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Expression from the human occludin promoter is affected by tumor necrosis factor α and interferon γ

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SUMMARY

The 65 kDa protein occludin is a membrane-spanning part of the epithelial tight junction, which is the main barrier of the paracellular pathway. The function of occludin as part of tight junctions is still poorly understood and even less is known about the regulatory mechanisms that influence occludin gene expression. This study aimed to identify the sequences essential in cis for genomic regulation of tight junction formation and to investigate their funcional role in cytokine-dependent tight junction regulation.

Using genome walking cloning of occludin-specific human genomic DNA sequences, a 1853 bp DNA fragment containing the transcription start point of occludin cDNA sequences was amplified and sequenced. Subcloning of this fragment in front of the luciferase reporter gene revealed strong expression of enzymatic activity after transfection of the human intestinal cell line HT-29/B6. With subsequent deletions of parts of the promoter fragment, its size was reduced to 280 bp that are necessary and sufficient to mediate promoter activity. Tumor necrosis factor α and another cytokine involved in inflammation, interferon γ, reduced transepithelial resistance in HT-29/B6 cells, which was preceded by a decrease in occludin mRNA expression as revealed by northern blot analysis. Tumor necrosis factor α and interferon γ diminished occludin promoter activity alone and even synergistically, suggesting a genomic regulation of alterations of the paracellular barrier.

In conclusion, proinflammatory cytokines such as tumor necrosis factor α and interferon γ can downregulate the expression of the transmembrane tight junction strand protein occludin, paralleling the barrier disturbance detected electrophysiologically. This could be an important mechanism in gastrointestinal diseases accompanied by barrier defects, for example inflammatory bowel diseases.

Key words: Tight junction, Gene regulation, Cytokine, Occludin

INTRODUCTION

Epithelial and endothelial cells serve in both selective transport and barrier function. The vectorial transport of solutes and water involves transcellular and paracellular transport pathways. Whereas transcellular transport is mediated with high molecular specificity by transmembranal proteins or by vesicular transport, the flow through the paracellular space is determined by a belt of anastomosing intramembranous fibrils, the so-called tight junction strands (Fanning et al., 1999). The tight junction strands are formed by integral membrane proteins and surround the lateral membrane of epithelial and endothelial cells. Besides serving as a gate for passive transport, the tight junction also serves as a fence against the lateral diffusion of proteins within the cell membrane, thus maintaining apical and basolateral polarization.

A number of transmembrane proteins associated with the tight junction have been identified recently including occludin (Furuse et al., 1993), junctional adhesion molecule (JAM) (Martin-Padura et al., 1998) and the claudin family (Furuse et al., 1998). The tight junction protein occludin was first identified in adherens junction fractions from chicken liver (Furuse et al., 1993). In 1996, the same group isolated occludin cDNA in three mammalian species, including human (Ando-Akatsuka et al., 1996). These sequences are very closely related and share an overall sequence homology of 90%. Occludin is a 65 kDa protein containing four transmembrane domains forming two extracellular loops, which are supposed to be involved in intercellular strand formation (Furuse et al., 1993). Freeze-fracture electron micrographs with antibodies directed against occludin revealed an intrastrand localization (Fujimoto, 1995) as well as a minor and less phosphorylated fraction distributed along the lateral membrane (Cordenonsi et al., 1997; Sakakibara et al., 1997). Occludin also contains a large cytoplasmic tail that interacts with the cytoplasmic tight junction-associated protein ZO-1 (Furuse et al., 1994). There is experimental evidence in transfection assays that this interaction is essential for targeting occludin to the tight junction in cells lacking endogenous occludin (Fanning et al., 1999), although C-terminally truncated occludins are inserted into tight junctions that contain intact occludin, presumably by lateral association (Balda et al., 1996).

In addition to the physiological evidence, pathophysiology provides further information about altered tight junction formation, as it plays a role in inflammatory bowel disease. Previous work from our laboratory has shown that ulcerative colitis is associated with altered tight junction strand formation (Schmitz et al., 1999a). Moreover, the proinflammatory cytokines tumor necrosis factor α (TNFα) and interferon γ (IFNγ) influence the epithelial barrier by altered tight junction strand formation in the human intestinal cell line HT-29/B6 (Schmitz et al., 1999b). Tight junction downregulation can give rise to increased back-leak of solutes and fluid into the lumen, manifesting the clinical symptom of leak flux (Fasano et al., 1991; Levine et al., 1988).

