Recombinant Human Interleukin-11 Restores Smooth Muscle Function in the Jejunum and Colon of Human Leukocyte Antigen-B27 Rats with Intestinal Inflammation

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ABSTRACT

Recombinant human interleukin (rhIL)-11 has anti-inflammatory and protective effects in models of intestinal mucosal injury. Our aim was to investigate whether oral treatment with rhIL-11 reverses functional abnormalities in intestinal muscle contractility resulting from human leukocyte antigen (HLA)-B27-dependent gut inflammation. Isometric contractions were studied in jejunal and colonic longitudinal muscles. Muscle strips were isolated from HLA-B27 transgenic rats with spontaneous inflammation of the intestine mediated by activation of immunocytes and the release of inflammatory mediators. Inflammation of the intestine mediated by activation of immunocytes and the release of inflammatory mediators has been altered to induce an abnormal immunoresponse. The discovery that interleukins and growth factors may have a critical role in the pathogenesis of IBD led to the search for more specific therapies using antibodies to pro-inflammatory cytokines and growth factors or anti-inflammatory cytokines manufactured by recombinant gene technology. Recombinant human IL-11 (rhIL-11) has exhibited potent anti-inflammatory activity in a number of animal models, including a rat model of gastrointestinal inflammation (Peterson et al., 1998). A therapy based on subcutaneous administration of rhIL-11 supplementing the continuous oral application of aminosalicylates and corticosteroids during 3 weeks of treatment showed clinical benefit and was well tolerated in patients with active Crohn's disease (Sands et al., 1999). Most recently, rhIL-11 was developed as an enteric-coated multiparticulate formulation for oral dosing in patients with IBD.

IL-11 is a 19-kDa monomer, originally detected in the medium of a bone marrow stromal cell line and cloned from human fetal lung fibroblasts (Paul et al., 1990, 1991). IL-11 belongs to a family of cytokines including IL-6, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, and cardiotrophin 1, which show overlapping effects mediated by the common signal-transducing gp130 receptor subunit. Initially characterized by its potent hematopoietic activity, IL-11 was also defined as an anti-inflammatory cytokine inhibiting the production of inflammatory mediators, such as TNF-α, IL-1β, and nitric oxide (Trepicchio et al., 1996, 1997; Castagliulo et al., 1997), and acting directly to reduce proliferation of gastrointestinal epithelial cells (Peter-

Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory disease of the intestine mediated by activation of immune cells and the release of inflammatory mediators. Increased tissue production of interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) has been found during the episodes of active IBD in patients with ulcerative colitis or Crohn's disease (Isaacs et al., 1992), indicating that the balance between pro-inflammatory and immunosuppressive mediators has been altered to induce an abnormal immune response. The discovery that interleukins and growth factors may have a critical role in the pathogenesis of IBD led to the search for more specific therapies using antibodies to pro-inflammatory cytokines and growth factors or anti-inflammatory cytokines manufactured by recombinant gene technology. Recombinant human IL-11 (rhIL-11) has exhibited potent anti-inflammatory activity in a number of animal models, including a rat model of gastrointestinal inflammation (Peterson et al., 1998). A therapy based on subcutaneous administration of rhIL-11 supplementing the continuous oral application of aminosalicylates and corticosteroids during 3 weeks of treatment showed clinical benefit and was well tolerated in patients with active Crohn's disease (Sands et al., 1999). Most recently, rhIL-11 was developed as an enteric-coated multiparticulate formulation for oral dosing in patients with IBD.

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ABBREVIATIONS: IBD, inflammatory bowel disease; IL, interleukin; TNF, tumor necrosis factor; rhIL-11, recombinant human IL-11; HLA, human leukocyte antigen; MPO, myeloperoxidase; EFS, electrical field stimulation; CSA, cross-sectional area; NANC, nonadrenergic, noncholinergic.
son et al., 1996). Consequently, the effects of treatment with rhIL-11, produced by DNA technology in Escherichia coli, have been studied in vivo in a variety of models of gut damage. Treatment with rhIL-11 proved to be protective in models of radiation-induced mucositis, reducing mortality due to sepsis following severe gastrointestinal mucosal damage (Du et al., 1994). A cytoprotective effect of rhIL-11 on gastrointestinal mucosal injury has been found in rat models of TNBS-induced colitis (Qiu et al., 1996) or ischemic bowel necrosis (Du et al., 1997). Furthermore, in HLA-B27 transgenic rats with chronic colitis, rhIL-11 treatment caused significant amelioration of the symptoms of diarrhea, decreased tissue inflammation, and improved the histology in the colon (Keith et al., 1994; Peterson et al., 1998; Greenwood-Van Meerveld et al., 2000). The beneficial effects of rhIL-11 in the HLA-B27 model of colitis coincided with down-regulated expression of the proinflammatory cytokines TNF-α, IL-1β, and interferon-γ (Peterson et al., 1998).

