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ORAL TREATMENT WITH RECOMBINANT HUMAN INTERLEUKIN 11 IMPROVES MUCOSAL TRANSPORT IN THE COLON OF HUMAN LEUKOCYTE ANTIGEN-B27 TRANSGENIC RATS

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ABSTRACT Recombinant human interleukin 11 (rhIL-11) is a pleiotropic cytokine with anti-inflammatory activity. The objective of the study was to investigate whether oral treatment with rhIL-11 improves colonic epithelial dysfunction in the HLA-B27 transgenic rat model of spontaneous chronic inflammation. Experiments were performed using adult male HLA-B27 rats, while healthy non-transgenic F344 rats served as controls. Enteric-coated rhIL-11 multi-particles (equivalent to 500 µg/kg rhIL11) or placebo (formulation lacking rhIL-11) were administrated orally on alternate days for 2 weeks to HLA-B27 or F344 rats. Stool character was observed daily during the treatment period. Animals were euthanized at the end of treatment and colonic inflammation was evaluated be measuring tissue myeloperoxidase (MPO) activity. Epithelial transport in isolated colonic mucosal sheets was studied in modified Ussing chambers. Oral treatment of HLA-B27 rats with rhIL-11 reduced MPO activity in the colon and suppressed the clinical signs of diarrhea. The electrophysiological characteristics of mucosal transport were improved in the HLA-B27 rats treated with rhIL-11 compared to placebo. Following rhIL-11 treatment the basal transepithelial resistance and the estimated paracellular resistance were significantly increased, neurally mediated secretory responses to electrical field stimulation were improved and cholinoceptor sensitivity was normalized. Treatment with rhIL-11 had no significant effect on basal Isc and the maximal secretory response to carbachol or substance P. Our data demonstrate that oral rhIL-11 therapy is associated with suppression of mucosal inflammation and a concomitant improvement of epithelial resistance and neurally mediated secretion in a model of chronic HLA-B27 colitis.

Interleukin-11 (IL-11) is a pleiotropic cytokine with biological effects on different cell types in a variety of tissues. The functional activities of IL-11 are associated with activation of the gp130 signal-transduction unit and involve hematopoietic, immunomodulatory and epithelial effects (Schwertschlag et al., 1999). Since recombinant human IL-11 (rhIL-11) has been manufactured by DNA technology in *Escherichia coli*, treatment of chronic gastrointestinal inflammation became the focus of preclinical and clinical studies investigating the effect of subcutaneously administered rhIL-11 on gastrointestinal inflammation and injury. RhIL-11 has been found to suppress inflammation by reducing the expression of pro-inflammatory mediators such as INF-γ, TNF-α, IL-1β, IL-6 and iNOS in the colon of HLA-B27 rats with chronic colitis (Peterson et al., 1998). In addition to having an anti-inflammatory effect, rhIL-11 was found to be an epithelial growth factor increasing survival after cytoablative therapy and irradiation in mice by preventing apoptosis, promoting recovery of small intestinal epithelial cells and restoring the intestinal mucosal barrier (Du et al., 1994; Orazi et al., 1996). *In vitro* studies indicate that IL-11 modulates epithelial dynamics in untransformed intestinal epithelial cells, suggesting that IL-11 may be part of the normal growth control and repair in the intestinal epithelium (Booth and Potten, 1995).

Although the effects of rhIL-11 on mucosal inflammation and histology in animal models of colitis have been well documented (Keith et al., 1994; Qiu et al., 1996), few studies have investigated the possible effects of rhIL-11 on intestinal transport and contractility. Subcutaneous

administration of rhIL-11 was associated with partial normalization of the colonic smooth muscle dysfunction in a rabbit model of colitis (Depoortere et al., 2000), and normalization of basal transepithelial potential difference (PD) in mucosal sheets isolated from the jejunum of HLA-B27 rats with chronic inflammatory disease (Greenwood-Van Meerveld et al., 2000). Furthermore, the oral treatment of HLA-B27 rats with a new enteric-coated formulation of rhIL-11 reversed the symptoms of colitis and normalized intestinal muscle function. When rhIL-11 was applied directly to isolated intestinal mucosal sheets studied in modified Ussing chambers, it caused a reduction of transepithelial PD (Greenwood-Van Meerveld et al., 2001), suggesting that IL-11 receptors expressed on the luminal surface of intestinal epithelial cells (Grosfeld et al., 1999; Opal et al., 2003) may be functionally activated. Such epithelial IL-11 receptors are likely to represent a site of specific interaction with orally administered rhIL-11. This suggestion is supported by a recent study (Ropeleski et al., 2003) demonstrating that IL-11 induces the expression of heat shock protein 25 in intestinal epithelial cells, which presents a cytoprotective mechanism against oxidative stress and increases epithelial viability. However, it remains unclear whether oral treatment with rhIL-11 can normalize abnormalities in intestinal epithelial transport.