The tight junction is assumed to be a complex meshwork of different transmembrane proteins that show differential expression in varying tissues. So far, little is known about the factors influencing genomic regulation of tight junction protein expression, and in order to investigate this we cloned and sequenced regulatory sequences essential in cis for the *occludin* gene. To clarify whether the alteration in strand counts after addition of inflammatory cytokines corresponds to an altered expression of tight junction molecules, we performed northern blot analysis to correlate gene expression with altered electrophysiological behavior of cultured epithelial cells. Finally, luciferase reporter gene assays revealed that these effects can be explained by an altered occludin promoter activity.

MATERIALS AND METHODS

Cell culture and chemicals

HT-29/B6 cells, a subclone of the human intestinal cell line HT-29 (Kreusel et al., 1991), were maintained in RPMI1640 supplemented with 10% foetal calf serum (FCS) and antibiotics (penicillin, streptomycin). TNFα and IFNγ were purchased from Biomol (Hamburg, Germany). Gene-specific oligodeoxynucleotide primers OCLN1 (5′-CTGCGTCCTAGACCGGCTCC-3′) and OCLN2 (5′-GCGCTCTGGACCTGGCTCTC-3′) were obtained from Metabion (Martinsried, Germany).

Molecular cloning of genomic DNA

Human genomic DNA encompassing the occludin transcriptional start point and upstream regions was cloned using the genome walking system (Clontech, Palo Alto, USA). In brief, amplification of occludin-specific DNA was done by polymerase chain reaction (PCR) with gene-specific primers and adaptor primers (AP1 and AP2), which anneal to adaptor sequences ligated to the ends of restriction enzyme-digested human genomic DNA. Products of the first round of amplification with the primers OCLN1 and AP1 were diluted 1:50 and amplified in a nested PCR with OCLN2 and AP2. Reaction products were analyzed by electrophoresis on 1.5% agarose gels and occludin-specific bands were verified by blotting and hybridizing with an occludin-specific probe (data not shown). Bands that were positive in hybridization analysis were excised from the gel, extracted and ligated to the pGEM-T cloning vector (Promega, Madison, USA). After transformation into *E. coli* TOP10 F′ bacterial cells, plasmids were screened by digestion with restriction endonucleases. DNA sequencing of the inserted fragments was done employing the dideoxynucleotide method with dye terminators. Sequencing products were analyzed on an ABI 310 sequencing system (PE Applied Biosystems, Foster City, USA).

Reporter gene constructs

pOCLNproluc7 was constructed by cutting the 1853 bp genomic fragment from pgOCLN1-3 by flanking restriction enzymes and ligating it to pGL3-Basic (Promega, Madison, USA). For subsequent deletion of sequences from the 1853 bp fragment, pOCLNprolucC+/– (480 bp fragment, nucleotides 1374-1853), pOCLNprolucD+/– (594 bp fragment, nucleotides 943-1536), pOCLNprolucE+/– (200 bp fragment, nucleotides 1374-1573), and pOCLNprolucF+/– (280 bp fragment, nucleotides 1574-1853) were constructed by cloning the subfragments indicated in parentheses either in the original (+) or reverse (–) orientation into pGL3-Basic. Amplified plasmids were screened by restriction digestion and the sequences were confirmed by DNA sequencing.

