Functional Expression of the Interleukin-11 Receptor α-Chain and Evidence of Antiapoptotic Effects in Human Colonic Epithelial Cells

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A tissue-protective effect of interleukin-11 (IL-11) for the intestinal mucosa has been postulated from animal models of inflammatory bowel disease (IBD). Despite the fact that the clinical usefulness of the anti-inflammatory effects of this cytokine is presently investigated in patients with IBD, there are no data available regarding the target cells of IL-11 action and the mechanisms of tissue protection within the human colonic mucosa. IL-11 responsiveness is restricted to cells that express the interleukin-11 receptor α-chain (IL-11Rα) and an additional signal-transducing subunit (gp130). In this study, we identified the target cells for IL-11 within the human colon with a new IL-11Rα monoclonal antibody and investigated the functional expression of the receptor and downstream effects of IL-11-induced signaling. Immunohistochemistry revealed expression of the IL-11Rα selectively on colonic epithelial cells. HT-29 and colonic epithelial cells (CEC) constitutively expressed IL-11Rα mRNA and protein. Co-expression of the signal-transducing subunit gp130 was also demonstrated. IL-11 induced signaling through triggering activation of the Jak-STAT pathway without inducing anti-inflammatory or proliferative effects in colonic epithelial cells. However, IL-11 stimulation resulted in a dose-dependent tyrosine phosphorylation of Akt, a decreased activation of caspase-9, and a reduced induction of apoptosis in cultured CEC. In HLA-B27 transgenic rats treated with IL-11, a reduction of apoptotic cell numbers was found. This study demonstrates functional expression of the IL-11Rα restricted on CEC within the human colonic mucosa. IL-11 induced signaling through triggering activation of the Jak-STAT pathway, without inducing anti-inflammatory or proliferative effects. The beneficial effects of IL-11 therapy are likely to be mediated by CEC via activation of the Akt-survival pathway, mediating anti-apoptotic effects to support mucosal integrity.

Interleukin-11 (IL-11) is initially cloned as a mediator of plasmacytoma cell proliferation (1) and was later found to exhibit a wide variety of biological effects in neural cells as well as in the hematopoietic and the immune system (for a review, see Ref. 2). IL-11 is a member of a family of cytokines that includes interleukin-6 (IL-6), leukemia inhibitory factor, oncostatin M, and ciliary neurotropic factor (3, 4). These so-called IL-6-type cytokines drive the assembly of a multisubunit receptor complex that initiates intracellular signaling by association with the transmembrane signal transducer glycoprotein gp130 (5–8).

IL-11 is specifically bound to a unique interleukin-11 receptor α-chain (IL-11Rα) (9, 10). The human IL-11Rα was initially cloned from a bone marrow cDNA library and shares 85% nucleotide and 84% amino acid identity with the murine IL-11Rα, which was first cloned in 1994 (9, 11, 12).

Recent studies show that IL-11-induced signaling is mediated by the formation of a hexameric receptor complex, composed of two molecules each of IL-11, IL-11Rα, and gp130 (13). An important signaling system activated by the IL-11Rα and other members of this receptor family is the Janus kinase signal transducer and activator of transcription (Jak-STAT) pathway (8, 14). Specific receptor-binding of IL-11 triggers dimerization of gp130 and transient activation via transphosphorylation of tyrosine kinases of the Jak family. IL-11 has been shown to induce activation of Jak1 and Jak2 receptor kinases downstream of IL-11Rα/gp130 activation (15, 16). Jak phosphorelay tyrosine residues in the cytoplasmic regions of gp130, which in turn serve as docking site for STAT1 and STAT3 proteins (17). These signaling molecules are members of the STAT family of transcription factors (18, 19). Recruited STAT proteins undergo tyrosine phosphorylation and dissociate from gp130, dimerize (20), and translocate into the nucleus, where they act as transcriptional activators of cytokine-responsive genes (14, 21).

IL-11 has been shown to be an important mediator in sys-
tems other than the hematopoietic system (22). IL-11 reduces the expression of a number of proinflammatory cytokines in several cell culture systems. Preclinical in vivo and in vitro studies have demonstrated that IL-11 inhibits the secretion of tumor necrosis factor (TNF), interleukin-12 (IL-12), interleukin-1β (IL-1β), and nitric oxide from activated macrophages as well as interferon-γ and IL-12 from activated T cells (29–36).

In addition, IL-11 exerts cytoprotective effects on the intestinal mucosa (27–30). IL-11 treatment reduced mucosal damage after chemotherapy and radiation (31–33). Remarkably, IL-11 ameliorated disease severity in colitis models, such as HLA-B27 transgenic rats (34–36) and attenuated cell death after intestinal ischemia-reperfusion (37). Because of its therapeutic effectiveness in several animal models, IL-11 was considered to be a promising drug candidate for the treatment of inflammatory bowel diseases (IBD). Recently, Sands et al. (38) in a randomized, controlled trial revealed that treatment with recombinant human interleukin-11 in patients with active Crohn's disease was well tolerated. However, there was only a trend to a decreased Crohn's disease activity index in the recombinant human IL-11 group versus placebo. Despite the fact that the clinical utility of the anti-inflammatory properties and beneficial effects of this cytokine are presently being investigated in clinical studies in patients with Crohn's disease (27, 39, 40), there are no data regarding the target cells of IL-11 action and the mechanisms of tissue protection within the human colonic mucosa.

