Dose-Dependent Effects of Recombinant Human Interleukin-11 on Contractile Properties in Rabbit 2,4,6-Trinitrobenzene Sulfonic Acid Colitis

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ABSTRACT

We studied the effect of recombinant human interleukin-11 (rhIL-11), a cytokine with protective effects against injury to the intestinal mucosa, on inflammatory changes in the muscle layers of the gut, in rabbits with colitis. A single dose of rhIL-11 (4, 40, or 720 μg/kg) was given 1 h before colitis was induced with 135 mg/kg 2,4,6-trinitrobenzene sulfonic acid (TNBS), followed by a continuous s.c. administration of 4, 40, or 720 μg/kg · day rhIL-11 or saline for 5 days. Colitis affected mucosal architecture, general mechanical properties (passive tension increased with 12.3 g/mm², optimal stretch decreased with 26%), and collagen content (decreased from 366 ± 25 to 237 ± 13 μg/mg of protein). Changes in passive tension and collagen content were normalized by the highest and lowest dose of rhIL-11, respectively, but neither dose could normalize the optimal stretch. Colitis also decreased maximal contractile tension in response to acetylcholine (ACh), motilin, substance P (SP), K⁺, and prostaglandin E₂ but this was normalized with 40 μg/kg · day (motilin, SP) and 720 μg/kg · day (ACh, K⁺) rhIL-11 but not for prostaglandin E₂. For motilin and SP, receptor density was decreased in colitis and normalized in treated rabbits. Colitis also increased the contractile potency toward ACh, an effect already reversed by rhIL-11, 4 μg/kg · day. In conclusion, rhIL-11 partially normalizes disturbed tension generation in experimental colitis. The use of this cytokine in the treatment of irritable bowel disease may contribute to the restoration of motor dysfunction.

Interleukin (IL)-11 was originally discovered as the factor present in the supernatants of transformed stromal cells that stimulated the proliferation of IL-6-dependent plasmacytoma cells (Paul et al., 1990). In accordance with this finding, most studies of IL-11 have focused on its role in hematopoiesis (Du and Williams, 1994). IL-11 has, however, been shown to have a variety of other effects, including the ability to stimulate the acute phase response, to augment the production of metalloproteinase inhibitors, to stimulate the proliferation of hippocampal neuronal progenitor cells, to increase Ig production, to inhibit the genesis of adipocytes, to activate osteoclasts, to regulate proinflammatory cytokines (particularly tumor necrosis factor-α (TNF-α)], and to regulate epithelial cell proliferation (for review, see Du and Williams, 1997).

IL-11 treatment also has protective effects on intestinal mucosa. It has been shown to prevent or reverse mucosal damage caused by experimental ischemic bowel necrosis (Du et al., 1997) and burn in mice (Schindel et al., 1997). It was also effective in a rat short-bowel model (Liu et al., 1996) and in a hamster model of mucositis induced by chemotherapy (Sonis et al., 1995).

Recently, a protective effect of IL-11 was demonstrated in acute and chronic inflammatory models of colitis in rats. IL-11 reduced damage caused by intracolonic administration of acetic acid (Keith et al., 1994) and 2,4,6-trinitrobenzene sulfonic acid (TNBS; Qiu et al., 1996) and reduced the severity of colitis in HLA-B27 transgenic rats (Keith et al., 1994). In a rabbit model of endotoxemia, IL-11 treatment prevented damage of the intestinal mucosa induced by lipopolysaccharide (Misra et al., 1996).