Transfection and luciferase assay

HT-29/B6 cells were seeded to 6-well plates 24 hours before transfection at a density of 2×10^6 cells/well. For transfection, a total of 2 μg DNA was mixed with 200 μl RPMI1640 (without FCS and antibiotics) and 12 μl Plusreagent (Life Technologies, Karlsruhe, Germany). To normalize transfection efficiency, 1 μg of pRL-TK or pRL-SV40 (Promega, Madison, USA) was included in the transfection procedure. After 15 minutes incubation at room temperature (RT), a mixture of 200 μl RPMI1640 (without FCS and antibiotics) and 8 μl Lipofectamine (Life Technologies, Karlsruhe, Germany) was added, followed by an additional 15 minutes of incubation at RT. Cells were washed twice with phosphate-buffered saline and 1.6 ml RPMI1640 was added. After addition of the DNA/Lipofectamine mixture, cells were incubated for 3 hours at 37°C and 5% CO_2. After this, 4 ml RPMI 1640 (with 10% FCS and antibiotics) were added. In experiments with cytokines, TNFα and IFNγ alone or in combination were added to a final concentration of 10 or 100 ng/ml and 10 or 100 U/ml, respectively. Cells were incubated for 21 hours at 37°C and 5% CO_2. Measurement of both firefly and *Renilla* luciferase activity was performed with the Dual-Luciferase Assay kit (Promega, Madison, USA) according to the manufacturer's instructions. Cells were washed once with phosphate-buffered saline and harvested with 1 ml lysis buffer. Protein concentration was estimated by a standard BCA protein assay (Pierce, Rockford, USA). 10 μl of cell lysate were used for assaying the enzymatic activities, using a LB9507 luminometer with dual injector (Berthold, Bad Wildbad, Germany). Each lysate was measured twice. Promoter activities were expressed as relative light units (RLU), normalized for the protein content and the activity of *Renilla* luciferase in each extract. The data were calculated as the mean of three identical setups. In experiments where cytokines were added, the normalization was made in the group of three identical setups to exclude cytokine effects on coreporter-expression (Farr and Roman, 1992).

Monitoring of transepithelial resistance

HT-29/B6 cells were seeded on Millicell PCF filters (0.6 cm^2, Millipore, Eschborn, Germany) to a density of 7×10^5 cells/cm^2. 3-4 filters were put together into one 60 mm cell culture dish. The apical and basolateral compartments were filled with 500 μl and 10 ml of cell culture medium, respectively. Under standard conditions, incubation led to confluence within 7 days. Transepithelial resistance (R^t) was measured as described (Schmitz et al., 1999b). In brief, two fixed pairs of electrodes (STX-2, World Precision Instruments, USA) were connected with an impedance meter (D. Sorgenfrei, Inst. Klinische Physiologie). R^t was calculated from the voltage deflections caused by an external ±10 μA, 21 Hz rectangular current. Depth of immersion and position of the filters was standardized mechanically. Temperature was maintained constant at 37°C by a temperature-controlled warming plate. Resistance values were corrected for the resistance of the empty filter and of the bathing solution. Experiments were performed after confluency was reached, i.e. when R^t was between 400 and 500 $\Omega\cdot cm^2$. For cytokine delivery in R^t experiments, TNFα was added to the basolateral part of the filter compartment at a concentration of 100 ng/ml. Cells were incubated in the presence or

absence of TNFα for 6 hours and 24 hours. All values are given as percentage of the initial resistance (means ± s.e.m.). The unpaired two-tailed *t*-test was used to determine the significance of differences. $P<0.05$ was considered significant.

RNA isolation and northern blotting

Filters with HT-29/B6 cells were washed extensively with phosphate-buffered saline. Cells were scraped from the filter in the presence of 250 μl RNAzol (WAK Chemie, Bad Soden, Germany). The cell lysate was mixed with 0.1 volume chloroform by vortexing for 15 seconds, followed by an incubation at 4°C for 15 minutes. After centrifugation at 12,000 ***g***, RNA was precipitated from the aqueous phase by the addition of 1 volume isopropanol and incubation at 4°C for 30 minutes. The pellet obtained after centrifugation was washed with 70% ethanol, dried and resuspended in RNAse-free H_2O. 20 μg of total RNA was separated in a 1% denaturing formaldehyde agarose gel along with a digoxigenin-labeled RNA molecular mass standard, transferred to a nylon membrane and hybridized with a gene-specific digoxigenin-labeled DNA probe, representing nucleotides 919-1315 of the human occludin cDNA (GenBank accession number U49184) (Ando-Akatsuka et al., 1996). To normalize for the amount of RNA, a digoxigenin-labeled probe directed against glyceraldehyde-3-phosphate dehydrogenase was included. The hybridized probe was visualized with alkaline phosphatase-conjugated anti-digoxigenin antibodies and the chemoluminiscence substrate CDPstar (Roche, Mannheim, Germany). Chemoluminiscence was detected by the LAS-1000 luminiscence image analyzer (Fuji, Japan) and quantified with the AIDA software package (Raytest, Straubenhard, Germany).