In patients with IBD, gut inflammation is associated with intestinal muscle dysfunction (Snape et al., 1991; Vermillion et al., 1993) and increased mass of muscle cells and collagen in the intestinal wall (Graham et al., 1988). These observations have been confirmed in a variety of animal models of experimental intestinal inflammation (Grossi et al., 1993; Martinolle et al., 1997), showing that smooth muscle dysfunction is linked to the inflammatory reaction and can be prevented by corticosteroid therapy. In transgenic rats expressing human leukocyte antigen (HLA)-B27 and microglobulin β2, intestinal inflammation is associated with decreased contractility of the longitudinal muscle in both the small and large intestine (Venkova et al., 2000). Subcutaneous administration of rhIL-11 to HLA-B27 rats suppressed the intestinal inflammation and repaired mucosal damage in the colon (Keith et al., 1994; Peterson et al., 1998). However, it is unclear whether the suppression of inflammation correlates with a recovery of normal intestinal muscle function. The aim of the present study was 2-fold: 1) to prove the efficacy of a more convenient oral administration of an enteric-coated formulation of rhIL-11 in reducing intestinal inflammation and ameliorating the symptoms of IBD; and 2) to establish whether oral administration of rhIL-11 to HLA-B27 transgenic rats stimulates the recovery of normal intestinal muscle contractility. The effects of a 2-week therapy with enteric-coated rhIL-11 multiparticulates (equivalent to 500 μg/kg rhIL-11 or placebo multiparticulates) administered orally at 48-h intervals were investigated. The dose and time course of treatment were based on preliminary data showing that rhIL-11 suppresses the diarrhea and heals the inflammation in the colon of HLA-B27 rats with chronic intestinal inflammation.

Materials and Methods

Experimental Animals. Fisher 344 rats genetically engineered to carry high-copy numbers of the human major histocompatibility complex class 1 allele B27 and β2-microglobulin genes were used in this study. Male transgenic HLA-B27 rats were purchased from Taconic Farms (Germantown, NY) and were housed individually under controlled conditions (21°C; 50 ± 10% humidity; 12-h light/dark cycle). The HLA-B27 rats were obtained at 10 weeks of age and were housed in the animal facility until the age of 40 weeks. Fisher 344 rats were obtained from Charles River Laboratories Inc. (Wilmington, MA) and served as nontransgenic controls. The Fisher 344 rats were purchased at 36 weeks of age and were housed in the animal facility under the same conditions for an additional 3 to 4 weeks. Prior to the experiments, no significant difference was found between the body weight of the HLA-B27 transgenic rats (350 ± 40 g, n = 12) and the age-matched F344 control rats (370 ± 20 g, n = 6). All animals were fed a standard laboratory diet and showed normal eating and drinking habits. The experimental protocol used in the study was approved by the Oklahoma City Veterans Affairs Medical Center Animal Care Committee.

Administration of rhIL-11. Experiments were designed to study the effects of oral administration of enteric-coated rhIL-11 multiparticulates to HLA-B27 transgenic rats with chronic gastrointestinal inflammation. In the present experiments, rhIL-11 multiparticulates were coated with an enteric polymer that dissolves at pH < 5.5, ensuring that the active compound passes the stomach and is released in the intestine. The test compound contained approximately 1 mg of rhIL-11 per 100 mg of multiparticulates, whereas sucrose multiparticulates served as placebo controls. The cumulative effect of single oral doses of enteric-coated rhIL-11 multiparticulates equivalent to 500 μg/kg rhIL-11 was followed during the 2 weeks of treatment by observing the symptoms of diarrhea. The dose and time course of treatment were selected based on preliminary experiments using subcutaneous administration of 33 μg/kg (Greenwood-Van Meerveld et al., 2000) or oral administration of 330 μg/kg nonformulated rhIL-11 (unpublished observations). With both dosing regimens, although rhIL-11 significantly decreased intestinal inflammation and improved stool consistency, neither of the treatments was sufficient for the complete normalization of stool consistency and the full recovery of intestinal epithelial and smooth muscle function. Thus a higher oral dose of 500 μg/kg given on alternate days during 2 weeks was selected in the present study. The intestinal inflammation, histological damage, and muscle contractility were studied at the end of the treatment period. Three groups of animals were involved in the study: a test group consisting of HLA-B27 rats (n = 6) treated with rhIL-11; the vehicle-control group consisting of HLA-B27 rats (n = 6) treated with placebo; and a healthy control group consisting of age-matched F344 rats (n = 6) treated with placebo. The animals were weighed daily during the 2 weeks of oral administration of rhIL-11, and there was no significant change in body weight induced by either rhIL-11 or the placebo. All animals were euthanized 4 h after the last administration of rhIL-11 or placebo, and the jejunum and colon were isolated immediately.

