratio to actin and control. **c** PCR for Claudin-1 in IEC-18 cells. There is minimal detectable claudin-1 at baseline. With 5 ng/ml of TNFα there is a marked increase in claudin-1 transcription and an even further increase at 50 ng/ml

5 ng/ml of TNFα treatment, there was a marked increase in claudin-1 transcription. There was a further increase in claudin-1 transcription with 50 ng/ml. These changes were seen with as little as 4 h of TNFα treatment (Fig. 1c). PCR for claudin-1 at 8, 12 and 24 h revealed the same pattern (not shown). These findings correspond with the Western blot data shown above.

TNFα Decreases Expression of Occludin

Western blot was performed for occludin on IEC-18 TNFα-treated and -untreated cell monolayers. Occludin was expressed in all monolayers. There was, however, a significant decrease in occludin expression with both 5 and 50 ng/ml of TNFα (Fig. 2a, b). These data show that, unlike claudin-1, which was induced with TNFα, a different transmembrane TJ protein such as occludin is decreased.

TNFα Alters Barrier Function in IEC-18 Cells

TER was measured daily from the time of cell plating until 48 h post-treatment (day 2) when assays were performed. TER was stable in all plates from day −1 to day 0 (treatment day) suggesting functionally stable TJ at the time of treatment. TNFα was then added to the basolateral membrane. There was a decrease in TER with increasing dose of TNFα across IEC-18 monolayers (Fig. 3a).

Permeability was measured at 48 h after TNFα treatment. There was an increase in permeability in the IEC-18 monolayer with increasing doses of TNFα. When percent change from untreated was calculated there was a significant increase in permeability with 50, 100, and 200 ng/ml of TNFα (Fig. 3b). The increase in permeability corresponds to the decrease in TER seen, and suggests a "loosening" of the monolayer. This may be due to changes in the TJ, such as loss of occludin, or possibly to apoptosis induced by the TNFα, or both. The lack of TJ localized claudin-1 may explain why the increase in claudin-1 did not lead to a rise in TER or a decrease in permeability.

Claudin:Occludin Ratio Differs Between Active and Inactive UC and Between UC and CD

Mucosa from TI and colon of patients with CD, UC, and non-inflammatory diseases (ND) was collected from operative specimens. The CD and UC tissue were further subdivided into samples that had grossly normal mucosa (designation 0) and those that had grossly diseased mucosa (designation 1). ND tissue was all grossly normal by definition. This designation was made when the sample was collected and prior to assay. Western blot was then performed for occludin and claudin-1. Overall, we observed a decrease in the amount of occludin and an increase in the amount of claudin-1 in the diseased tissues. Figure 4a is a representative Western blot showing these changes in two different UC patients. The samples that showed the greatest loss of occludin had the highest increase in claudin-1, and therefore a ratio of claudin-1 to occludin (C:O) was calculated. Figure 4b shows a graphic representation of the mean C:O ratio for group of tissues.

When we looked first at the UC tissue we found that there was a significant increase in the C:O ratio in grossly diseased UC colon compared to grossly normal UC colon and ND colon ($P < 0.001$ and $P < 0.01$, respectively). This was due to both a loss of occludin and an increase in claudin-1. There was also a significant difference in the C:O ratio between diseased UC colon and UC TI ($P < 0.01$) which is by definition without IBD. One would expect similar findings in the tissues from CD patients;