The mechanism by which IL-11 reduces epithelial damage in these models of intestinal injury remains largely un-

ABBREVIATIONS: IL, interleukin; TNF-α, tumor necrosis factor-α; TNBS, 2,4,6-trinitrobenzene sulfonic acid; CD, Crohn’s disease; rhIL-11, recombinant human interleukin-11; ACh, acetylcholine; SP, substance P; PGE₂, prostaglandin E₂; IBD, irritable bowel disease; IL-RA, interleukin receptor antagonist; COX-2, cyclooxygenase-2; PCR, polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; UC, ulcerative colitis; NK, neurokinin.
known. Orazi et al. (1996) showed that IL-11 exerts its potent effect on the recovery of the small intestinal mucosa of mice, after treatment with chemotherapy and radiation, by increasing proliferation and by suppressing apoptosis of the crypt cells. Castagliuolo et al. (1997) found that the protective effect of IL-11 on the inflammatory diarrhea induced by Clostridium difficile toxin A in rats may be due to an inhibition of the release of inflammatory mediators from mucosal mast cells and macrophages. In accordance with this hypothesis, Trepicchio et al. (1996) demonstrated that IL-11 reduced the lipopolysaccharide-induced production of proinflammatory cytokines (TNF-α, IL-1β, IL-12, and interferon-γ) and of nitric oxide from macrophages. These observations have prompted further studies toward the clinical application of the protective effects of the cytokine against injury to the intestinal mucosa in Crohn’s disease (CD). It was reported recently that short-term treatment with recombinant human interleukin-11 (rhIL-11) is well tolerated and appears effective in inducing remission in a subset of patients with active CD (Sands et al., 1999).

Besides mucosal damage, inflammation is known to affect the deeper, neuromuscular layers of the gut wall as well, but no data are available on the effects of IL-11 on this process. We have recently shown that TNBS-induced colitis in rabbits changes the general mechanical properties of the smooth muscle but also decreases the contractile response of the strips by affecting receptor-mediated pathways (Depoortere et al., 1999). The aim of this study was to evaluate the effect of IL-11 on the inflammatory lesions and on the changes in contractile response toward receptor-specific [motilin, acetylcholine (ACh), substance P (SP), prostaglandin E2 (PGE2)] and receptor-independent (KCl) stimuli. ACh is the classical excitatory neurotransmitter in the gut. SP was chosen because it may act as a mediator of neurogenic inflammation during inflammatory bowel disease (IBD) and motilin because it is an important endocrine regulator of gastrointestinal motility. For motilin and SP the effect of IL-11 treatment on contractile activity was correlated with changes in receptor density. In addition the effect of IL-11 treatment on changes in the contractile response to an inflammatory mediator with direct contractile effects, PGE2, also was evaluated.

Materials and Methods

Experimental Design

Twenty-five New Zealand White rabbits of either gender (2.5−3 kg) were randomly divided into five groups of five animals each. In four groups, colitis was induced by TNBS and the animals were treated with saline, or 4, 40, or 720 μg·kg⁻¹·day recombinant human IL-11 (rhIL-11). The fifth group served as a control group. rhIL-11 was kindly provided by Dr. J. Keith from the Genetics Institute (Andover, MA).

Animal Treatment

One hour before the induction of colitis, rabbits received an s.c. injection of 4, 40, or 720 μg·kg⁻¹ rhIL-11 or saline. Rabbits were anesthetized and colitis was induced by intrarectal administration of a dialysis bag filled with 135 mg/kg TNBS (Fluka, Buchs, Switzerland) in 50% ethanol for 1 h according to the method described previously (Percy et al., 1993). During the anesthesia an osmotic pump (Alza Corporation, Palo Alto, CA) filled with rhIL-11 or saline was implanted s.c. to deliver rhIL-11 or saline continuously for 5 days. At the end of the treatment period rabbits were sacrificed. The distal colon was removed and rinsed with 0.9% NaCl. All procedures were approved by the Ethical Committee for Animal Experiments of the Belgian Ministry of Agriculture (approval number LA1210225).

Histological Evaluation of Colitis

Segments of the distal colon taken at 5 and 40 cm from the rectum were snap-frozen in 2-methylbutanate at −30°C. Cryostat sections (5 μm) were stained with H&E.