Evaluation of Stool Character. All HLA-B27 rats showed clinical symptoms of colitis. The stool character was observed daily and characterized as normal, soft, or diarrhea. Scores of 0 for normal, 1 for soft with pellets formed, 2 for soft with no pellet formation, and 3 for diarrhea, were given daily before and during treatment of HLA-B27 rats with rhIL-11 or placebo. Average daily scores were calculated to characterize stool consistency.

Intestinal Inflammation. Myeloperoxidase (MPO), specifically expressed by neutrophils, is considered a marker of inflammatory cell infiltration. Our previous observations in HLA-B27 rats showed that treatment with rhIL-11 results in a decrease in MPO activity accompanied by normalization of mucosal histology in the jejunum and colon (Keith et al., 1994; Greenwood-Van Meerveld et al., 2000). Thus, the activity of MPO in intestinal tissue extracts was used as an index of inflammation in the present study. Full-thickness jejunal and colonic samples (100–150 mg) were taken from the tissue isolated for the contractile experiments and were immediately frozen in liquid nitrogen. The samples were stored at −80°C and MPO activity was assayed simultaneously for the whole set of experiments. Homogenization and extraction of MPO from the homogenate were carried out in hexadecyl-trimethylammonium bromide phosphate buffer (pH 6) according to a modification of the procedure described by Castro et al. (1974). MPO activity was tested in 10-μl samples using 3,3’-5,5’-tetramethyl benzidine Microwell peroxidase substrate system (Sigma Chemical Co., St. Louis, MO) and horseradish peroxidase as a relative standard. MPO activity was expressed as equiv-
alent to the activity of the relative standard (nanograms of horse-
radish peroxidase) converting the same amount of 3,3’,5,5’-
tetramethylbenzidine substrate for 10 min at room temperature. The
data were expressed in nanograms and normalized per gram wet
weight of the tissue.

Histological Evaluation. Jejunal and colonic tissue samples
were harvested from HLA-B27 rats following the oral administration
of rhIL-11 or placebo. The specimens were immersed in 10% neutral-
buffered formalin, processed, embedded in paraffin, and sectioned at
5-μm thickness. Slide-mounted sections were stained with hematoxy-
ilin and eosin and investigated by light microscopy for the presence
of ulceration, inflammatory infiltrates, transmural lesions, and fi-
brosis. The slides were examined in a blinded fashion, and each
parameter was scored as follows: 0 to 2 for ulceration and fibrosis; 0
to 3 for inflammation and depth of lesions. The absence of pathology
was scored as zero. A total score was calculated according to the
method described by Boughton-Smith et al. (1988) as the sum of
the scores of individual parameters (maximum was 10).

Intestinal Muscle Contractility. Segments of the jejunum (ap-
proximately 5 cm distal to the ligament of Treitz) and the colon
(approximately 4 cm distal to the ileocecal junction) were harvested
and placed in ice-cold oxygenated Krebs’ bicarbonate solution. Lon-
gitudinal muscle strips were dissected from the intestinal segments
by gently peeling the muscle in longitudinal direction. Muscle strips
(10–12 mm long) were excised following the direction of the muscle
with the help of a dissecting microscope, and both ends were secured
with silk surgical suture (size 3–0). The strips were mounted verti-
cally in 10-ml organ baths with one end fixed and the other attached
to an isometric force transducer (Radnoti Glass Technology Inc.,
Monrovia, CA). The baths were filled with Krebs’ bicarbonate solu-
tion, maintained at 37°C and aerated with 95% O2 and 5% CO2. The
solution was changed by perfusion at 30-min intervals. Each smooth
muscle strip was allowed to equilibrate at zero tension for 20 min,
followed by consecutive loading with 0.20g force increments until a
level of optimal resting tension was achieved. Resting tension was
considered optimal when the contractile response to 80 mM KCl
closed to increase with loading. Strips were allowed an additional 20
min of equilibration. All experiments were performed at optimal
tension and isometric contractions were recorded using a MacLab
data acquisition system (AD Instruments Ltd., Castle Hill, Aus-
tralia).