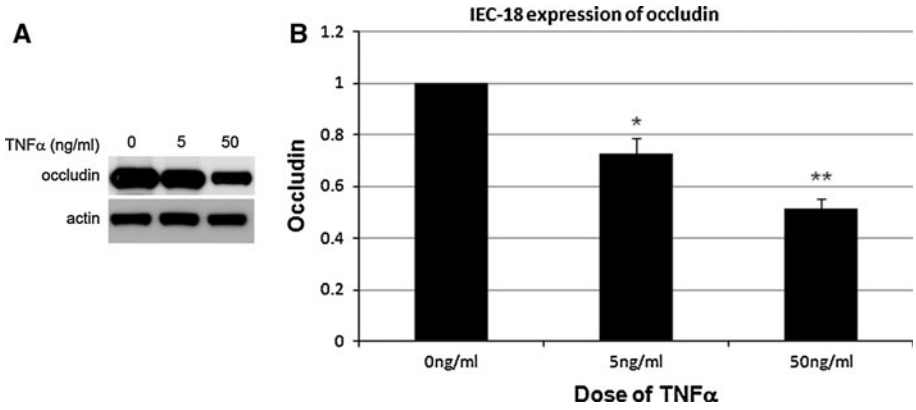

Fig. 2 TNFα decreases occludin expression in IEC-18 cells: IEC-18 monolayers were treated on the basolateral side of the monolayer with increasing doses of TNFα. **a** Sample Western blot showing decrease in occludin with TNFα treatment. Actin confirms equivalent lane loading. **b** Graphical summary of IEC-18 Western blots showing mean occludin expression ± standard error. There was a significant decrease in occludin by Western Blot starting at 5 ng/ml of TNFα, $n = 4$. $^{*}P < 0.01$ versus 0 ng/ml, $^{**}P < 0.001$ versus 0 ng/ml. Occludin is expressed as a ratio to actin, and untreated cells

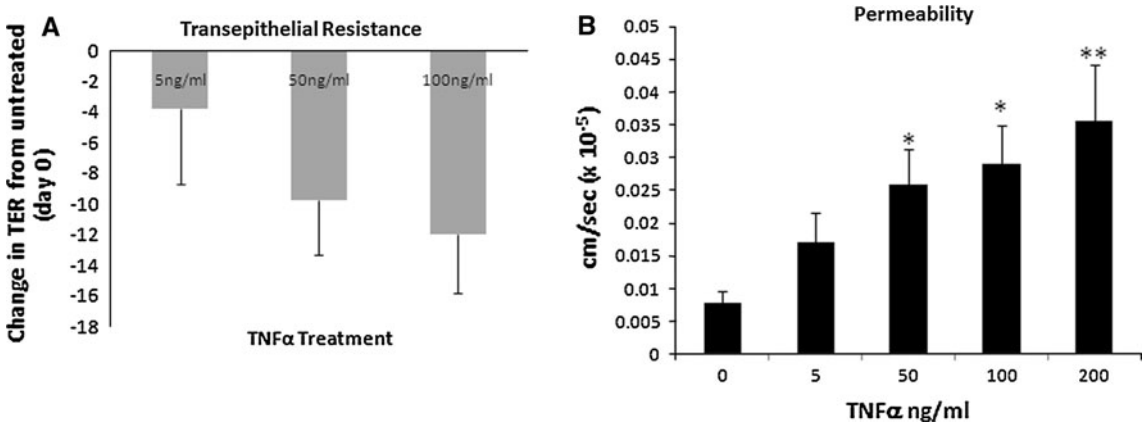

Fig. 3 TNFα alters barrier function in IEC-18 cells. **a** Change in TER from day 0 to day 2 was calculated for TNFα-treated and -untreated monolayers. There was a decrease in TER with increasing dose of TNFα. Values are mean ± standard error, $n = 13$. **b** There was an increase in permeability of the IEC-18 cell monolayer to 70,000 RITC dextran with increasing doses of TNFα applied to the basolateral side of the monolayer. There was a significant increase in permeability with 50, 100, and 200 ng/ml of TNFα. This increase corresponds to the decrease in TER. Values are mean of 4 experiments ± standard error. $^{*}P < 0.05$ versus untreated monolayer, $^{**}P < 0.01$ versus untreated monolayer

however, that was not the case. We found an elevated C:O ratio in all CD tissue whether they appeared grossly diseased or grossly normal. There was also no difference in the C:O ratio between any of the healthy appearing CD tissue and the diseased appearing CD tissue in either the TI or the colon. There was, however, a significant increase in the C:O ratio in the diseased UC tissue compared to both healthy and diseased CD tissue, $P < 0.01$. These findings suggest that the mechanism of alteration of the TJ complex seen in IBD may be different in UC and CD. The changes in the TJ complex in UC mirror the severity of the disease and may be a result of the inflammatory process. However, in CD, the changes in the TJ complex occur regardless of disease severity suggesting that there may be an underlying TJ barrier defect in CD regardless of the degree of inflammation. This difference has the potential to help differentiate between CD and UC microscopically in cases where the distinction between these two forms of IBD is difficult to make.