Nucleotide sequence accession number

The sequence of the occludin promoter fragment described in this paper is published in GenBank (accession number AF246304).

RESULTS

Identification of the human occludin promoter

In order to investigate the role of genomic sequences upstream from the transcription start point for human occludin mRNA, a 1853 bp DNA fragment was cloned from human genomic DNA by the genome walking technique using occludin-specific oligonucleotide primers. The complete nucleotide sequence of this fragment was determined (Fig. 1). Close to the 3′ end of this fragment, sequence homology was found to the published cDNA sequences of the tight junction protein occludin (Ando-Akatsuka et al., 1996; Van Itallie and Anderson, 1997). A number of putative transcription factor binding sites, e.g. for TCF, NF-IL6 and AP2, were found in this fragment. When cloned into a luciferase reporter gene vector in the same orientation compared to the occludin open reading frame, this fragment could mediate gene expression after transient transfection of the human intestinal cell line HT-29/B6 (Fig. 2).

Deletion analysis of the occludin promoter

From this 1853 bp fragment a number of smaller fragments were tested for promoter activity using overlapping restriction enzyme sites for subcloning. Two fragments close to the right end of the sequence (C and F) were sufficient to mediate luciferase gene expression (Fig. 2). When inverting these

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ATTATGCTAAGTGAAAGAAGACAGACACAAAGGGCACATATTGTATTATCCATTCATATAAAATGTCCAG   70

AATAGGCAAATTAATAGAGACAGAAAGTACATTAGTGGTTGCCGAGGGATGAGGGGAGAGGGGAAATGGG  140

GAGTGACTATTAATGGGCATGGGGTTTCTTTTGGGGGCGATTAAAATGGAATTAGATGGTGGTGATGGTT  210

GTACAATCTGGTGAGTATACTAAAACCCATTGAATTGTACGTGCCCTTTAAAAGGGTGAATTTTATGGTA  280

TGTGAACTCTCTTTTATAAAGTGGAAAAAAACTCCTAAGATCTCTTCAGAACATGTCAAAATACATTATA  350

AAATAATAGACATGTGTTTACAAATCTGAGAGTATTAGGAAATGTTCCCTTGTTTAATTATAAACCAAAT  420
                                  NFIL6
GGAATGTTTGGAGATTTCAGTAATCTGACAGGGAACATTAAGGGGATTAACCTGACTTCCCCAGTGTTAA  490
            NFIL6
CAATACCAATTTAAACTGCATCATTCAAAAACTACATAGTCATATTAAAGCATTTGTAGCAATGACTTCC  560
                                                    NFIL6
ACGAAAAAAATACCAATTAAATTAATTACCCATTAAAGCTGCCATCATCTGAAATACCTCATATTTATAT  630

AGTGCTTTTACTTCCTCAAGTATGAATTGTGCCTTAAGATCTAATGTATGGGAGAGTCACATCTCTAACC  700

ATTTAATTAAAGGTAGAGAAGTGGGTGGGATTGGATAGAAATTTATTAGCAATGCTGACATTCCAGATTG  770

GAACACAAAGACAAGCAGGATGTAAGAAACCTAAAAGTTCGCTTTCAATGCAGATAGTTAAATGCCAAGA  840

ACTATAATTGCCACATCCTGGAGTACAATTAAAAAATATGTTGAAAAACAACCAACATACATAAAAATAT  910
                                  D
ACACAGTGTTAAGTGCAGACTATGAAATTTCCCTTGGAAACAGAATCCAGATCAAGAAATAGAATACAGC  980