Determination of Total Collagen Content

Collagen content was determined by a colorimetric method (Coats et al., 1996). A piece of colon (100 mg) taken 5 cm from the rectum was hydrolyzed in 6 N HCl for 22 h at 110°C. The hydrolysate was lyophilized and dissolved in citric-phosphate buffer. Chloramine T (75 μl, 0.177 M) and Erlich’s reagent (75 μl) were added to the sample (50 μl) in the well of an enzyme-linked immunosorbent assay plate. Standards of hydroxyproline (0−50 μg/ml) were treated similarly. The enzyme-linked immunosorbent assay plate was incubated at 75°C for 16 min under constant shaking. The absorbance of the samples was then read at 550 nm. The protein content of the hydrolysates was determined according to the method of Lowry et al. (1951). The ratio of the weight of collagen/weight of hydroxyproline was considered to be 7.46. Results are expressed as micrograms collagen per milligram of protein.

Expression of TNF-α, IL-1β, IL-Receptor Antagonist (IL-RA), and Cyclooxygenase-2 (COX-2) by Semiquantitative Reverse Transcription-Polymerase Chain Reaction (PCR)

Total RNA was prepared from the mucosa and the muscle layer of the colon with TRIzol Reagent (Life Technologies, Grand Island, NY). Single-stranded cDNA was synthesized with 200 U of superScript II RNase H− reverse transcriptase (Life Technologies) and oligo(dT) (25 μg/ml; Life Technologies) as primer. The obtained cDNA served as a template for the PCR, consisting of 30 (TNF-α), 29 (IL-1β), 27 (IL-RA), and 32 cycles (COX-2) of amplification (95°C for 1’, 58°C for 1’, 72°C for 1’ with a final extension duration of 10’ at 72°C) and with 1.5 U of Taq DNA polymerase (Pharmacia Biotech, Uppsala, Sweden). Primers for rabbit TNF-α (forward: 5’-CCACCATCTAGAAAACCTGGA-C-G-3’, reverse: 5’-GCTGAGCCTCCCAAATATAC-3’), for rabbit IL-1β (forward: 5’-TGAAGACGTGTCCTCAGGAC-3’, reverse: 5’-TGCGCGAATCTCAAATTCCAG-3’), for rabbit IL-RA (forward: 5’-CTGGGGATTTAAACAGGAGG-3’, reverse: 5’-AGTGAACATTTGTTGACCCAG-3’), and for rabbit COX-2 (forward: 5’-CCTACAATCAAGAGCTGGG-3’, reverse: 5’-ATCAAACCAGCCGACCA-G-3’) were selected on the basis of the sequences published by Ito et al. (1986), Mori et al. (1988), Goto et al. (1992), and Guan et al. (1997), respectively. For semiquantitative assessment, primers of the rabbit housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH; forward: 5’-TGATCCATTGACCTCC-3’, reverse: 5’-TGGATTTCCACCCG-TACTC-3’; Applequist et al., 1995) were added. PCR products were analyzed on a 1.2% agarose gel, visualized with ethidium bromide, and photographed. The density of the bands was determined by image analysis with the ImageMaster 1D software (Pharmacia Biotech). The results are expressed as the ratio of intensity of the compound of interest to the intensity of the housekeeping gene.

Contraction Studies

Approximately 8 cm from the rectum, a piece of colon of 5 cm was removed. Circular strips of 0.2 × 2.5 cm were cut, freed from mucosa, and suspended along their circular axis in a tissue bath filled with HEPES buffer (11.6 mM HEPES, 137 mM NaCl, 5.9 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂, 11.5 mM glucose) at pH 7.4 and the response of the strips was measured either isotonically or isometrically as previously described (Depoortere et al., 1999).
Isotonic Measurements. Cumulative dose-response curves toward ACh (10^{-5}–10^{-4} M), motilin (10^{-7}–10^{-5} M), SP (10^{-10}–10^{-6} M), and PGE_{2} (10^{-9}–10^{-5} M) were established. Results are expressed relative to a supramaximal dose of ACh (10^{-4} M) added at the end of the dose-response curves. Calculations were performed with the WINDAQ/EX playback software (Dataq Instruments, Akron, OH). The pEC_{50} values were derived from the concentration-response curves by linear interpolation.