IL-11 responsiveness is restricted to cells that express both the IL-11Rα subunit and the transmembrane signaling receptor gp130 on their surface (9, 12). Expression of gp130 protein has previously been described on many cells types and tissues (41); however, only preliminary data are available for the expression of IL-11Rα mRNA in murine intestinal cell lines (28) and murine tissue (42). A recently generated IL-11Rα mAb (clone E24.2) reacting with an epitope expressed in the extracellular region of human IL-11Rα (43) enabled us to investigate the expression of the IL-11Rα in mucosal tissue. A functional role for IL-11 and for its receptor moiety in the human colonic mucosa has not yet been established. In order to address these questions, we investigated the expression of IL-11Rα mRNA and protein in the colon cancer cell line HT-29 and in human primary colonic epithelial cells (CEC). We further analyzed signaling via tyrosine phosphorylation of downstream molecules upon treatment with IL-11 and possible effector mechanisms.

In this study, colonic epithelial cells are shown to be the prime target of IL-11 in the human mucosa, since the functional surface expression of the receptor in conjunction with the gp130 protein could be demonstrated. Furthermore, our data provide evidence that IL-11 mediates activation of the Jak-STAT pathway within colonic epithelial cells, causing anti-apoptotic but not anti-inflammatory or proliferative effects. In vivo studies in HLA-B27 transgenic rats revealed a reduction of apoptotic CEC in IL-11-treated animals.

MATERIALS AND METHODS

Preparation of Human Primary Colonic Epithelial Cells—Colonic tissue was obtained from patients undergoing surgical resection for colorectal carcinoma. The study was approved by the University of Regensburg Ethics Committee. Normal mucosa was taken at least 10 cm distant from the tumor. The mucosa was stripped from the submucosa within 30 min after bowel resection and rinsed several times with PBS at room temperature. The mucus was removed by incubating the specimens twice in 1 mM dithiothreitol (Sigma) for 15 min at 37 °C. After washing with PBS, the mucus was rotated in 2 mM EDTA in tissue (42). A recently generated IL-11Rα mAb as well as interferon-γ and IL-12 from activated T cells (29–36).

To separate CEC-containing crypts from contaminating single cells, the suspension was allowed to settle down for up to 5 min at room temperature. The sedimented crypts were collected, washed with PBS, and resuspended in minimal essential medium supplemented with Earle salts, 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml gentamycin, 2.5 μg/ml fungizone (JRH Bioscience, Lenexa, KS). Media were purchased from Biochrom (Berlin, Germany), and supplements were obtained from Sigma.

Cell Culture and Stimulation Conditions—HT-29 were obtained from American Type Culture Collection (Manassas, VA) and cultured in Dulbecco's modified Eagle's medium (Biochrom) supplemented with 10% FCS, 1% nonessential amino acids, and 1% sodium pyruvate (Biochrom) under standard tissue culture conditions (10% CO2 at 37 °C). HT-29 cells were cultured on collagen-coated nylon filters according to the method described earlier (44). For the detection of tyrosine phosphorylation of Jak1 and STAT3 upon treatment with IL-11, HT-29 cells were cultured in Dulbecco's modified Eagle's medium containing 0.5% FCS for 48 h to reduce basal levels of intracellular phosphorylation. Subsequently, the medium was removed, and cells were incubated in medium without serum for 2 h and then treated with serum-free medium containing various concentrations of recombinant IL-11 (Genetics Institute, Cambridge, MA) for different periods of time.

Since CECS rapidly undergo apoptosis after loss of anchorage from the mucosa (45), freshly EDTA-isolated crypts were immediately resuspended in minimal essential medium without FCS and centrifuged (3 min at 1000 rpm) to retain cell-cell contact. Thereafter, sedimented CEC were starved in serum-free minimal essential medium for 2 h at 37 °C, resuspended in serum-free medium containing various concentrations of IL-11, and finally incubated at 37 °C for 15 min.

For the investigation of antiapoptotic properties of IL-11 in CEC, cells were pretreated with different concentrations of IL-11 and kept in suspension by shaking to induce detachment-induced apoptosis.

RT-PCR and Northern Blot Analysis—Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and reverse transcribed into first-strand cDNA. Primers specific for the human IL-11Rα (forward, 5'-GCTGAAAGCTGT-GTTGCTCTG-3'; reverse, 5'-GCTCTAGACCTGTCCTCTC-3') and human glyceraldehyde-3-phosphate dehydrogenase (internal control) (forward, 5'-TTAGCACCCGTCGAAAGG-3'; reverse, 5'-CTTACCTCTGAGGCGGCAAGT-3') were used for PCR (Hotstart; Qiagen). The sizes of the PCR products are 339 bp for IL-11Rα and 529 bp for G3PDH. PCR conditions for both were as follows: 95 °C for 15 min for denaturation with Hotstart polymerase, 94 °C for 30 s, 55 °C for 1 min for 35 cycles for IL-11Rα and 25 cycles for G3PDH, followed by 72 °C for 10 min.