ACCCGGAACTAAGGTGCTTTTTCGTTTTCTTAAAAAAAAAAAAGTAAACCAAGTTTATTTTGCTTTTTAA 1050

GTAGTGTTGTTCTTAAAGCACTACAGGTTGGTAAACAACGTATAAGGTGCTTTTTCTCAGGCCAAAGAGC 1120

CATAATAGTTTAAATTTCCCGGTCCTCAAGAGCGGACCGGGTGGGCAGGAGAGGACCCTGGGGTGGTGAT 1190

GTGTAAACTGTATTATGCACTTTAGCCTGCTGGATGGCAACTAACACCTACAGTAGTTCACCCTCATTTT 1260

AACCCCTCTAAGTAATTGTCTCTTATTCTGAATCTAGAGATTCAGAAACAGCGCCAATGTTTACACACGA 1330
                  Myc                   C,E
CTTTTGAAATTTTCCCAGGAGTCTTTCGTTGGAGCAATACATCTAGATGCCTTTTTCCAGCAACAGTTTA 1400
 NFIL6                    NFIL6
ATCAAATTCTGGAAGCAGAAAAGTGTCCTGTGAGGACGTGCCTTTCCTATCAAAGTGCTGAGTGCCTGGA 1470
       NFIL6                          ◆       TCF1          D
CCCTCTTTCCGGAGGAAACAGTCCCCTCTGGACCTCGTTCGGCCTCTCTCCATCAGACACCCCAAGGTTC 1540
                                  E   F
CATCCGAAGCAGGCGGAGCACCGAACGCACCCCGGGGTGGTCAGGGACCCCCATCCGTGCTGTCCCCTAG 1610
                          AP2                 AP2
GAGCCCGCGCCTCTCCTCTGCGCCCCGCCTCTCGGGCCGCAACATCGCGCGGTTCCTTTAACAGTGCGCT 1680

GGCAGGGTGTGGGAAGCAGGACCGCGTCCTCCCGCCCCCTCCCATCCGAGTTTCAGGTGAATTGGTCACC 1750
       NFIL6                  □     AP2   AP2
GAGGGAGGAGGCCGACACACCACACCTACACTCCCGCGTCCACCTCTCCCTCCCTGCTTCCTCTGGCGGA 1820
                          C,F
GGCGGCAGGAACCGAGAGCCAGGTCCAGAGCGC                                       1853
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Fig. 1. The complete sequence of the 1853 bp genomic DNA fragment. Positions of a putative transcription start point, as published in Van Itallie and Anderson (1997) (GenBank accession number U53823) and Ando-Akatsuka et al. (1996) (GenBank accession no. U49184) are marked by ◆ and □, respectively, above the sequence. Putative transcription factor binding sites are underlined. The borders of the fragments used for subcloning into the luciferase reporter gene vector are indicated by vertical bars and capital letters.

fragments relative to the reporter gene, luciferase activity was abolished, thus fulfilling the rule for the unidirectional use of a promoter sequence. The smallest fragment showing promoter activity, F, contains 280 bp of human genomic DNA, from which 208 bp are located directly upstream from the occludin cDNA sequence (Ando-Akatsuka et al., 1996).

Transepithelial resistance and occludin mRNA expression are affected by TNFα

To clarify whether the alteration in strand counts observed in freeze-fracture electron micrographs of TNFα-treated HT-29/B6 cells (Schmitz et al., 1999b) can be explained by an altered expression of tight junction molecules, we performed occludin-specific northern blot analysis from filter grown HT-29/B6 cells, treated with TNFα for 6 hours and 24 hours from the basolateral side. During this time, transepithelial resistance as a functional assay for the effect of TNFα on the epithelial barrier was monitored to correlate the altered barrier function with occludin gene expression in the selfsame cells. 6 hours after incubation the untreated controls showed only a slight change in transepithelial resistance (105±2% of initial resistance), whereas addition of 100 ng/ml TNFα led to a significant reduction in transepithelial resistance after this time (86±3% of initial resistance, $P<0.001$, $n=9$, Fig. 3A). This became more evident 24 hours after addition of TNFα when transepithelial resistance was reduced to 32±2% of initial resistance compared to a smaller decrease in the untreated control (87±2% of initial resistance, $P<0.001$, $n=9$, Fig. 3A). Northern blot analysis with RNA extracted from these cells using occludin-specific probes showed a reduction of occludin mRNA to 69% by 6 hours after addition of TNFα, and to 87% after 24 hours incubation compared to the untreated controls (Fig. 3B,C).