Solutions and Drugs. The modified Krebs bicarbonate solution
contained (in mM): NaCl 120, KCl 6, MgCl2 1.2, NaH2PO4 1.2, CaCl2
2.5, NaHCO3 14.4, and glucose 11.5. The solution was continuously
gassed with 95% O2 and 5% CO2 (v/v), and the pH ranged from 7.2 to
7.3. Carbamylcholine chloride, atropine sulfate, guanethidine, and
tetrodotoxin were obtained from Sigma Chemical Co. and were dis-
solved in distilled water. All drugs were added to the baths in
volumes less that 1% of the total bath volume. The enteric-coated
formulation of rhIL-11 and the enteric-coated sucrose multiparticu-
lates (placebo) were kindly supplied by Wyeth/Genetics Institute,
Inc. (Andover, MA).

Data Analysis and Statistics. Contractions induced by high
KCl, carbachol, or electrical field stimulation (EFS) were measured
as changes in basal tension (mN) and normalized per mm2 of cross-
sectional area (CSA) for each muscle strip. The CSA was calculated
using the following equation: CSA (mm2) = tissue wet weight (mg)/
tissue length (mm) × tissue density (mg/mm3). The tissue length was
measured at optimal tension at the beginning of each experiment,
while tissue wet weight was measured upon completion of the exper-
iment. Specific smooth muscle tissue density was assumed to be
1.05 mg/mm3 (Gordon and Siegman, 1971). Complete concentration-
response curves to carbachol were used to evaluate maximal choliner
tic tension and sensitivity of smooth muscle muscarinic cholinore-
ceptors. The concentration of carbachol producing 50% of the
maximal effect (EC50) was calculated by regression analysis of the
linear portion (20–80% of maximal effect) of the curve. Differences
between regression lines (slope and position) were assessed by anal-
ysis of covariance of multiple regression lines (Kenakin, 1993).

The results were expressed as mean ± S.E.M. for each of the
groups. A maximum of two preparations isolated from one animal
was used in individual experiments. Prior to selecting a statistical
test for independent group comparison, the data were subjected to
assumption testing of normality and equal variances to define
whether a parametric or a nonparametric test was required. Differ-
ences between mean values obtained in placebo-treated Fisher 344,
placebo-treated HLA-B27 rats, and HLA-B27 rats treated with
rhIL-11 were assessed for statistical significance using one way
analysis of variance followed by a two-tailed unpaired t test or an
alternative Mann-Whitney rank sum test where appropriate. Statis-
tical significance was inferred when p < 0.05. Computer analysis of
the data was performed using StatView software (SAS Institute Inc.,
Cary, NC).

Results

Effect of rhIL-11 Treatment on Chronic Diarrhea in
HLA-B27 Rats. The HLA-B27 rats developed spontaneous
inflammation with age. At the age of 40 weeks, the trans-
genic rats had intestinal inflammation manifested by chronic
diarrhea. Age-matched nontransgenic Fisher 344 rats ap-
peared to be healthy, and the stool consistency was normal.
Loose stools without pellet formation and diarrhea were ob-
erved in all HLA-B27 rats prior to administration of rhIL-
11. Oral administration of rhIL-11 resulted in significant
inhibition of the symptoms of diarrhea, i.e., following the first
9 days of treatment the stool character changed toward nor-
mal with soft but normally formed pellets (Fig. 1). No
changes in stool character were observed in HLA-B27 rats
receiving placebo. Likewise, placebo treatment had no effect
on the normal stool character in healthy F344 rats (data not
shown).