The goal of the present study was to examine the effect of oral treatment with rhIL-11 on abnormalities in colonic electrogenic transport in HLA-B27 transgenic rats with chronic colitis.

HLA-B27 transgenic rats were used as a model of colitis because they develop spontaneous chronic inflammation of the gastrointestinal tract that closely resembles inflammatory bowel disease (IBD) in humans (Hammer et al., 1990). Non-transgenic Fisher 344 (F344) rats served as controls. The rats were treated with an oral formulation of rhIL-11, that was specifically designed to pass through the stomach and deliver rhIL-11 within the lumen of the intestine without detectable systemic absorption (Tseng et al., 2000). Electrogenic mucosal transport was studied in modified Ussing chambers by measuring basal short circuit current (Isc) and changes in epithelial secretion and transepithelial electrical resistance in colonic mucosal sheets. The therapeutic effect of oral dosing of rhIL-11 (500 µg/kg given on alternate days for 2 weeks) was followed *in vivo* as changes in stool character during the treatment period, while tissue myeloperoxidase (MPO) activity and mucosal transport were measured *in vitro* in colonic preparations isolated at the end of the treatment.

METHODS

Experimental animals.

Male Fischer 344 rats genetically engineered to express human HLA-B27 and β_2-microglobulin were used as a model of chronic inflammation reminiscent of human inflammatory bowel disease (IBD). Age-matched non-transgenic Fisher 344 rats were used as controls. HLA-B27 transgenic rats (n=12) or Fisher 344 rats (n=12) were purchased from Taconic (Germantown, New York) at 10-12 weeks of age and were housed in the animal facility until the age 36-40 weeks. All rats were housed in individual cages at room temperature of 21^{o}C, standard humidity and a 12-h light/dark cycle. Conventional laboratory rodent chow and tap water were provided *ad libitum*. The V.A. Animal Care and Use Sub-Committee, Oklahoma City, Oklahoma approved the experimental procedures used in the study.

Treatment with rhIL-11.

The study was designed to investigate whether oral treatment with enteric-coated rhIL-11 multi-particles or placebo can improve the impaired mucosal ion transport in the colon of HLA-B27 transgenic rats with chronic colitis. The dose and time-course of rhIL-11 treatment were selected from a previous study (Greenwood et al., 2001) showing normalization of mucosal histology and a reversal of intestinal smooth muscle dysfunction in the colon of HLA-B27 rats. The formulation of rhIL-11 for oral administration consisted of multi-particles coated with an enteric

polymer that dissolves at pH >5.5, thus ensuring that the coating is insoluble in the stomach but does dissolve in the small intestine. The test article contained approximately 1.0 mg rhIL-11 per 100 mg/multi-particles. The control article (placebo) consisted of enteric polymer coated sucrose multi-particles. A daily oral dose of enteric-coated rhIL-11 or placebo was given to the animals at 8 - 9 A.M. every other day during 2 weeks. The rats were euthanized 3 h after receiving the last oral dose and the colon was isolated for *in vitro* investigation. The rats were randomly assigned to four groups that received different treatment as follows: a test group of HLA-B27 transgenic rats (n=6) treated with enteric-coated rhIL-11; a placebo-treated group of HLA-B27 transgenic rats (n=6) receiving enteric-coated sucrose multi-particles; a test group of non-transgenic F344 rats (n=6) treated with enteric-coated rhIL-11; and a placebo-treated group of non-transgenic F344 rats (n=6) receiving enteric-coated sucrose multi-particles.

Evaluation of stool character

The stool character was observed and scored daily during the two weeks of treatment. Specifically, the stool was scored as 0 for normal, 1 for soft with formed pellets, 2 for soft with no pellet formation and 3 for watery diarrhea. Average daily scores were calculated for each experimental group.

Myeloperoxidase (MPO) activity.