TNFα and IFNγ suppress occludin promoter activity

These experiments gave us a first hint that TNFα affects occludin gene expression at the transcriptional level. Therefore we examined the effect of TNFα and a second cytokine involved in inflammation, interferon γ, on occludin promoter activity. Reporter gene assays were performed in the absence or presence of different concentrations and combinations of both cytokines (Fig. 4). Addition of 10 ng/ml TNFα reduced promoter activity by the 1853 bp fragment to 34±5% ($P<0.001$, $n=3$). Higher concentrations (100 ng/ml) of TNFα did not augment this effect significantly. The addition of 10 U/ml IFNγ also affected promoter activity by diminishing it to 56±7% ($P<0.003$, $n=3$). The reduction of gene expression became more evident with 100 U/ml IFNγ. If both cytokines were added simultaneously at concentrations of 10 ng/ml for TNFα and 10 U/ml for IFNγ, occludin promotor activity was reduced in an overadditive effect to 6.8±0.3% ($P<0.001$, $n=3$) compared to the untreated controls. Smaller fragments containing the essential elements for occludin promoter activity (C and F, Fig. 1) gave similar results under these conditions (data not shown).

DISCUSSION

To analyze the regulation of tight junction protein expression at the molecular level, we cloned and sequenced a 1853 bp fragment from human genomic DNA with occludin-specific oligonucleotides. Sequence analysis revealed that this fragment contains the putative transcription start point for occludin mRNA, and a number of transcription factor binding sites have been identified in close proximity. We verified promoter activity of the 1853 bp fragment by cloning it into a luciferase reporter gene vector. Deletions from this fragment narrowed the essential elements necessary for promoter activity in cis to 208 bp upstream from the putative transcription start point for occludin mRNA. Inversion of fragments relative to the luciferase open reading frame abolished enzyme activity nearly completely, as expected. Fragments that include the cDNA start of the sequence published by another group (Van Itallie and Anderson, 1997) showed no promoter activity when the