Fig. 1. Effect of oral treatment with enteric-coated rhIL-11 on stool
character in HLA-B27 transgenic rats with chronic gastrointestinal
inflammation. The stool character was observed daily during a 2-week
therapy with orally administered enteric-coated rhIL-11 (500 μg/kg given
at 48-h intervals) (closed symbols) or enteric-coated placebo multiparticu-
lates (open symbols). Animals were housed individually and stool consis-
tency was scored daily for each animal (0 = normal, 1 = soft with pellet
formation, 2 = soft with no pellet formation, 3 = diarrhea). The data are
means ± S.E.M. of the scores. *p < 0.05 compared with placebo-treated
HLA-B27 rats.
Effect of rhIL-11 Treatment of HLA-B27 Rats on Intestinal Inflammation. Our results indicated a 2.3-fold increase of MPO activity in the small intestine and a 3.8-fold increase of MPO activity in the colon of HLA-B27 rats treated with placebo in comparison with placebo-treated nontransgenic Fisher 344 rats. Treatment of HLA-B27 rats with rhIL-11 significantly reduced the activity of MPO in both the jejunum and colon (Fig. 2). At the end of the 2-week treatment with rhIL-11, MPO activity was reduced to levels that resembled those in nontransgenic Fisher 344 rats. In contrast, the same course of treatment with placebo showed no significant decrease in MPO activity in the jejunum and colon from HLA-B27 rats.

The improvement of stool character was associated with healing of colonic mucosa. Alternate day therapy with enteric coated rhIL-11 resulted in reduction of the histological lesion scores in sections isolated from the colon of animals receiving rhIL-11 (Table 1).

Acute Effect of rhIL-11 on Basal Contractile Activity. In the jejunal longitudinal muscle of F344 control rats, basal activity recorded at optimal tension was characterized by low resting tension (3.1 ± 0.8 nM/mm²) and spontaneous low-amplitude contractions appearing at a frequency of 18 ± 5 cycles/min. There was no significant difference between the basal activity recorded in muscles isolated from placebo-treated F344, placebo-treated HLA-B27 rats, or HLA-B27 rats treated with rhIL-11. When rhIL-11 (1–10,000 ng/ml) was added to the bathing solution, no significant changes in background activity were found in jejunal muscles isolated from both Fisher 344 or HLA-B27 rats. Accordingly, contractions induced by carbachol (0.1 μM) were not altered when rhIL-11 (1–10,000 ng/ml) was present into the bathing solution (data not shown).

Colonic longitudinal muscles isolated from placebo-treated control F344 rats showed low resting tension (2.4 ± 0.3 mN/mm²) with or without occurrence of spontaneous contractions. Resting tension and spontaneous contractions were similar in muscles from F344 and HLA-B27 rats receiving placebo or rhIL-11. The addition of rhIL-11 (1–10,000 ng/ml) to the bathing solution showed no acute effects on spontaneous contractility or contractile responses to carbachol (1 μM) in the colon of Fisher 344 rats or HLA-B27 rats (data not shown).

Effects of rhIL-11 Treatment on Receptor-Independent Intestinal Muscle Contraction. Increasing the concentration of KCl in the bathing solution induced receptor-independent membrane depolarization and muscle contraction. Concentrations of 60 to 80 mM KCl were required to elicit maximal contractions in jejunal or colonic muscle strips isolated from both Fisher 344 and HLA-B27 rats. However, the active tension generated by muscles from placebo-treated HLA-B27 rats was lower compared with that generated by muscles from placebo-treated Fisher 344 rats. Treatment of HLA-B27 rats with rhIL-11 increased the maximal contraction induced by high KCl in both the jejunum and colon (Fig. 4). Moreover, there was no significant difference between the responses to high KCl in muscles isolated from HLA-B27 rats treated with rhIL-11 compared with placebo-treated Fisher 344 rats.

Effects of rhIL-11 Treatment on Cholinergic Intestinal Muscle Contraction. Complete dose-response curves to carbachol were obtained in jejunal and colonic longitudinal muscles (Fig. 5) and the summarized results from the regression analysis are presented in Table 2. Longitudinal muscles isolated from the jejunum of HLA-B27 rats showed abnormal contractile responses. The maximal active tension generated in response to increasing concentrations of carbachol (1 nM–10 μM) was significantly lower in the muscles isolated from placebo-treated HLA-B27 rats compared with placebo-treated nontransgenic Fisher 344 rats (Fig. 5A). The reduction in contractile responses was accompanied by a shift of the dose-response curve to lower carbachol concentrations. Accordingly, the EC50 for carbachol in jejunal muscles from placebo-treated HLA-B27 rats is significantly lower compared with the EC50 value obtained in the jejunum of Fisher 344 rats. The treatment of HLA-B27 transgenic rats with rhIL-11 resulted in a significant increase in carbachol-induced maximal tension.