MPO is a granule-associated peroxidase primarily contained in polymorphonuclear neutrophils infiltrating the inflamed tissue. MPO activity, measured in colonic tissue was considered a biochemical marker of inflammation. Tissue samples (150-200 mg) from both HLA-B27 and F344 rats were collected from the mid to proximal colon isolating small segments from regions adjacent to those used for the Ussing chamber preparations. Full thickness colonic samples were immediately frozen in liquid nitrogen and stored at –80^{o} C. The samples were analyzed simultaneously as described previously (Venkova et al., 2000). Briefly, homogenization and MPO extraction was performed in hexadodecyl-trimethylamonium bromide (HTAB) phosphate buffer (pH 6). MPO activity was measured in 10 µl samples using 3,3',5,5'-tetramethylbenzidine TMB Microwell Peroxidase Substrate System (Sigma Chem. Co., St. Louis, MO) and horseradish peroxidase (HRP) as a relative standard. MPO activity was expressed as equivalent to the activity of the relative standard (ng of HRP) converting the same amount of TMB substrate for 10 min at room temperature. Mean values are given in ng normalized per g wet weight of the tissue.

Ussing chamber experiments

The colon was harvested, the luminal contents were removed by washing with modified Krebs solution and the clean tissue was placed in ice-cold modified Krebs buffer aerated with 95% O_2

and 5% CO_2. Isolated mucosal sheets were obtained following removal of the outer muscle layer from segments (15 mm long) cut from the mid portion of the colon (1 cm of the most distal and proximal colon were discarded). Four to six mucosal sheets obtained from each animal were mounted in Ussing chambers (1 cm^2 window opening) and electrogenic mucosal transport was measured as previously described (Greenwood-Van Meerveld et al., 2000). The luminal and serosal side were bathed with modified Krebs buffer maintained at 37^o C and continuously aerated with 95% O_2 and 5% CO_2. Potential difference (PD) and short circuit current (Isc) were recorded via two pairs of agar-salt bridge electrodes connected to an EVC 4000 voltage/current clamp apparatus (World Precision Instruments, Sarasota, FL). Resistance was calculated using Ohm's law from the open circuit PD and the Isc. Throughout the experiment Isc was recorded using a MacLab data acquisition system (AD Instruments Ltd., Castle Hill, Australia). Basal PD was lumen negative and measured in mV, basal Isc was measured in µA and normalized for the mucosal surface area (µA/cm^2). Drug-induced changes in Isc were presented as the difference between the basal value measured before drug administration and the maximum of the drug-induced effect. Neurally mediated changes in Isc were induced by electrical field stimulation (EFS) applied via a pair of foil electrodes positioned on the serosal surface. Electrical stimuli were generated by a Grass S88 stimulator (Grass Institute Division, West Warwick, RI) and were passed to the tissue through a stimulus isolation unit Grass SIU5. Rectangular electrical pulses of 0.5 ms pulse duration were applied at a frequency of 5 Hz in trains with 5 s train-duration. In

each of the experiments the electromotor force was gradually increased (40 mV -90 mV) to induce a maximal response.

Paracellular electrical resistance

Both paracellular and transcellular pathways contribute to the transepithelial electrical resistance of a mucosal sheet measured in the Ussing chamber. To determine the paracellular resistance we used the approach of Parkos et al. (1992) and Riegler et al. (1999). The response to SP was selected as a model system to investigate the relationship between Isc and conductance because SP is known to induce a transient increase in Isc without altering paracellular resistance (Reigler et al., 1999). In our experiments transepithelial PD and Isc were measured at different time points during the ascending (0-2 min) and descending (2-20 min) phase of the response to SP (1 µM) applied to the serosal side. Electrical conductance (mS/cm^2) was calculated for each time-point and was plotted against the Isc ($\mu A/cm^2$). Separate plots were constructed for the ascending and descending phase of the SP response. The value of conductance at the x-axis intercept (Isc equals zero) was determined by linear regression analysis of the plots for the ascending and descending phase of the SP response. The paracellular resistance was calculated as reciprocal of the conductance at zero Isc (resistance (Ω/cm^2) = 1/conductance (mS/cm^2) x 1000). Because the secretory response to SP involves changes in transcellular resistance alone, the paracellular resistance has the same value for the ascending and descending phase of the response SP.

Solutions and drugs

The experiments were performed using modified Krebs bicarbonate solution of the following composition (in mM): NaCl 120, KCl 6, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 14.4 and glucose 11.5. The solution was continuously gassed with 95% O_2 and 5% CO_2 (vol/vol) and the pH at room temperature ranged from 7.2 to 7.3. Carbamylcholine chloride (carbachol), atropine sulfate, guanethidine and substance P were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO) and were dissolved 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 rhIL11 and the enteric-coated sucrose multiparticles (placebo) were provided by Wyeth Research (Cambridge, MA).