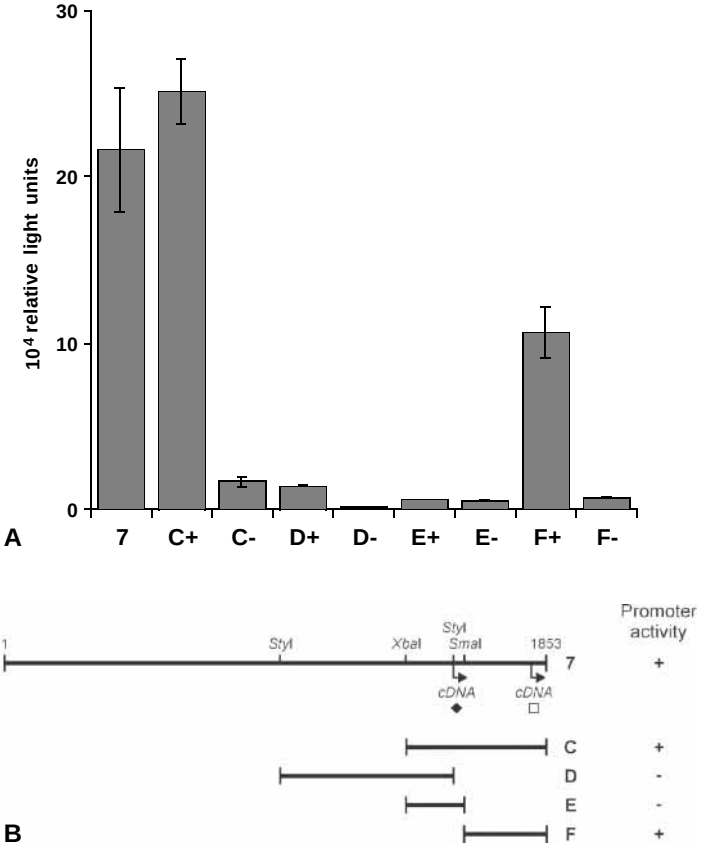

Fig. 2. Effect of subsequent deletions on the occludin promoter activity. (A) Genomic fragment 7 and subsequent restriction fragments (B) were cloned in front of a luciferase reporter gene either in the original (+) or the inverse (–) orientation compared to the occludin open reading frame. After transfection of HT-29/B6 cells, extracts were analyzed for luciferase activity. Chemoluminiscence was monitored as relative light units. Values are normalized for protein content and expression of cotransfected *Renilla* luciferase reporter gene. Values are means ± s.e.m. ($n=3$). (B) General scheme of genomic DNA fragments used for promoter analysis. Relative positions of restriction enzymes used for subcloning are indicated as vertical bars. Locations of the 5′ ends of cDNA as published elsewhere are marked by arrows for ◆ (Van Itallie and Anderson, 1997) and □ (Ando-Akatsuka et al., 1996). Expression of promoter activity when cloned into a luciferase reporter gene vector in the correct orientation with respect to the genomic situation is indicated by +.

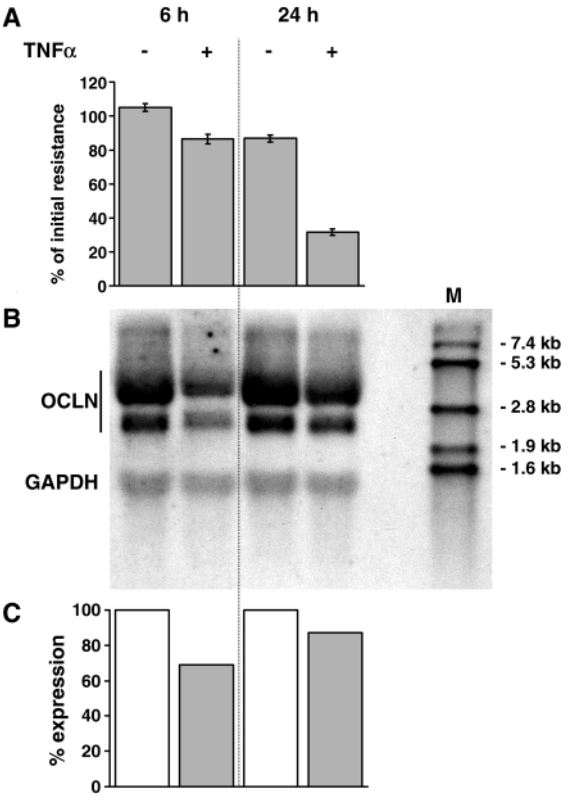

Fig. 3. Effect of TNFα on R^t and occludin mRNA expression in HT-29/B6 cells. Filter grown HT-29/B6 cells were incubated in the absence (–) or presence (+) of 100 ng/ml TNFα for 6 hours or 24 hours from the basolateral side (*n*=9 for each time point). (A) Course of transepithelial resistance is shown as a percentage of initial resistance. (B) RNA from the selfsame cells was subjected to northern hybridization with occludin (OCLN)- and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-specific DNA probes. (C) Quantitation of OCLN-specific signals (filled columns) is shown as percentage expression compared to the untreated cells (open columns) after normalization to GAPDH.