For Northern blot analysis, 15 μg of total RNA were size-fractionated on a 1.0% formaldehyde-agarose gel, transferred to a nylon membrane (Hybond™; Amersham Biosciences) and UV-cross-linked in a UV Stratalinker 1800 (Stratagene, Amsterdam, The Netherlands). The am- phose DNA probes encoding the human IL-11 and GAPDH were labeled with [32P]dATP (3000 Ci/mmol) (Amersham Biosciences) using a random primed DNA labeling kit (Roche Applied Science). The membranes were hybridized with the labeled probes for 1 h at 65 °C in Quikhyb solution (Stratagene). After extensive washing, the membranes were exposed to Kodak X-Omat films (Eastman Kodak Co.) at −80 °C using an intensifying screen.

Flow Cytometric Analysis—To obtain a single cell suspension from the isolated crypts, CEC were incubated in dispase (1.2 mg/ml in Hanks' balanced salt solution; Roche Applied Science) for 2 min at 37 °C followed by vigorous shaking. Enzyme activity was stopped by the addition of EDTA (1 mM), and cells were fixed in 70% methanol at −20 °C until analysis. HT-29 cells were detached from culture flasks with accutase (Sigma), washed with PBS, pelleted at 4000 rpm, and resuspended in PBS plus 2% FCS. Following blocking for 30 min on ice, cells were incubated with specific anti-IL-11Rα mAb E24.2, anti-gp130 Ab (Upstate Biotechnology, Eching, Germany), or isotype control (mouse IgG1; Sigma) for 1 h on ice. Thereafter, cells were washed twice with PBS containing 2% FCS, and IL-11-expressing CEC were stained by PE-labeled rabbit anti-mouse Ab (DAKO, Hamburg, Germany) for 1 h. Samples were washed twice with PBS and resuspended in 500 μl of PBS for flow cytometric analysis. For phenotypic staining, cells were incubated on ice for 1 h with FITC-labeled mouse anti-epithelial antigen mAb EP-4 (clone Ber-EP-4; DAKO) or IgG1 FITC (Coulter Immunotech, Hemburg, Germany) for 1 h. Cells were then washed and stained as isotype control. The sample was then run on an EPICS XE-MCL flow cytometer (Coulter Immunotech). Based on their forward and side scatter characteristics, living cells were gated.

For flow cytometric cell cycle analysis and detection of DNA fragmentation, ~106 cells were fixed in ice-cold methanol (70%) for 1 h on
ice, washed twice with PBS, and resuspended in PBS. Fixed cells were treated with RNase (0.01 mg/ml; Roche Molecular Biochemicals) for 30 min at 37 °C, stained with 50 μg/ml propidium iodine (PI; Sigma), and kept in the dark on ice for 30 min before analysis. Cell cycle analysis was carried out using an EPICS XL-MCL flow cytometer (Coulter Immunotech). Data were analyzed with the Multicycle software (Becton Dickinson, Heidelberg, Germany).

**Immunohistochemistry**—Colonic specimens were immediately frozen and cut into 5-µm sections. HT-29 cells were seeded onto glass tissue slides at different states of confluence. Before staining, slides were fixed in 100% methanol for 15 min and then rinsed twice with PBS. Endogenous peroxidase was quenched for 30 min with 0.3% hydrogen peroxide in PBS buffer. After blocking nonspecific binding sites with normal goat serum (Sigma) for 30 min, the slides were incubated with anti-IL-11R antibodies, anti-EP-4 mAb, or anti-mouse IgG1 as negative control for 1 h. Slides were then rinsed with PBS and incubated with biotinylated rabbit anti-mouse IgG (DAKO) for 30 min. After rinsing twice with PBS, slides were treated with streptavidin-conjugated peroxidase (Vector ABC Elite Kit; Vector Laboratories, Burlingame, CA) for 30 min at room temperature. Slides were incubated for 5–10 min in 3,3'-diaminobenzidine solution (Vector Laboratories) at room temperature to allow color development and rinsed with distilled water to quench the reaction. Mayer’s hematoxylin was used as a counterstain.

**Immunoblotting**—Cells were resuspended in ice-cold radiolabeled precipitation assay buffer (1× PBS; 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3VO4, 50 mM NaF, and one tablet of complete protease inhibitor mixture (Roche Applied Science) per 50 ml) for 10 min, sonicated on ice, and centrifuged (12,000 × g, 15 min at 4 °C). Protein concentration of the supernatant (protein fraction) was determined by Bradford protein assay (Bio-Rad). An aliquot of 35–75 μg of protein was mixed with an equivalent volume of 2× protein loading buffer containing 2-mercaptoethanol and boiled for 5 min before loading onto an SDS-polyacrylamide gel. After electrophoresis, proteins were transferred onto nitrocellulose membranes using the XCell Blot Module (Invitrogen BV/NOVEX, Groningen, The Netherlands) and blocked in TBST (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20) containing 5% nonfat dry milk powder or 3% bovine serum albumin for anti-phosphotyrosine antibodies, and protein bands were visualized using a commercial peroxidase substrate (ECL Plus; Amersham Biosciences) as described by the manufacturer.