Isometric Measurements. Length-tension relationships of the strips were established. Muscle strips were stretched with 5% increments of the initial length to a maximum stretch of 100% of the initial length. The passive tension that developed in response to stretch was measured for each increment in length. A contraction was then elicited by addition to the bathing medium of either maximally effective doses of ACh (10^{-4} M), motilin (10^{-7} M), SP (10^{-7} M), or K^{+} (140 mM). This contraction, above the passive tension, was taken as the active tension. Optimal stretch (L_{o}) was defined as the degree of stretch that gave the maximum response to a contractile agent. The magnitude of the tension was expressed in grams and normalized for the cross-sectional area of the strip.

Receptor-Binding Studies

Motilin. Colonic smooth muscle tissue freed from mucosa and serosa was finely minced and homogenized in sucrose buffer with inhibitors. Binding of ^{125}I-Nle^{13}-porcine motilin was studied in washed 1000g fractions of the tissue homogenates as previously described (Bormans et al., 1986).

SP. The procedure of Burcher et al. (1986) was followed to determine SP binding. The supernatant of the 1,000g fractions used for motilin receptor-binding studies was further centrifuged at 48,000g and the pellet was resuspended in 50 mM Tris-HCl (pH 7.4, 10 mM EDTA, 300 mM KCl) for 1 h at 4°C. After centrifugation the pellets were washed and resuspended in incubation buffer (50 mM Tris-HCl, 300 mM KCl) for 1 h at 4°C. Attachment of the ligand to tissue homogenates was measured as described (Bormans et al., 1986).

Binding of ^{125}I-Nle^{13}-motilin was studied in tissue homogenates as previously described (Bormans et al., 1986).

Statistical Analysis

Data are represented as mean ± S.E. Dose-dependent effects of rhIL-11 were compared with one-way ANOVA. Specific comparisons were made by calculating appropriate \( t \) values. Significance was accepted at the 5% level.

**Results**

**Effect of rhIL-11 Treatment on Appearance of Colitis**

Induction of colitis caused a loss of body weight that amounted to 333 ± 28 g (12.5% of their initial weight). Treatment with rhIL-11 could not reverse this effect, in contrast the highest dose of rhIL-11 (720 µg/kg · day) significantly (\( P < .0005 \)) enhanced body weight loss to 483 ± 17 g compared with nontreated inflamed rabbits (Fig. 1). Inflammation increased the cross-sectional area of the distal colon (5 cm from the rectum) from 31.6 ± 2.6 to 66.2 ± 3.1 mm². A similar increase in cross-sectional area was observed in the second, more proximal part, of the distal colon (40 cm from the rectum). rhIL-11 treatment dose dependently decreased this effect to normal values in both parts (Fig. 1). However, in the more proximal part the effect was already normalized with the dose of 40 µg/kg · day rhIL-11, whereas in the distal part a dose of 720 µg/kg · day was needed.

**Histology.** Figure 2 shows an H&E staining of colonic sections from inflamed rabbits treated with saline, 4 µg/kg · day, 40 µg/kg · day, or 720 µg/kg · day of rhIL-11. The sections of rabbits treated with saline and with the lowest dose of rhIL-11 show a loss of mucosal haustration and folding and a distortion of the architecture of the mucosa. The lamina propria cellular infiltrate is increased in intensity and is transmucosal in location. Also apparent in these sections is the submucosal edema and the segmentation of the circular muscle layer. These characteristics resemble those previously described in inflamed rabbits (Depoortere et al., 1999). Treatment of rabbits with 40 µg/kg · day rhIL-11 restores the haustration and folding of the mucosa but increases the height of the crypts. There is still evidence of an increased cellular infiltrate, basal in location, and of submucosal edema. However, this dose of rhIL-11 restores the smooth muscle layer to a normal continuous layer. Treatment with the highest dose of rhIL-11 results in histological sections with a normal appearance.

**Collagen Content.** Collagen content in tissue from non-inflamed rabbits was 366 ± 25 µg/mg of protein. Inflammation significantly (\( P < .0005 \)) decreased collagen content to 237 ± 13 µg/mg of protein. Collagen content was already normalized by the lowest dose of rhIL-11 (Fig. 3).