Statistical Analysis

The data presented in the study are mean ± SEM of 6 animals for each experimental group. In the Ussing chamber experiments 4-6 values from individual mucosal sheets were obtained from each rat. MPO activity was measured in triplicate in single tissue samples isolated from each animal. Regression analysis of complete concentration-response curves was used to calculate the concentration of carbachol causing 50% of the maximal effect (EC_{50}) and the 95% CI. Homogeneity of variances i.e. no significant difference between SD among the groups was verified using Bartlett's test. Comparison between multiple groups was made using ordinary one-

way ANOVA followed by Bonferroni post-test. Differences were considered significant at $p<0.05$. Statistical analysis and curve fitting was performed using InStat and Prism software (GraphPad Software, Inc., San Diego, CA).

RESULTS

Effect of rhIL-11 treatment on stool character

With the advance of age, all HLA-B27 rats used in the study spontaneously developed colitis. The symptoms of loose stool without pellet formation alternating with long-lasting episodes of watery diarrhea became evident at the age of 22-28 weeks and persisted for 4-6 weeks before initiation of the rhIL-11 or placebo treatment. At the beginning of rhIL-11 or placebo treatment, the stool consistency scores were 2 and 3 in all HLA-B27 rats. In contrast, the age-matched F344 rats appeared to be healthy and had normal stools (stool consistency score 0). In HLA-B27 rats, oral treatment with rhIL-11 caused a significant improvement is stool consistency compared to the lack of effect in the placebo treated group. At the end of the treatment period the stool character observed in the group of HLA-B27 rats receiving rhIL-11 was changed towards normal i.e. soft but normally formed pellets (score 1 and 2), while the group of HLA-B27 rats receiving placebo showed no change in the pattern of chronic diarrhea (score 2 and 3). The groups of healthy F344 rats receiving either rhIL-11 or placebo treatment had normal stools throughout the treatment period (score 0).

Effect of rhIL-11treatment on colonic MPO activity

The activity of MPO was measured in whole tissue samples isolated from the colon of HLA-B27 transgenic rats or F344 rats receiving either rhIL-11 or placebo treatment. As shown in Figure 1,

MPO activity was significantly higher in the colon of HLA-B27 rats receiving placebo compared to placebo-treated healthy F344 rats. Treatment with rhIL-11 significantly reduced MPO activity in the colon of HLA-B27 rats, while having no significant effect in the colon of F344 rats. However, the decreased level of MPO activity observed in the HLA-B27 group treated with rhIL-11 was still significantly higher compared to the levels of MPO measured in the F344 groups.

Effect of rhIL-11 treatment on basal electrogenic transport

Basal electrogenic transport in the isolated colonic mucosal sheets was characterized by measuring Isc as a marker of active electrogenic transport and by transepithelial resistance as an electrophysiological correlate including transcellular and paracellular permeability for water and electrolytes. The HLA-B27 rats receiving placebo showed significantly lower values of basal Isc (Figure 2 A) and transepithelial resistance (Figure 2 B) compared to the placebo-treated F344 group. Treatment of HLA-B27 rats with rhIL-11 resulted in a significant increase in transepithelial resistance compared to placebo-treated HLA-B27 rats (Figure 2 B), while rhIL-11 had no significant effect on basal Isc (Figure 2 A). A relatively small but statistically significant increase in resistance was found in mucosal sheets isolated from the colon of F344 rats treated with rhIL-11 compared to placebo-treated F344 rats.

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Effects of rhIL-11 treatment on SP-induced response and paracellular mucosal resistance

A set of experiments was designed to study the secretory response to SP (1 μM) and to determine the paracellular resistance during the ascending and descending phase the response. The increase in Isc induced by SP in mucosal preparations isolated from HLA-B27 rats was of lower amplitude compared to F344 rats, while there was no significant difference between the SP-induced increase in Isc in placebo-treated or rhIL-11 treated rats (Figure 3 A,B). Specifically, the maximal Isc induced by SP in the colon of HLA-B27 rats was 61±9 $\mu A/cm^2$ for the placebo-treated rats and 67±7 $\mu A/cm^2$ for the rhIL-11-treated rats, while the maximal response measured in F344 rats was significantly higher ($p<0.01$), reaching 166±16 $\mu A/cm^2$ and 143±25 $\mu A/cm^2$, respectively. Concurrent with the increase in Isc, SP induced a decrease in transepithelial resistance in the colon of F344 rats, while no substantial changes of resistance were observed following SP administration to the colonic mucosa from HLA-B27 rats (Figure 3 C,D). The Isc and conductance measured during the ascending and descending phase of the SP effect were used to estimate paracellular resistance. In accordance with previous studies (Reigler et al.,1999) we found that in each group the paracellular resistance calculated for the ascending and descending phase of the SP response had the same value (Table 1) indicating that paracellular resistance is not affected by changes in transepithelial conductance driven by active Cl^- secretion. By comparing the results found in colonic mucosa from placebo-treated HLA-B27 rats with these in preparations from placebo-treated healthy F344 rats we defined that paracellular