**Determination of IL-8 Protein**—Supernatants of control and TNF/IL-11-treated cell cultures were collected after 24 h and centrifuged. IL-8 concentration in the incubation medium was quantified by enzyme-linked immunosorbent assay (Biotrak-Amersham, Braunschweig, Germany) according to the manufacturer’s protocol.

**Caspase-9 Fluorometric Assay**—CEC in medium with or without IL-11 were kept in suspension by shaking and lysed after the indicated time points. The cell lysate was kept for protease activity by the addition of the caspase-9 specific substrate peptide LEHD (R&D Systems). The cleavage of the peptide by caspase-9 releases the fluorochrome that upon excitation at 480 nm emits light at 505 nm. The level of caspase-9 enzymatic activity in the cell lysate is directly proportional to the fluorescence signal detected with a fluorescence microplate reader.

**In Vivo Studies in HLA-B27 Transgenic Rats**—HLA-B27 (I-Ek microglobulin deficient) transgenic rats were obtained from Taconic Inc. (Germantown, WI) and housed in isolated ventilated cages on standard bedding. All rats were fed standard rat chow ad libitum.

Recombinant human IL-11 (R&D Systems) was dissolved in sterile PBS containing 0.1% bovine serum albumin to a concentration of 14 μg/ml cytokine.

HLA-B27 transgenic rats were divided into two groups (n = 5) and treated either with recombinant human IL-11 or with placebo (0.5 ml subcutaneously, ~33 μg/kg), in a double blinded fashion, every other day for 2 weeks. The last injection was administered 4 h prior to sacrifice.

**RESULTS**

**IL-11Ra Is Mainly Expressed on Epithelial Cells within the Human Colon**—Since the site of IL-11 action within the intestinal tract had not yet been elucidated, the first aim of our study was to determine the localization of IL-11Ra expression in normal human colonic mucosa with the anti-IL-11Ra mAb, IgG1 isotype staining as negative controls (C and D), and epithelial cell characterization with the EP-4 mAb (E and F) in tissue specimens obtained from normal mucosa. Immunohistochemistry revealed expression of the IL-11Ra selectively on epithelial cells within the human colon. IL-11Ra is also expressed by colonic epithelial cell lines: IL-11Ra staining (G) and IgG1 isotype as negative control on HT-29 (H). Magnification was ×400.

A TUNEL staining on paraffin-embedded specimen of colonic mucosa was performed as described earlier (46, 47). The number of apoptotic cells was determined microscopically. 1000 epithelial cells were evaluated in three different microscopic fields per animal.

Cells were isolated from mucosal specimens as described above (44). Flow cytometrical cell cycle analysis was performed as described for human CEC. Acidine orange staining was performed according to standard procedures.
The immunohistochemical findings prompted us to investigate receptor expression in the colon cancer cell line HT-29. Cells grown on glass slides at different states of confluence demonstrated a clear signal for gp130 expression in HT-29 and freshly isolated CEC (Fig. 2A). No detectable staining was obtained when a mouse IgG1 isotype control was used as a negative control (Fig. 1H).

**Constitutive IL-11Ra mRNA Expression in HT-29 and CEC**—The immunohistochemical findings prompted us to investigate transcription of IL-11Ra mRNA. Total RNA extracted from HT-29 and CEC was subjected to RT-PCR to screen for IL-11Ra mRNA expression. Signals of the appropriate size were detected in HT-29 and freshly isolated CEC (Fig. 2A). RT-PCR analysis of G3PDH mRNA expression confirmed the quality of all RNA preparations used for cDNA amplification (Fig. 2A). We could not detect IL-11Ra mRNA expression in other mucosal cells (purified intestinal macrophages, colonic myofibroblast cultures) with RT-PCR analysis (data not shown).

We also performed Northern blot analysis to confirm the presence of IL-11Ra mRNA in colonic epithelial cells. As shown in Fig. 2B, both HT-29 and CEC revealed expression of a 1.8-kb transcript for the IL-11Ra. These results demonstrate that human colonic epithelial cells constitutively express IL-11Ra mRNA.

**IL-11Ra Protein and Cell Surface Expression in HT-29 and CEC**—We further determined whether translation of IL-11Ra mRNA occurred constitutively in these cells as well. IL-11Ra cell surface expression in HT-29 and freshly isolated CEC was analyzed by flow cytometry. As shown in Fig. 3A, there was no staining of HT-29 with an IgG1 isotype control. In contrast, more than 90% of the analyzed cells showed a bright staining for the IL-11Ra.

The purity of the CEC preparation from mucosal tissue was assessed by flow cytometrical analysis. To ensure single cell analysis, isolated crypts were briefly treated with dispase prior to staining with the FITC-labeled EP-4 mAb antigen. 98% of the EDTA-isolated cells were EP-4-positive, indicating epithelial origin (Fig. 3B). The amounts of contaminating nonepithelial cells were less than 1%. Hardly any positive staining for lymphocytes (CD3), macrophages (CD68), mast cells (CD117), or myofibroblasts (specific myofibroblast marker AS02) could be detected among the isolated cells (data not shown), demonstrating that the epithelial cell preparation method ensured isolation of highly purified CEC. Staining of freshly isolated CEC resulted in 93% IL-11Ra-positive cells (Fig. 3B). Using the two-parameter analysis of EP-4/FITC and IL-11Ra/PE on the isolated mucosal cells, expression of the α-chain of the receptor exclusively on CEC was confirmed (Fig. 3B).