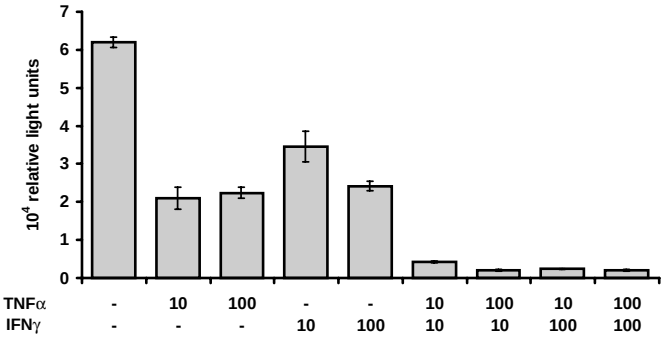

Fig. 4. Effect of TNFα and IFNγ on occludin promoter activity. HT-29/B6 cells transiently transfected with pOCLNproluc7 were treated for 21 hours with 10 or 100 ng/ml TNFα and 10 or 100 U/ml IFNγ. Untreated cells served as a control. Occludin promoter-mediated luciferase expression was monitored 24 hours after transfection in a chemoluminiscence assay and is indicated as relative light units. Values are normalized for protein content and expression of cotransfected *Renilla* luciferase reporter gene and are means ± s.e.m. (*n*=3).

downstream promoter element was deleted. Thus, it cannot be excluded that a second promoter is located in the 5′ direction of this putative transcription start. This also would explain the existence of occludin-specific mRNA molecules with different sizes not only by alternative splicing but also by differential gene expression from distinct promoters.

TNFα and IFNγ are proinflammatory cytokines that alter tight junction strand formation in a dose-dependent manner (Schmitz et al., 1999a, 1999b). To elucidate their effect on occludin gene expression, the human intestinal cell line HT-29/B6 was incubated with different concentrations of both cytokines. HT-29/B6 cells are a suitable model for analyzing paracellular barrier effects because they grow as a highly differentiated polarized epithelial cell layer and exhibit a transepithelial resistance comparable to that of native colon (Kreusel et al., 1991). The decrease in transepithelial resistance after incubation of HT-29/B6 cells with TNFα from the basolateral side is preceded by a reduction of occludin-specific mRNA expression. These experiments gave us a first hint that TNFα-dependent barrier impairment is caused at the transcriptional level of occludin gene expression.

Therefore we examined the effect of TNFα and IFNγ on occludin promoter activity. The results clearly show, that TNFα and IFNγ reduce gene expression mediated by the human occludin promoter. This suggests that TNFα impairs barrier function by affecting gene expression of tight junction proteins. The synergistic effect of IFNγ may be due to IFNγ-dependent upregulation of TNF receptors or metabolic effects enhancing TNFα susceptibility. These results correlate with electrophysiological investigations, in which the addition of both cytokines resulted in an over-additive decrease in transepithelial resistance of HT-29/B6 cells (Schmitz et al., 1999b). In close proximity to the predicted transcription start points of occludin mRNA were found a number of potential binding sites for nuclear factor interleukin-6 (NF-IL6). Transcription factor NF-IL6 was suggested to be involved in TNFα-mediated gene expression (Yamamoto et al., 1995) as well as in the regulation of TNFα gene expression itself (Zagariya et al., 1998). The intracellular pathways that mediate TNFα-dependent transcription of genes for tight junction proteins remain to be elucidated. However, altered gene expression of the tight junction protein occludin may lead to alterations in tight junction strand formation and, subsequently, may influence barrier function.

Recent studies have shown that occludin is not the only integral membrane protein involved in tight junction strand formation. Embryonic stem cells, in which occludin gene expression was knocked out experimentally, can differentiate into polarized cells (Saitou et al., 1998). The claudin gene family seems to contribute in a tissue specific manner to the assembly of tight junctions (Morita et al., 1999). A third member of membrane proteins thought to be associated with the tight junction, junctional adhesion molecule (JAM), was shown to be redistributed from intercellular junctions after a combined treatment with TNFα and IFNγ (Ozaki et al., 1999). These data suggest that the composition of the tight junction is highly regulated and that the loss or redistribution of one member of the intercellular junction may be compensated through the upregulation of another tight junction protein. Thus, even if an occludin knockout approach is not accompanied by loss of barrier function (Saitou et al., 1998),

downregulation of occludin by proinflammatory cytokines may be important when playing in concert with cytokine effects on other tight junction strand components.

The influence of cytokines that are involved in inflammation may be important for barrier dysfunction in many intestinal disorders including inflammatory bowel disease, celiac sprue (Schulzke et al., 1998, 1995) and infectious bowel diseases (Stockmann et al., 1998), in which altered tight junction formation and elevated cytokine levels have been observed.

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