**Inflammatory Mediators.** TNBS colitis increased the expression of COX-2, IL-1β, and IL-RA mRNA in the colon (Fig. 4). IL-1β and IL-RA mRNA were increased 4.3- and 7.9-fold, respectively, in the mucosa but not COX-2 mRNA. In the muscle layer COX-2 expression was increased 2.3-fold and a small but significant increase also was observed for IL-1β and IL-RA. In contrast, inflammation induced a small decrease of TNF-α mRNA expression in both the colonic mucosa and muscle layer. During rhIL-11 treatment COX-2 mRNA and IL-1β expression remained increased in the muscle layer and the mucosa, respectively, whereas IL-RA mRNA expression was further enhanced in the mucosa (Fig. 4). Both IL-1β and IL-RA mRNA expression disappeared in the muscle layer, whereas TNF-α mRNA levels were normalized during rhIL-11 treatment.

**Effect of rhIL-11 Treatment on Contractile Properties of Colitic Strips**

**Passive Tension.** In inflamed colonic strips passive tension was increased from 4.2 ± 0.9 to 16.5 ± 6.5 g/mm². This
Increased tension was normalized by 720 μg/kg · day but not by 4 or 40 μg/kg · day rhIL-11 (Fig. 5).

**Length-Tension Relationships to ACh, Motilin, SP, and KCl.** As reported previously (Depoortere et al., 1999), inflammation markedly affected the length-tension relationship. Maximal tension, and the stretch required to reach it, were markedly reduced. Treatment with rhIL-11 reversed the effect on maximal tension but not on stretch and this was observed for ACh (10^{-4} M), motilin (10^{-7} M), SP (10^{-7} M), and KCl (140 mM) but not for PGE_{2}.

As an example, Fig. 6 shows the results obtained with ACh. In inflamed strips, maximal active tension ($T_{\text{max}}$) was decreased from 13.4 ± 1.0 to 5.1 ± 0.6 g/mm², and the optimal stretch ($L_{o}$) needed to achieve it was reduced from 62 ± 6 to 46 ± 5%. The decreased $T_{\text{max}}$ could be normalized by treatment with 720 μg/kg · day rhIL-11 but neither dose of rhIL-11 could shift the decreased $L_{o}$ toward control values.

Similar length-tension relationship curves were obtained if motilin, SP, or KCl were used as contractile agents. Figure 7 summarizes the changes in optimal stretch needed to induce maximum active tension in response to the different contractile stimuli during treatment with rhIL-11. For all compounds, the optimal stretch remained decreased with all doses of rhIL-11 studied. As in the case of ACh, maximal active tension generated in response to motilin, SP, and KCl could be normalized by rhIL-11 treatment; however, the dose of rhIL-11 needed to restore the maximal active tension was dependent on the contractile agent used (Fig. 8). For motilin
and SP, T_max was normalized after treatment with 40 μg/kg • day rhIL-11, whereas for ACh and KCl a dose of 720 μg/kg • day rhIL-11 was needed.

**Contractile Potency.** Inflammation increased the contractile potency (pEC50) for ACh from 5.90 ± 0.12 to 6.43 ± 0.08 and already the smallest dose of rhIL-11 normalized the increased affinity for ACh. No changes were observed of the potencies for motilin and SP (Table 1).

**Effect on Response to PGE2.** In noninflamed tissue, the response to PGE2 consisted of a small contraction amounting to 11 ± 4% of the response to a supramaximal dose of ACh, followed by a relaxation of the contraction by 22 ± 3%. In inflamed tissue the contraction was not significantly affected but the relaxation was reduced to 4 ± 2%. rhIL-11 treatment could not reestablish the PGE2-induced relaxation.

**Effect of rhIL-11 Treatment on Motilin and SP Receptor Density**

For motilin and SP the changes in maximal active tension after treatment with rhIL-11 were related to changes in receptor density. Inflammation per se decreased motilin receptor density and maximal active tension by 71% (from...
Effect of rhIL-11 treatment (\(\mu g/kg\) · day) of inflamed rabbits on the contractile potency (\(pEC_{50}\) values) calculated from contraction dose-response curves in response to ACh, motilin, and SP.