resistance is lower in the HLA-B27 rats. Treatment of HLA-B27 rats with rhIL-11 resulted in a significant increase in paracellular resistance to values that are not different from these in placebo-treated F344 rats (Table 1).

Effect of rhIL-11 treatment on mucosal responses to EFS

In a separate series of experiments EFS (0.5ms, 5 Hz) was applied to the mucosal sheets to induce a maximal secretory response measured as an increase in Isc. The response to EFS was completely inhibited by TTX (1 μM) indicating that it is due to intramural nerve stimulation (data not shown). Colonic mucosal sheets isolated from HLA-B27 or F344 rats treated with either rhIL-11 or placebo were studied and mean values of the maximal increase in Isc induced by EFS were calculated for each group. As illustrated in Figure 4A, the EFS-induced increase in Isc was significantly lower in mucosal sheets isolated from placebo-treated HLA-B27 rats compared to placebo-treated F344 rats. However, when HLA-B27 rats received rhIL-11 treatment, the response to EFS was significantly increased and was no longer different from the response obtained in placebo-treated F344 rats. No significant changes in EFS-induced Isc were found for the group of F344 rats treated with rhIL-11. Following the initial recording of maximal responses to EFS, the mucosal sheets were treated with atropine (1 μM) and guanethidine (10 μM) applied to the serosal side and responses to non-adrenergic non-cholinergic (NANC) nerve stimulation were investigated (Figure 4 B). While the NANC responses were of lower amplitude

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compared to the initial maximal responses in all experimental groups, the EFS-induced responses in mucosal sheets isolated from the HLA-B27 or F344 rats treated with either rhIL-11 or placebo showed the same differences as the initial responses in untreated preparations. That is the NANC-mediated Isc was lower in the colon of HLA-B27 rats compared to F344 rats and treatment with rhIL-11 induced a significant increase in NANC-induced responses in the colon of HLA-B27 rats.

Effect of rhIL-11 treatment on carbachol-induced Isc

The increase in Isc induced by activation of muscarinic cholinoceptors was studied by following the response to carbachol applied to the serosal bathing solution. Cumulative administration of increasing concentrations of carbachol resulted in a concentration-dependent increase in Isc until a maximal response was achieved (Figure 5). The maximal carbachol-induced response in mucosal sheets isolated from placebo-treated HLA-B27 rats was of significantly lower amplitude compared to placebo-treated F344 rats (28±6 $\mu A/cm^2$ vs. 98±10 $\mu A/cm^2$, $p<0.001$). Treatment with rhIL-11 did not cause a significant change in the maximal response to carbachol in either the HLA-B27 or F344 rats. The EC_{50} values calculated from the concentration-response curves (Table 2) showed that mucosal sheets isolated from placebo-treated HLA-B27 rats yielded an EC_{50} that was significantly higher compared to placebo-treated F344 rats. However, in both

HLA-B27 or F344 rats treated with rhIL-11 the carbachol EC_{50} value was significantly lower compared to the respective group of placebo-treated rats.

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DISCUSSION

Intestinal inflammation in patients with IBD is associated with loss of tolerance to resident enteric bacteria (Duchmann et al., 1995), activation of immune cells, and infiltration of leukocytes coordinated by the production of chemokines, proinflammatory monokines, and Th1 cytokines (Fiocchi, 1998). Therapies based on the antagonist activity of antibodies against TNFα have recently been introduced as an alternative to steroids in the treatment of IBD (Sandborn and Hanauer, 2002). In this respect, the potential therapeutic use of rhIL-11 presents an unique approach to the treatment of IBD combining the effects of a multipotent protein of mesenchymal origin (Dorner et al., 1997) acting both as an anti-inflammatory cytokine (Trepicchio et al., 1999) and as an epithelial growth factor restoring the integrity of intestinal epithelium. Furthermore, experiments performed *in vitro* using specimens of human colonic mucosa (Valenick et al., 1998) have indicated that rhIL-11applied to the mucosal or serosal solution prevents the typical increase in mucosal permeability induced by *C. difficile* toxin, thus suggesting a topical protective effect of IL-11. In clinical studies, weekly subcutaneous injection with rhIL-11 was safe and effective in inducing remission in patients with active Crohn's disease (Sands et al., 1999; Sands et al., 2002). In addition rhIL-11 was found to reduce bacteraemia in patients undergoing chemotherapy by preserving mucosal integrity in the gastrointestinal tract (Ellis et al., 2003).