Discussion

In the present study, we saw a decrease in TER and an increase in permeability of the IEC-18 cell monolayer with the application of TNFα. In most studies using colonic epithelial cells, similar changes in permeability and TER are attributed to loss of key TJ proteins as a result of cytokine or other agent application [22, 23, 25]. This was not the case in our study. In addition to the loss of occludin

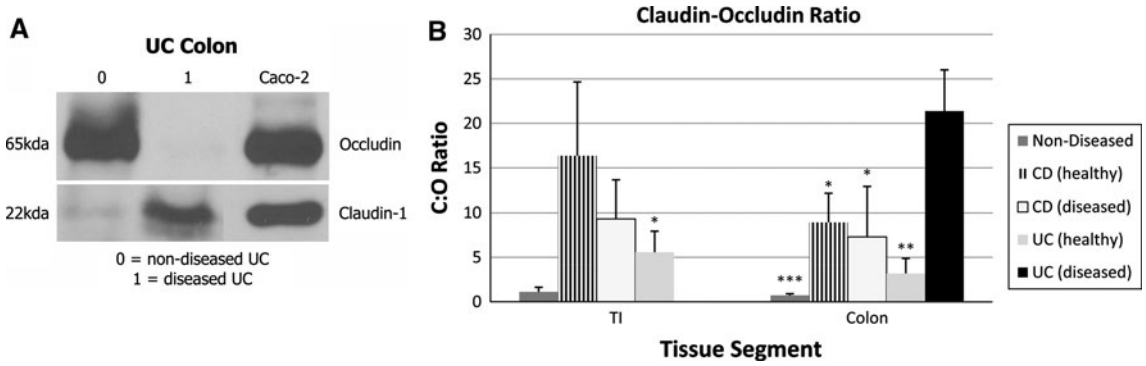

Fig. 4 Claudin:occludin ratio distinguishes active UC from inactive and UC from CD. Western blot was performed on the mucosa of operative specimens from patients with CD, UC, and non-inflammatory diseased. **a** Western blot for occludin and claudin-1 in representative UC patients. In UC patients with healthy appearing mucosa (*lane 0*), there was a *dark band* showing a large amount of occludin present, but minimal claudin-1 leading to a low claudin-1 to occludin ratio. In diseased appearing UC mucosa (*lane 1*), the opposite was seen, with minimal to no occludin and a *dark band* at claudin-1. This results in a significantly higher claudin-1 to occludin ratio. Caco-2 cells are shown as a positive control for both proteins. **b** Graphical summary showing the mean C:O ratio in all samples segregated by disease and tissue state. There is a significant increase in the C:O ratio in diseased UC tissue compared to healthy UC and ND ($^{**}P < 0.001$, and $^{***}P < 0.01$, respectively). There is no difference in the C:O ratio amongst any of the CD tissue regardless of disease state. There is also a significant increase in the C:O ratio in diseased UC tissue compared to healthy and diseased CD tissue. Values are mean ± standard error. $^{*}P < 0.01$ versus disease UC [1]. $n \geq 11$ for ND, $n \geq 9$ for CD, $n \geq 16$ for UC

that was consistent with colonic studies, we found a marked increase in claudin-1 by PCR and Western blot. Despite this increase, we had a decrease in barrier function. One would expect that an increase in one of the TJ transmembrane proteins would lead to an increase in TJ integrity manifested by a decrease in permeability and an increase in TER. There are several possible explanations for our seemingly contradictory findings. One is that claudin-1 has a disruptive effect on the TJ in this model system. However, we do not believe this to be the case because in other systems the addition of claudin-1 has resulted in an increase in TER and a decrease in permeability [26]. The other possible, and more likely explanation, is that in these experiments claudin-1 is not contributing to barrier function, and that the loss of barrier function is due to the loss of occludin. Although we have shown an increase in claudin-1 by Western blot and PCR, we have also found by immunofluorescence that the claudin-1 does not localize to the TJ (data not shown). In fact, claudin-1 appears to be present in the cytoplasm and the nucleus as opposed to on the cell membrane in the position of a usual component of the TJ. Therefore, it is unlikely that the claudin-1 is contributing to the barrier function in this model. It appears that TNFα is able to induce the synthesis of claudin-1 but that something else may be needed to direct claudin-1 to its appropriate location on the cell membrane. This could explain why the increase we have seen in claudin-1 did not lead to an increase in barrier function. Other TJ proteins besides occludin and claudin-1, that were studied here, could also contribute to the decrease in barrier function.

In our human samples, we found a significant increase in the C:O ratio in the diseased UC colon compared to grossly normal appearing UC colon and compared to colon from patients with non-inflammatory diseases. This resulted from both an increase in claudin-1 and a decrease in occludin in the diseased UC tissue. The healthy appearing UC tissue had a C:O ratio that was indistinguishable from the non-IBD tissue, suggesting in UC that the C:O ratio correlated with the disease state. The fact that the C:O ratio was only increased in the grossly diseased UC tissue and not the healthy appearing UC tissue suggests that the inflammatory process in UC may lead to the disruption in the TJ complex that was seen in our study.