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Fig. 2. MPO activity in jejunal (A) and colonic (B) tissue isolated from nontransgenic F344 rats or HLA-B27 transgenic rats. The F344 rats received placebo, whereas HLA-B27 rats received oral doses of either placebo or enteric-coated rhIL-11 (500 μg/kg at 48-h intervals) during a 2-week treatment. Data are mean ± S.E.M. (n = 6 for each group). a, p < 0.001 compared with placebo-treated F344 rats; b, p < 0.001 compared with placebo-treated HLA-B27 rats.
generated by the jejunal muscle. Besides the significant increase, the amplitude of maximal response remained lower than the maximal contraction in muscles from placebo-treated Fisher 344 rats. The EC\textsubscript{50} for carbachol in the jejunum of HLA-B27 rats treated with rhIL-11 was significantly reduced compared with placebo-treated HLA-B27 rats and was similar to the EC\textsubscript{50} in the jejunum of Fisher 344 rats. The maximal active tension generated in response to carbachol by colonic muscles from placebo-treated HLA-B27 rats was lower than that generated by muscles from placebo-treated Fisher 344 rats (Fig. 5B; Table 2). Following rhIL-11
therapy, the maximal tension induced by carbachol in colonic muscles from rhIL-11-treated HLA-B27 rats was significantly increased compared with placebo-treated HLA-B27 rats and was similar to that in the colon of placebo-treated Fisher 344 rats. In contrast to the jejunum, the concentration-effect curves for carbachol obtained in colonic muscles from F344 or HLA-B27 rats treated with placebo, as well as from HLA-B27 rats treated with rhIL-11, had similar position and did not show significant difference between EC$_{50}$ values.

**Effect of rhIL-11 Treatment on Neurally Mediated Intestinal Muscle Contractions.** In the longitudinal muscle of the jejunum, EFS (0.5-ms pulse duration, 5 Hz, 5-s train duration) induced contractile responses. The increase in tension reached maximum during stimulation and decreased to the resting level after the end of the stimulus train. Responses to EFS were reproducible throughout the experiment. In the presence of atropine (1 μM) and guanethidine (10 μM), EFS induced nonadrenergic, noncholinergic (NANC) contractile responses of lower amplitude. No relaxation was observed. Guanethidine alone had no effect on EFS-induced contractions; thus, the difference between the control response and the NANC component represented a cholinergic (atropine-sensitive) component of the EFS-in-
Although the links between HLA-B27 and microglobulin expression and increases with age (Taurog et al., 1993, 1994). Normal luminal bacteria are associated with chronic and TNF-α/H9251ous inflammation is T-cell-dependent and involves increased evidence that rhIL-11 administered subcutaneously to HLA-B27 rats with chronic inflammation indicates that NANC contractions. In colonic muscles, EFS induced a contractile response, which was partially inhibited by atropine and guanethidine, revealing a NANC contraction. Similar to the jejunum, the colonic muscles maintained a relatively low level of resting tension, and no relaxatory responses were observed. In muscles from placebo-treated HLA-B27 rats, the control response to EFS was reduced compared with placebo-treated F344 rats. In contrast to the jejunum, there was also a significant reduction in the amplitude of NANC contractions. Treatment of HLA-B27 rats with rhIL-11 significantly increased the amplitude of control EFS-induced contraction and normalized the NANC response (Fig. 6A). Tetrodotoxin (1 μM) completely abolished both control and NANC responses to EFS, indicating that they result from activation of enteric neurons.

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**Discussion**

Intestinal inflammation in the HLA-B27 rat model involves a genetically altered inflammatory response due to overexpression of the major histocompatibility complex class I allele B27 and microglobulin β2 genes (Hammer et al., 1990). Normal luminal bacteria are associated with chronic gastrointestinal inflammation in the HLA-B27 rats (Rath et al., 1996), and it has been reported that susceptibility to the inflammatory disease correlates with the levels of B27 expression and increases with age (Taurog et al., 1993, 1994). Although the links between HLA-B27 and microglobulin β2 expression and gastrointestinal inflammation remain to be established, it is evident that the development of spontaneous inflammation is T-cell-dependent and involves increased production of the pro-inflammatory cytokines IL-1α, IL-1β, and TNF-α (Breban et al., 1993, 1996). Previous studies comparing RNA for many cytokines in colonic tissue from HLA-B27 rats and Fisher 344 rats have shown elevated levels of mRNA for interferon-γ, TNF-α, IL-1α, IL-1β, IL-6, IL-12, and inducible nitric-oxide synthase in the HLA-B27 rats.