<table>
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<tr>
<th></th>
<th>ACh</th>
<th>Motilin</th>
<th>SP</th>
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<tbody>
<tr>
<td>Control</td>
<td>5.90 ± 0.12</td>
<td>8.13 ± 0.13</td>
<td>8.23 ± 0.18</td>
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<tr>
<td>TNBS</td>
<td>6.43 ± 0.08*</td>
<td>8.03 ± 0.17</td>
<td>8.08 ± 0.12</td>
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<tr>
<td>4 (\mu g/kg) · day rhIL-11</td>
<td>6.18 ± 0.21</td>
<td>8.11 ± 0.09</td>
<td>8.45 ± 0.25</td>
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<tr>
<td>40 (\mu g/kg) · day rhIL-11</td>
<td>6.07 ± 0.16</td>
<td>7.95 ± 0.12</td>
<td>7.99 ± 0.05</td>
</tr>
<tr>
<td>720 (\mu g/kg) · day rhIL-11</td>
<td>5.72 ± 0.07</td>
<td>7.99 ± 0.08</td>
<td>7.86 ± 0.27</td>
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* \(P < .005\) versus control.

Fig. 8. Maximum active tension (\(T_{max}\)) generated by inflamed colonic strips after treatment with increasing doses of rhIL-11 (\(\mu g/kg\) · day) in response to 10\(^{-5}\) M ACh (\(\bullet\)), 140 nM KCl (\(\circ\)) (left), 10\(^{-7}\) M motilin (\(\square\)), and 10\(^{-7}\) M SP (\(\triangle\)) (right). Results are mean ± S.E. of five determinations. \(*P < .05\), \(**P < .0005\) versus control.

Fig. 9. Effect of saline or rhIL-11 treatment (\(\mu g/kg\) · day) of inflamed rabbits, in comparison with control rabbits, on motilin receptor density (\(B_{max}\), fmol/mg of protein; \(\square\)) and maximal active tension (g/mm²; line) induced by motilin. Points are mean ± S.E. of five rabbits. \(*P < .05\), \(**P < .005\).

128 ± 21 to 37 ± 7 fmol/mg of protein) and 58% (from 9.1 ± 0.6 to 3.8 ± 0.7 g/mm²), respectively (Fig. 9). Treatment with 4 \(\mu g/kg\) · day rhIL-11 was without effect, but 40 \(\mu g/kg\) · day rhIL-11 normalized both motilin receptor density and maximal active tension. Motilin receptor affinity was not affected by the inflammatory process nor by rhIL-11 treatment.

For SP, inflammation decreased the density of the high-affinity receptor-binding site from 13.0 ± 2.9 to 1.6 ± 0.8 fmol/mg of protein and increased the affinity (\(pK_d\)) from 10.04 ± 0.08 to 10.83 ± 0.06. In contrast the affinity for the low-affinity receptor-binding site was not affected (\(pK_d\): 8.00 ± 0.28 (control) versus 8.40 ± 0.18 (TNBS)) but the receptor density also was decreased [522 ± 214 (control) versus 24 ± 11 (TNBS) fmol/mg of protein]. Binding parameters were restored after treatment with 40 \(\mu g/kg\) · day of rhIL-11, in parallel with the maximal active tension induced by SP (Fig. 10).

Discussion

We investigated the effect of treatment with rhIL-11 on the inflammatory changes caused by TNBS-induced colitis on the neuromuscular layer of the colonic wall in rabbit. rhIL-11 improved the development of active tension toward contractile agents, which is decreased by inflammation. The dose of 40 \(\mu g/kg\) · day normalized the response to motilin and SP, and the highest dose (720 \(\mu g/kg\) · day) the response to ACh and KCl. The response to PGE2 was not normalized by rhIL-11. For motilin and SP changes in tension paralleled changes in receptor density. rhIL-11 also normalized the increased contractile potency to ACh. However, rhIL-11 was unable to ameliorate markedly the effect of inflammation on the general mechanical properties of the strips, despite the fact that collagen content was normalized by the lowest dose of rhIL-11. The increased passive tension was only reduced with the highest dose (720 \(\mu g/kg\) · day) of rhIL-11, whereas neither dose affected the reduced optimal stretch.