Western blot analysis of IL-11Ra expression in colonic epithelial cells demonstrated three specific bands with protein extracts from HT-29 and CEC (Fig. 3C). The 49-kDa band, detected by the IL-11Ra mAb, indicated a molecular mass in agreement with the one of the polypeptide backbone. We could detect two additional immunoreactive bands of 33 and 36 kDa with this mAb (Fig. 3C), which may reflect the described isoform of the IL-11Ra that lacks the cytoplasmatic region and different states of N-glycosylation sites of this isoform (11, 48). Immunoprecipitation of IL-11Ra protein from cell lysates and detection with another polyclonal goat anti-mouse IL-11Ra Ab after SDS-PAGE revealed the same pattern of immunoreactive bands as was obtained with the mAb (data not shown). These findings indicate the translation of IL-11Ra mRNA into protein and localization of the receptor α-chain to the cell membrane of HT-29 and CEC.

**Function of IL-11Ra in Colonic Epithelial Cells**—The functional receptor complex for IL-11 is a multiheterodimer composed of the specific ligand-binding α-chain of the IL-11 receptor and the signal-transducing subunit gp130. To investigate gp130 co-expression, we performed Western blotting of lysates obtained from HT-29 and freshly isolated CEC. As shown in Fig. 4A, gp130 protein is present in HT-29 and to a lesser extent in CEC. β-Actin was used as a control to verify equal loading of the gel lanes.

Cell surface expression of the transmembrane glycoprotein gp130 was analyzed by flow cytometry. HT-29 cells revealed a homogeneous gp130 expression with 94% positively stained cells as compared with the IgG1 isotype control (Fig. 4B). Cell surface gp130 expression was also detectable in CEC with two populations showing different staining intensity, indicating different receptor densities along the colonic crypts (Fig. 4B). These findings reveal the constitutive expression of the signal transducing subunit gp130 in colonic epithelial cells.

**Jak1 and STAT3 Phosphorylation Induced by IL-11 in Colonic Epithelial Cells Is Dose- and Time-dependent**—In order to elucidate functional expression of the IL-11Ra in colonic epithelial cells, we assessed activation of Jak1 and STAT3 in HT-29 and CEC upon treatment with IL-11. In these experiments, an Ab specific to tyrosine-phosphorylated (Tyr1022/1023) Jak1 and phosphorylated (Tyr705) STAT3 was used to determine the activated form of Jak1 and STAT3. The IL-11-induced Jak1 tyrosine phosphorylation pattern is presented in Fig. 5A. A weak basal activation of Jak1 could be detected in unstimulated HT-29 despite serum starvation of the cells. Treatment with increasing amounts of IL-11 for 15 min induced Jak1 activation in a concentration-dependent manner, peaking at 100 ng/ml IL-11. Immunoblot analysis of the same sample with anti-Jak1 mAb indicated that the total levels of Jak1 protein were not changed by IL-11 treatment (Fig. 5A).

The time course of Jak1 tyrosine phosphorylation induced by 100 ng/ml IL-11 in HT-29 is shown in Fig. 5B. Jak1 activation could be assessed as early as 1 min after stimulation. A maximal response was observed within 15 min, declining during the next 45 min. In CEC, a dose-dependent increase of tyrosine-phosphorylated Jak1 could also be detected (Fig. 5C).

Without stimulation, immunoreactivity specific for phosphotyrosine-STAT3 was not detectable in immunoblotted cell ly-
**FIG. 3.** Cell surface IL-11Rα protein expression in human colonic epithelial cells. HT-29 cells were stained with an IgG1 isotype control or the anti-IL-11Rα mAb, followed by treatment with a secondary, PE-labeled anti-mouse IgG Ab for flow cytometric analysis (A). B, staining of the isolated mucosal cells. Epithelial cells were characterized with IgG1-FITC as isotype control or FITC-labeled anti-EP-4 mAb. IgG1 isotype control or IL-11Rα PE staining was conducted to analyze cell surface expression. Two-parameter analysis was performed to characterize IL-11Rα expression exclusively on CEC. Western blot analysis of lysates was conducted with the IL-11Rα mAb to assess IL-11Rα expression in HT-29 and CEC (C).
sates of HT-29 (Fig. 5D). After 15 min of cytokine treatment, activation of STAT3 was induced by 10 ng/ml IL-11 and increased in a dose-dependent manner to maximal levels at 200 ng/ml IL-11. Levels of STAT3 protein remained unchanged by IL-11 stimulation (Fig. 5D). In time course experiments, activation of STAT3 was detected within 5 min, reaching a peak at 15 min and declining within the next 45 min (Fig. 5E). In untreated CEC, a constitutive STAT3 activation was observed, despite starving the freshly isolated cells for 2 h in serum-free minimal essential medium (Fig. 5F). Stimulation of CEC with varying amounts of IL-11 caused an increasing tyrosine phosphorylation of STAT3. These data indicate that the cell surface expression of the receptor α-chain in colonic epithelial cells was shown to be functional, since stimulation with its cognate ligand IL-11 induced activation of Jak1 and STAT3.