In the present study we found that oral treatment of HLA-B27 rats with rhIL-11 reverses inflammation and heals the histological damage to the colonic mucosa. The reduction of intestinal MPO activity following oral administration of enteric-coated rhIL-11 at a dose of 500 μg/kg was comparable with that described previously in HLA-B27 rats receiving subcutaneous injections of rhIL-11 at a dose of 36 μg/kg (Peterson et al., 1998). The similarity between the anti-inflammatory effect of oral and subcutaneous administration of rhIL-11 is of particular interest, since a recent study of rhIL-11 pharmacokinetics showed that the interleukin is not systemically bioavailable following oral administration in HLA-B27 rats (Tseng et al., 2000), strongly suggesting a topical mechanism of action. Although the exact mechanism of action of topically applied rhIL-11 is unclear, there is evidence that rhIL-11 administered subcutaneously to HLA-B27 rats down-regulates the production of pro-inflammatory cytokines (TNF-α, transforming growth factor-β1, IL-1α, IL-6) and inducible nitric-oxide synthase (Peterson et al., 1998), most probably acting through the inhibition of transcription nuclear factor-κB (Trepicchio et al., 1997). Thus, we speculate that the therapeutic effect of orally administered rhIL-11 is due to its ability to regulate the cascade of inflammatory reactions in the colon. The evidence that oral treatment with rhIL-11 is effective against colonic inflammation in HLA-B27 rats suggests that an oral therapy could prove effective in patients with ulcerative colitis or Crohn’s disease.

Earlier, we established that the spontaneous chronic inflammation in transgenic HLA-B27 rats is accompanied by a decrease in contractile responses of the longitudinal muscles of the jejenum and colon to enteric nerve stimulation, muscarinic cholinoreceptor activation, or receptor-independent membrane depolarization induced by KCl (Venkova et al., 2000). The lack of difference in neurally mediated NANC contractions in the jejunum of control F344 rats and HLA-B27 rats with chronic inflammation indicates that NANC excitatory pathways remain functionally intact in the inflamed jejunum, whereas they are impaired in the colon of HLA-B27 rats. In the present study, we found that the treatment of HLA-B27 rats with rhIL-11 restores the ability of jejunal and colonic muscles to develop active tension. In the jejunum, the recovery of maximal response to carbachol was not significant between the amplitude of NANC contractions. Treatment of HLA-B27 rats with rhIL-11 reverses inflammation and heals the histological damage to the colonic mucosa. The reduction of intestinal MPO activity following oral administration of enteric-coated rhIL-11 at a dose of 500 μg/kg was comparable with that described previously in HLA-B27 rats receiving subcutaneous injections of rhIL-11 at a dose of 36 μg/kg (Peterson et al., 1998). The similarity between the anti-inflammatory effect of oral and subcutaneous administration of rhIL-11 is of particular interest, since a recent study of rhIL-11 pharmacokinetics showed that the interleukin is not systemically bioavailable following oral administration in HLA-B27 rats (Tseng et al., 2000), strongly suggesting a topical mechanism of action. Although the exact mechanism of action of topically applied rhIL-11 is unclear, there is evidence that rhIL-11 administered subcutaneously to HLA-B27 rats down-regulates the production of pro-inflammatory cytokines (TNF-α, transforming growth factor-β1, IL-1α, IL-6) and inducible nitric-oxide synthase (Peterson et al., 1998), most probably acting through the inhibition of transcription nuclear factor-κB (Trepicchio et al., 1997). Thus, we speculate that the therapeutic effect of orally administered rhIL-11 is due to its ability to regulate the cascade of inflammatory reactions in the colon. The evidence that oral treatment with rhIL-11 is effective against colonic inflammation in HLA-B27 rats suggests that an oral therapy could prove effective in patients with ulcerative colitis or Crohn’s disease.