We also monitored mucosal parameters and inflammatory mediators to document the inflammatory process. Earlier studies have shown that rhIL-11 prevents mucosal damage in several inflammatory models and in agreement with these studies we found that rhIL-11 treatment restored the normal mucosal architecture. It was noted that rhIL-11 treatment was not able to overcome the loss of body weight induced by inflammation. In fact, the effect was enhanced with the highest dose of rhIL-11. This is in contrast to studies in hamsters...
and rats where IL-11 treatment diminished body weight loss induced by chemotherapy-induced mucositis and by small bowel resection, respectively (Sonis et al., 1995; Liu et al., 1996). Weight loss is probably caused by a lack of appetite, and TNF-α and ILs are established mediators of inflammation-induced anorexia (Plata-Salaman et al., 1988). However, because TNF-α mRNA expression was decreased in rabbits with TNBS colitis it is unlikely that TNF-α is the major inducer of the weight loss and rhIL-11 treatment normalized the decrease but did not further enhance it. IL-1β expression was increased by inflammation and remained increased during rhIL-11 therapy, but IL-RA, which may counteract the effect of IL-1β, also was increased and enhanced by rhIL-11 therapy, so that a role of IL-1β seems unlikely. The more pronounced weight loss observed with the highest dose of rhIL-11 is therefore unexplained and requires further study. It was shown by Plata-Salaman (1996) that IL-6 subfamily members, including IL-11, which are activators of the signal transducer gp 130, may act centrally to decrease feeding. Another hypothesis is that rhIL-11 might increase leptin levels as has been reported in vivo for IL-1β and TNF-α (Ballinger, 1999).

Inflammation induced an increase in passive tension, which opposes and restores the original length of the muscle after release. Changes in the extracellular matrix (edema, collagen, elastin fibers, proteoglycans) to which smooth muscle fibers are attached may contribute to changes in passive tension but in this study the decrease in collagen content was already normalized by the lowest dose of rhIL-11, whereas passive tension was only restored by the highest dose. Thus, collagen content does not seem to be the major factor determining the change of passive tension. However, our histological observations show that the increased edema only disappears with higher doses of rhIL-11 and therefore parallels the changes in passive tension. Edema is caused by increased vascular permeability and this may be related to an increase in inducible nitric oxide (Boughton-Smith et al., 1993; Miller et al., 1995; Ribbons et al., 1995). Because IL-11 can reduce the production of nitric oxide, also in rabbits (Misra et al., 1996; Trepicchio et al., 1986), through inhibition of nuclear factor-κB-dependent transcriptional activation (Trepicchio et al., 1997), this may be the mechanism responsible for the gradual decrease in edema and restoration of passive tension with increasing doses of rhIL-11.

It may be noted that a decrease in collagen content also has been described in the rectal mucosa from patients with ulcerative colitis (UC; Hendel et al., 1986; Graham et al., 1988), whereas in the ileum of patients with CD the opposite has been found (Graham et al., 1988). Changes in collagen content may be caused by either changes in collagen production or changes in collagen degradation. Interestingly, Matthes et al. (1992) demonstrated by in situ hybridization that the RNA transcript levels of procollagen types I, III, IV, and V were higher in UC compared with CD, yet the collagen deposition, as determined by immunohistology, was lower in UC than in CD. This points to a different metabolism of procollagen in UC and CD. Although IL-11 is a fibroblast-derived cytokine not much is known about its effect on collagen metabolism. Our study suggests that IL-11 increases collagen deposition because the decrease in collagen content caused by colitis was normalized by rhIL-11. This is in agreement with the observation that targeted expression of IL-11 in the mouse airway causes airway remodeling with increased types III and I collagen and local accumulation of fibroblasts (Tang et al., 1996). One way in which IL-11 could favor collagen deposition is by inducing the production of tissue inhibitors of metalloproteinases, the enzymes involved in collagen catabolism (Maier et al., 1993). This topic also deserves further study because the opposite effects on collagen content observed in patients with IBD may explain why in UC the bowel has the tendency to dilate and perforate, whereas structure formation is more common in CD.