**IL-11 Does Not Inhibit Activation of NF-κB Signaling in HT-29**—IL-11 up-regulates the synthesis of IκBa or IκBβ in mononuclear cell lines, resulting in diminished nuclear translocation of NF-κB and reduction in proinflammatory cytokine synthesis. TNF is the prototypic activator of the NF-κB pathway causing rapid degradation of IκB proteins. The effects of IL-11 on the NF-κB pathway were determined by analyzing the protein levels and potential degradation of IκBa, which is necessary for NF-κB activation. As shown in Fig. 6A, IL-11 (100
Furthermore, pretreatment of HT-29 with 100 ng/ml IL-11 for 30 min did not inhibit IL-8 secretion by HT-29 when left untreated or upon stimulation with 0.5 ng/ml TNF for an additional 16 h (Fig. 6B). These findings indicate that IL-11 does not act in an anti-inflammatory manner on HT-29 by inhibiting nuclear translocation of NF-κB.

**IL-11 Treatment Did Not Alter Proliferation in HT-29**—We further investigated whether exogenous IL-11 induces proliferative effects downstream to the STAT3 activation in colonic epithelial cells. HT-29 cells were cultured for 48 h with different concentrations of IL-11 in a subconfluent and a confluent state. Influences on the proliferation rate by IL-11 stimulation were analyzed by flow cytometry and cell cycle analysis of PI-stained cells. Treatment of HT-29 with different concentrations of IL-11 did not alter the amount of cells in S phase at different states of confluence (Fig. 7). These findings indicate that administration of IL-11 does not influence the growth of HT-29 cells.

**Bcl-2 Expression Is Increased upon Treatment with IL-11 Exclusively in CEC**—We determined whether IL-11 exerts antiapoptotic effects in colonic epithelial cells downstream to STAT3 activation by the induction or regulation of Bcl-2 protein. HT-29 and CEC were cultured for 24 h with increasing concentrations of IL-11, and protein expression was investigated by Western blotting. In HT-29, we did not detect expression of Bcl-2 protein even after stimulation with IL-11 (Fig. 8A). Treatment with IL-11 induced a dose-dependent increase of the Bcl-2 protein level in CEC (Fig. 8B). These data suggest an antiapoptotic effect of IL-11 via the regulation of Bcl-2 protein in CEC.

**Akt Phosphorylation Induced by IL-11 in Colonic Epithelial Cells Is Dose-dependent**—In order to elucidate alternative antiapoptotic effects of IL-11 in colonic epithelial cells, we assessed activation of the protein kinase Akt in HT-29 and CEC. In these experiments, an Ab specific to serine-phosphorylated (Ser473) Akt was used to determine the activated form of Akt. The IL-11-induced Akt phosphorylation pattern is presented in Fig. 9. A weak basal activation of Akt could be detected in unstimulated HT-29 despite serum starvation of the cells. Treatment with increasing amounts of IL-11 for 30 min induced Akt activation in a concentration-dependent manner, peaking at 200 ng/ml IL-11. Immunoblot analysis of the same sample with anti-Akt Ab indicated that the total levels of the protein were not changed by IL-11 treatment (Fig. 9A). In CEC, a weak increase of serine-phosphorylated Akt could also be detected (Fig. 9B).

**Antiapoptotic Effects by Decreased Caspase-9 Activation and Induction of Apoptosis in CEC upon Treatment with IL-11**—To assess whether IL-11 stimulation results in antiapoptotic effects downstream to Akt phosphorylation and Bcl-2 regulation, we analyzed the activation of caspase-9 induced by stimulation of CEC with IL-11. Freshly isolated CEC were treated with or without 200 ng/ml IL-11 and kept in suspension by shaking to trigger detachment-induced apoptosis. At the indicated time points, cells were lysed, and activation of caspase-9 was analyzed with a fluorometric assay. Stimulation with 200 ng/ml IL-11 resulted in a significantly decreased caspase-9 activity in CEC kept in suspension in comparison with untreated cells (Fig. 10A). Finally, we determined whether IL-11 stimulation influences spontaneous apoptosis of CEC in culture. Cells were cultured for 24 h with or without IL-11 (200 ng/ml) on collagen-coated filter inserts. The rate of apoptotic cells was measured by flow cytometry after PI staining. The percentages of apoptotic cells are depicted by the sub-G1 peak (M1) of fragmented DNA in Fig. 10B. Treatment of cultured CEC with IL-11 reduced the number of apoptotic cells from 39 to 30%. These data indicate that IL-11 mediates antiapoptotic effects in CEC un-
nder conditions of detachment-induced or spontaneous epithelial cell death.