In CD, we saw a very different pattern. All the tissue samples, from both TI and colon, and both grossly normal and grossly diseased, had an increased C:O ratio, and all the C:O ratios were approximately the same regardless of disease state. While the C:O ratio in the CD was elevated, it was still significantly lower than in the diseased UC tissue. This suggests that, unlike UC, the alteration in the TJ complex in CD did not correlate with the amount of gross inflammation seen. This lack of correlation with disease in CD can be possibly explained in two ways. One possibility is that the TJ complex becomes disrupted or abnormal early in the CD disease process before gross disease is clinically evident. The other possibility, and the more likely one, is that there is a disruption of the TJ complex in CD regardless of the presence of active inflammation, such that the disruption of the TJ complex is a prerequisite for the development of CD.

These findings suggest that there may be a significant difference in the underlying pathophysiology of the TJ complex in the two diseases. We have shown that the TJ is inherently abnormal in the CD patient regardless of severity of colonic/intestinal inflammation, which would point to a genetic predisposition being required for the development of the disease. This is consistent with findings of other investigators that there is increased intestinal permeability in patients with CD and increased intestinal permeability in unaffected relatives of patients with CD [27–30]. In UC, we have shown that the abnormality of the TJ may be from the inflammation and not a prerequisite for the development of the inflammation.

Several other investigators have shown that there is an abnormality in the TJ complex in IBD [10–19]. Looking at the structure of the TJ complex, it has been shown that there is a reduced number of strands, strand discontinuities, and reduced depth of the TJ complex in CD [16, 24, 31, 32]. Similar changes were also seen in UC. Changes in specific molecules within the TJ have also been seen. A decrease in claudin-2, 5, and 8, desmoplakin-1, desmoglein-2, and desmocollin-2 have been reported by other investigators [10, 32]. This is the first published study to look at the changes in the ratio of occludin and claudin-1. Also, this is the first published study to show a difference in the changes in claudin-1 and occludin in CD and UC.

TNFα is a key cytokine in the inflammatory processes in both CD and UC. Our cell culture model in IEC-18 cells showed that TNFα treatment leads to the upregulation of claudin-1 and loss of occludin. This corresponds to the increase in C:O ratio seen in our IBD tissues. Other investigators have shown that TNFα is increased in the serum and tissues of patients with IBD [1–3]. The increased tissue TNFα may contribute to the increase in claudin-1 and decrease in occludin seen in our human tissue specimens. Using the DSS colitis model of intestinal inflammation, we have previously shown that there is a loss of ZO-1 and occludin but an increase in claudin-1 [13, 19]. In this model, we also saw that claudin-1 was not confined to the cell membrane.

TNFα is known to induce apoptosis and it remains controversial as to what role that apoptosis has to do with the TNFα-induced changes in barrier function and in IBD. Several investigators have shown that TNFα-induced apoptosis is involved in barrier function changes [21, 33]. Bruewer et al. [34], on the other hand, demonstrated that barrier changes induced by TNFα were independent of apoptosis in T84 cell monolayers. The role for apoptosis in the pathophysiology of IBD is also controversial with some investigators suggesting that epithelial cell apoptosis plays a role in the development of IBD [35]. Infliximab, an anti-TNF monoclonal antibody used to treat IBD, has been shown to work by inducing apoptosis but in T cells [36–38]. There are no data on whether or not Infliximab induces apoptosis in epithelial cells. Overall the role of TNFα induced apoptosis in epithelial cells in the pathophysiology of IBD remains unclear.

Although claudin-1 is a transmembrane TJ protein, its role in inflammation is unclear, and it may have additional roles that have yet to be defined. In our cell culture model, we were able to induce the transcription and synthesis of claudin-1 with TNFα but it did not localize to the TJ. Claudin-1's presence in the cytoplasm and the nucleus may suggest that it has other functions as yet undescribed. For example, claudin-1 has been shown to be upregulated in colorectal cancer [39–42].

Conclusions

We have found that there is an induction of claudin-1 in IEC-18 cells when they are treated with TNFα. Despite the increase in claudin-1, there is a decrease in TER and an increase in permeability that may be due to the fact that claudin-1 is not located on the cell membrane. We have also found that there is a loss of occludin and an increase in claudin-1 in IBD resulting in an elevated claudin-1 to occludin ratio. In UC, this ratio is only elevated in tissue with grossly evident disease, but in CD, the ratio is elevated whether the tissue is diseased or not, suggesting an underlying TJ abnormality in CD but not UC.

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