Maximum tension occurred at smaller degrees of stretch in inflamed tissue. Optimal stretch is also a function of the elastic components of the muscle, the connective tissues, and the contractile filament content. rhIL-11 apparently did not restore the changes in these parameters.

The decreased maximal active tension to motilin and SP was normalized by treatment with 40 μg/kg · day rhIL-11. This dose also restores the continuity of the smooth muscle layer so that an optimal contact between neighboring smooth muscle cells is reached. However, changes in tension also were reflected by changes in motilin and SP receptor density, indicating effects at the receptor level and suggesting the presence of a cytokine response element in the promoter region of the motilin and SP receptor. For motilin the receptor affinity was not affected, but for SP the affinity for the high-affinity binding site was increased. These sites probably do not represent different subtypes of SP receptors because the binding can only be displaced by SP and the neurokinin (NK1 agonist [βAla4,Sar9,Met(O2)11]-SP(4-11) but not by the NK2 agonist [βAla8]-neurokinin A (4-10) or NK3 agonist [MePhe7]-neurokinin B (our unpublished data). Furthermore, Koelbel et al. (1989) have shown that the myogenic response of the distal colon of the rabbit appears to be mediated through NK1 receptors. It is rather likely that the high- and low-affinity binding site represent, respectively, neural and smooth muscle SP receptors. In rats with TNBS colitis SP receptors also are down-regulated (Evangelista et al., 1996), but in patients with IBD, SP receptors in the circular muscle are unaffected but are up-regulated in the lymphoid aggregates, small blood vessels, and myenteric neurons (Mantyh et al., 1995).

The response to KCl, which acts independent of a receptor, also was normalized by rhIL-11, suggesting that rhIL-11 interferes with the contractile apparatus itself. A higher dose of rhIL-11 was needed to reestablish the response to ACh and KCl than to motilin and SP, suggesting that general smooth muscle damage is not the main cause for the decreased contractility. However, the lowest dose of rhIL-11 already normalized the increased contractile potency to ACh. That different doses are required to restore different parameters may be due to the fact that factors involved in receptor regulation, transduction, or Ca2+ handling properties have a different susceptibility to IL-11, but are of different importance for the respective contractile agents. Furthermore, the magnitude of the tension that has to be restored is higher for ACh and KCl than for motilin and SP.

Whether the effects of rhIL-11 represent direct effects or involve other inflammatory mediators is not known. It has been shown that in rabbits with TNBS colitis PGE2 levels are significantly increased within 6 h of exposure to TNBS and remain elevated for at least 10 days (Mellman et al., 1990). We found that COX-2 mRNA expression was increased in the colonic...
muscle layer of inflamed rabbits and was not affected by rhIL-11 treatment. Desensitization may therefore be responsible for the reduced contractile response to PGE₂ observed in our study. Also in rats the protective effects of rhIL-11 on colonic damage induced by TNBS are not mediated by actions on the eicosanoid metabolism (Qiu et al., 1996). In the HLA-B27 rat, rhIL-11 treatment reduces clinical signs and histological lesions of the colon that are associated with down-regulation of the expression of inducible nitric oxide synthase, IL-6, transforming growth factor-β, and of multiple proinflammatory cytokines, including IL-12 p40, interferon-γ, IL-1β, TNF-α, and IL-1α (Peterson et al., 1998). In this study we demonstrated that in the colonic mucosa IL-RA expression was enhanced by rhIL-11 treatment and that in the colonic muscle layer IL-1β and IL-RA mRNA levels disappeared during therapy, supporting a role for IL-1β in the impaired contractility observed during colitis but not for TNF-α, which was investigated by the inflammatory process. It remains to be investigated which other cytokines are involved.

In conclusion, rhIL-11 partially normalizes changes in contractile response toward receptor-specific (motilin, ACh, SP) and receptor-independent (KCl) stimuli. These findings suggest that this cytokine may contribute to the restoration of motor dysfunction in patients with IBD.

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References


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