Effect of IL-11 Treatment on CEC Apoptosis in HLA-B27 Transgenic Rats—To determine whether IL-11 treatment has an effect on apoptosis in vivo, HLA-B27 rats (five per group), which spontaneously develop colitis, were treated with 33 μg/kg recombinant IL-11 subcutaneously every second day for 2 weeks. All analyses were performed blinded. The rats showed only a moderate degree of inflammation (histologic score, 2.4; range of score, 0–4). In contrast to recent publications (33, 34, 36), we did not detect a significant change in the histologic score (untreated, 2.4 ± 0.7; IL-11-treated, 2.3 ± 0.8) or weight change (untreated, −0.9 ± 2.0 g; IL-11-treated, +0.7 ± 8.0 g) in treated versus untreated animals despite a tendency to an improvement in the IL-11-treated group.

In IL-11-treated rats, the amount of apoptotic cells was significantly reduced compared with untreated rats as revealed by fluorescence-activated cell sorting analysis (Fig. 11A) (11.9 versus 5.9% of pre-G1 peak, p < 0.05). When a TUNEL assay was performed, the number of TUNEL-positive cells was generally low (Fig. 11B). Therefore, the number of apoptotic cells per 1000 CEC was calculated. The number of TUNEL-positive cells in IL-11-treated animals was reduced compared with untreated (p = 0.06) (Fig. 11B). Additional acridine orange staining also indicated higher rates of apoptosis in the CEC from

![Graph](image-url)
**Function of IL-11Ra in Colonic Epithelial Cells**

IL-11Ra was shown to be functional, since stimulation with its cognate ligand IL-11 induced Jak-STAT signaling. IL-11 stimulation was followed by a rapid and transient tyrosine phosphorylation of Jak1 and STAT3 in the cells analyzed. Since STAT3 protein phosphorylated at Tyr705 dimerizes, enters the nucleus, and induces transcription of target genes (7, 8, 19), signaling via the Jak-STAT pathway is a key event in IL-11-mediated gene regulation and downstream effects.

Whereas IL-11 induced a time- and concentration-dependent activation of STAT3 in colonic epithelial cells, the proinflammatory responses of HT-29 to TNF, such as IκBα degradation or IL-8 secretion, were not inhibited by this cytokine. Previous studies indicated IL-11 to be involved in the regulation of the production of proinflammatory cytokines in several cell types such as macrophages and activated T-cells (23, 25, 26). The beneficial effects of IL-11 in animal models of inflammation have been attributed to reduced mRNA levels of proinflammatory cytokines including IL-1β, interferon-γ, TNF, and IL-12 (34). These effects might have been mediated by the ability of IL-11 to inhibit NF-κB binding activity, a transcription factor pivotal to the inflammatory response (49). Transcription factors of the NF-κB family play an important role in the regulation of genes involved in inflammation. In IBD, proinflammatory cytokines known to be regulated by NF-κB are involved. Colonic epithelial cells as well as intestinal macrophages contain activated NF-κB, indicating an active role in the inflammatory process (50). However, the anti-inflammatory effects of IL-11 in animal models of endotoxemia also appear to be due to inhibition of proinflammatory mediators in macrophages (26). Our data do not support an anti-inflammatory effect of this cytokine in colonic epithelial cells by inhibiting nuclear translocation of NF-κB. A similar lack of anti-inflammatory effects of IL-11 has been shown in human vascular endothelium cells in which activation of IL-11 decreased immune-mediated injury (51).

Therefore, the salutary and cytoprotective effects on the barrier function of colonic mucosa by direct interaction with the colonic epithelial cells may be more important for the positive effects of IL-11 on the course of mucosal inflammation. One of the most important functions of epithelial cells in the human colon is the formation of a physiological barrier against antigens and potentially pathogenic organisms in the gut lumen (52–54).Remarkably, IL-11 exerts salutary effects on the barrier function of intestinal mucosa in a variety of animal models of IBD, after chemotherapy and radiation (29, 33–36, 55). The direct effects of IL-11 on intestinal epithelium are complex, including stimulation of crypt cell proliferation after injury and diminished apoptosis of enterocytes at higher levels on the crypt-villus axis (29). IL-11 can exert a potent effect on the recovery of the small intestinal mucosa of mice by its combined effects on proliferation and apoptosis of crypt cells. IL-11 may thus have potential clinical applications in limiting the intestinal toxicity that is associated with cytotoxic therapies. Therapeutic benefits of this IL-6-type cytokine have also been observed in several models of acute colitis and mucositis (33, 56, 57). In our studies, we were unable to assess any proliferative effects of IL-11 in colonic epithelial cells.

In our studies with HLA B27 transgenic rats, which spontaneously develop colitis and are regarded as a model for IBD, we could not find a significant effect of subcutaneously administrated recombinant IL-11 on the inflammatory score of the mucosa. This is in contrast to earlier publications, which reported that IL-11 therapy suppressed the symptoms of diarrhea, normalized myeloperoxidase activity, and healed mucosal injury (34, 36). This might be due to a relatively low inflammation present in the animals at the beginning of the experiment.

**Fig. 8.** Expression and regulation of Bcl-2 Protein in human colonic epithelial cells upon stimulation with IL-11. Cells were stimulated with different concentrations of IL-11 as indicated. After 24 h, cells were lysed, and Bcl-2 protein expression was investigated by Western blotting in HT-29 (A) and CEC (B). Detection of β-actin was used to verify equal loading of proteins.