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associated with a shift of the responses to lower concentrations, overlapping with the concentrations that induce responses in muscles from nontransgenic Fisher 344 rats. However, the present experiments could not identify whether the effects of rhIL-11 treatment on smooth muscle contractility are a late result of cessation of the inflammatory process and mucosal recovery and/or are the direct result of rhIL-11-induced improvements in the function of the contractile apparatus of smooth muscle cells. The recovery of normal intestinal muscle contractility induced by the oral treatment of HLA-B27 rats with rhIL-11 may be due to rhIL-11-induced inhibition of the expression of proinflammatory cytokines. Such mechanism of action is sustained with the significant decrease in the activity of MPO, and a reduction in the levels of inflammatory mediators in the colon has been demonstrated in HLA-B27 rats after subcutaneous administration of rhIL-11 (Peterson et al., 1998). The inhibition of IL-1β and TNF-α may be of particular importance for normalization of neuromuscular dysfunctions (Marzio et al., 1990) and the decrease of acetylcholine release from enteric nerves (Collins et al., 1992). Previous studies in models of acute intestinal inflammation have shown that corticosteroid therapy prevents the development of abnormal smooth muscle contractions (Marzio et al., 1990) and the decrease of acetylcholine and noradrenaline release from enteric nerves (Collins et al., 1989; Swain et al., 1991). Thus, the neuromuscular dysfunction was considered a consequence of the inflammatory response. In our experiments, the inhibition of chronic intestinal inflammation by oral administration of rhIL-11 to HLA-B27 rats resulted not only in the recovery of the normal ability of jejunal muscles to generate active tension in response to enteric nerve stimulation, carbachol activation of muscarinic cholinoreceptors, or receptor-independent depolarization of the smooth muscle membrane with KCl, but also in normalization of cholinoreceptor sensitivity (EC50) for carbachol, which was increased during inflammation. These findings imply that rhIL-11 therapy improves both inflammation-induced alterations in intestinal smooth muscle contractile mechanisms at a receptor-independent level, as well as at the level of enteric neuronal pathways regulating smooth muscle contraction.

Intestinal muscle cells are actively involved in the inflammatory reaction expressing the genes of cytokines IL-1β, IL-6, and TNF-α and secreting the proteins (Khan et al., 1995). Thus, during the treatment of chronic inflammation with rhIL-11, a reduced ability of smooth muscle cells to produce cytokines may be caused by a direct interaction between rhIL-11 and the intestinal muscle cells. Moreover, IL-1 and TNF-α have been shown to promote the proliferation of human intestinal smooth muscle cells in vitro, suggesting that these cytokines play a key role in the thickening of the bowel wall observed in animal models of intestinal inflammation and in patients with Crohn’s disease (Owens and Grisham, 1993). We suggest that IL-11 may regulate smooth muscle cell proliferation and/or collagen deposition in the small intestine and in the colon by inhibiting the expression of pro-inflammatory cytokines. In patients with Crohn’s disease, the thickening of the bowel wall is associated with smooth muscle cell hyperplasia and deposition of collagen (Graham et al., 1988; Stallmach et al., 1992), and it seems that rhIL-11 therapy may prove effective against the narrowing and stricturing of the intestine.

Finally, the amelioration of smooth muscle function by treatment with rhIL-11 is indirect evidence in support of the concept that endogenous IL-11 plays a protective role during the inflammatory remodeling of intestinal smooth muscle. It is reasonable to suggest that gastrointestinal muscle cells may express IL-11 genes in response to increasing concentrations of pro-inflammatory cytokines. This assumption is based on the recent finding that in vascular muscle IL-1,
transforming growth factor-β and TNF-α induce the expression of IL-11, which has a protective role against atherosclerotic lesions (Taki et al., 1999).

In summary, we have shown that oral administration of enteric-coated rhIL-11 to HLA-B27 rats with chronic intestinal inflammation reverses the inflammatory response and restores the ability of the longitudinal muscle to develop active tension in both the jejunum and colon. In HLA-B27 rats displaying the symptom of chronic diarrhea, the 2-week treatment with oral doses of rhIL-11 (500 µg/kg at 48-h intervals) showed a cumulative effect normalizing the stool character. These findings suggest that a relatively short-lasting oral therapy with enteric-coated rhIL-11 may have the advantage of low toxicity (topical versus systemic action) in the treatment of patients with IBD.

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References


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