**Fig. 9.** Akt serine phosphorylation upon IL-11 treatment in human colonic epithelial cells. Activated Akt was detected by Western blotting with an Ab specific to phosphorylated Akt (P-Akt) at serine residue 473. To confirm equal protein loading, each blot was reprobed with anti-Akt Ab (Akt). Dose-dependent effects on the phosphorylation of Akt induced by varying amounts of IL-11 for 30 min in HT-29 (A) and CEC (B) are shown.

untreated HLA-B27 transgenic rats (data not shown). However, since CEC showed clumping during the analysis, quantification of the amount of green fluorescent cells was not possible.

**DISCUSSION**

The recently generated IL-11Ra mAb E24.2 (43) allowed us to examine IL-11Ra expression in mucosal tissue. We demonstrate for the first time that IL-11Ra expression in the human mucosa is restricted to epithelial cells. No other cells of the lamina propria were markedly stained with this mAb in immunohistochemistry. We could further demonstrate constitutive IL-11Ra mRNA and cell surface protein expression in HT-29 and freshly isolated CEC, whereas IL-11Ra mRNA expression was absent in intestinal macrophages and myofibroblasts. In addition, we demonstrated co-expression of the signal-transducing subunit gp130 in colonic epithelial cells. The
As the targets of STAT3 activation in CEC are not fully understood, we further investigated effects of IL-11 on the apoptotic signaling cascade. The STAT transcription factor family has recently been reported to directly regulate the genes of the Bcl-2 family, which are key regulators of apoptosis (57). The levels of the antiapoptotic proteins of this family are increased in response to gp130-induced activation of STAT3, and IL-11 could thereby induce beneficial effects on the mucosal system by up-regulating antiapoptotic proteins. Members of the Bcl-2 family of proteins are known to be expressed in epithelial cells of the normal colonic crypts (59). Treatment with IL-11 induced a dose-dependent increase of the Bcl-2 protein level in CEC. Therefore, the up-regulation of the anti-apoptotic protein Bcl-2 could be considered as an important mechanism to protect colonic epithelial cells from cell death. Another key enzyme for regulating antiapoptotic events that

**Fig. 10. Antiapoptotic effects upon IL-11 stimulation in CEC.** CEC were treated with or without IL-11 (200 ng/ml) and kept in suspension by shaking to induce detachment-induced apoptosis. At the indicated time points, cells were lysed, and activation of caspase-9 was analyzed with a fluorometric assay (A). CEC were cultured for 24 h with or without IL-11 (200 ng/ml). Spontaneous apoptosis in adherent CEC was measured by flow cytometry after PI staining (B). The percentages of apoptotic cells are depicted by the sub-G1 peak (M1) of fragmented DNA.
is known to be essential in promoting cell survival is the serine/threonine kinase Akt (also known as protein kinase B) (60). Recently, phosphatidylinositol 3-kinase and subsequent Akt has been described to be activated by IL-6 and by certain other gp130 cytokines (61, 62). In the current study, we found that IL-11 stimulates Akt phosphorylation at Ser473 in colonic epithelial cells. Given the importance of Akt in inhibiting apoptosis, we hypothesize that this kinase is a critical target of IL-11-induced cytoprotective effects and mediates cell survival in CEC. Although previous studies do not indicate an activation of Akt by IL-11 in human umbilical vein endothelial cells (63), our data are consistent with the idea that IL-11-induced activation of STAT3 might regulate the expression of a different set of genes, depending on the cell type investigated.

Further, we examined the influences of IL-11 on the apoptotic signaling cascade. We found that IL-11 stimulation decreased the activation of caspase-9 and reduced spontaneous apoptosis in cultured CEC.

Despite the very moderate effect on mucosal inflammation, we found an effect on the number of apoptotic cells in vivo in HLA-B27 transgenic rats. An increase in the number of apoptotic CEC in IBD has been shown before (47), which may be more important in ulcerative colitis than in Crohn’s disease. Therefore, a decrease in CEC apoptosis may well be of importance in vivo and in patients with ulcerative colitis.

Another mechanism that could be involved in the protection of CEC resulting in decreased rates of apoptosis has been described recently (64). Ropeleski et al. (64) found an induction of heat shock protein 25 by IL-11 protecting CEC from oxidative stress. Since oxidative stress is known to induce apoptosis, this might be another mechanism besides Akt involvement contributing to the CEC protective effects of IL-11.

In summary, our results indicate functional expression of the IL-11Ra mainly on the epithelial cells within the human colon. IL-11 signals through activation of the Jak1-STAT3 pathway, without inducing anti-inflammatory or proliferative effects in colonic epithelial cells. The known beneficial effects of IL-11 therapy in animal models of bowel diseases are likely to be mediated by epithelial cells via activation of the Akt survival pathway, mediating antiapoptotic effects to ensure mucosal
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integration. In this study, we provided evidence of direct cytoprotective effects of IL-11 downstream to the activation of the transcription factor STAT3 in human colonic epithelial cells. We established a link between IL-11 treatment and antiapoptotic effects in CEC. Future clinical studies with IL-11 therefore should focus more on potential effects on mucosal restoration than on direct anti-inflammatory treatment effects.

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