The present study investigated the ability of a new formulation for local delivery of rhIL-11 into the intestine to suppress colonic inflammation and reverse epithelial dysfunction in a rat model of chronic colitis. This strategy has the advantage that rhIL-11 delivery may be directly targeted to the site of inflammation. A previous study (Tseng et al., 2000) indicated no measurable levels of systemic rhIL 11 in normal Sprague Dawley or Fisher rats or in diseased HLA-B27 transgenic rats. Moreover, to determine whether a small amount of rhIL-11 has reached the portal blood and has been subsequently removed by first-pass hepatic uptake, the gene expression of hepatic acute phase proteins was investigated. The results found no changes in the expression of rhIL-11 responsive hepatic genes indicating no hepatic or systemic exposure to the cytokine following oral administration of the formulation. Experiments following the distribution of radiolabeled enteric coated rhIL-11 confirmed intraluminal levels of rhIL-11 in the terminal ileum and proximal colon (unpublished data, Wyeth Research) Taken together these results imply that the enteric coated formulation designed for oral administration of rhIL-11 may provide topical action while preventing hematopoetic effects associated with systemic rhIL-11 administration. The use of HLA-B27 transgenic rats co-expressing the human major histocompatibility class I allele HLA-B27 and β_2-microglobulin provided a model of T-cell-dependent chronic multiorgan inflammation that is reminiscent of B27-associated spondyloarthropathy and IBD in humans (Hammer et al., 1990). Our results confirmed that a two-week therapy of either oral administration of enteric-coated rhIL-11 (Greenwood-Van Meerveld et al., 2001) or

subcutaneous rhIL-11 injection (Keith et al., 1994; Greenwood-Van Meerveld et al., 2000) in HLA-B27 rats with chronic colitis caused similar improvement in stool character and equivalent suppression of colonic inflammation evaluated histologically or biochemically via a decrease in MPO activity. Moreover, we demonstrated that colonic inflammation in the HLA-B27 rat was associated with decreased transepithelial resistance, dysregulation of epithelial secretion of water and electrolytes and epithelial hyporesponsiveness to secretagogues. Subsequently, oral administration of rhIL-11 to HLA-B27 rats resulted in restoration of transepithelial resistance and a significant improvement of neurally mediated secretory responses in the colon, while epithelial hyporesponsiveness to secretagogues was not affected by the treatment. Previous findings in healthy Sprague Dawley or diseased HLA-B27 rats (Greenwood-Van Meerveld et al., 2000) showed that *in vitro* application of rhIL-11 to either the mucosal (intraluminal) or the serosal bathing solution reduced PD in the jejunum and Isc in the colon, implying that an IL-11 receptor may be located on both the apical and basolateral surface of the enterocyte. Similar to the observation with oral rhIL-11 treatment, subcutaneous rhIL-11 reduced colonic inflammation in the HLA-B27 rats but did not significantly colonic improve active transport (basal Isc and induced Isc). However, the low basal transepithelial PD in the jejunum was restored after 2 weeks subcutaneous treatment with rhIL-11 suggesting that normalization of the mucosal transport in the small intestine may contribute to the suppression of diarrhea and the improvement of neurally mediated secretion found in the colon of HLA-B27 rats following oral

rhIL-11 treatment. In accordance with other studies (Tseng et al., 2000) indicating no measurable levels of rhIL-11 in F344 or diseased HLA-B27 rats following oral administration of the enteric coated rhIL-11 formulation, our data strongly suggests that local mechanisms of interaction between rhIL-11 and epithelial and immune cells and/or submucosal neurons within the intestinal wall can promote healing of HLA-B27 associated colitis.

Epithelial pathophysiology during inflammation is characterized by increased permeability and abnormal secretion and/or absorption of water and electrolytes (Santos and Perdue, 1998). Therefore, understanding the clinical relevance of these findings is important for the development of new approaches in the therapy of immune-regulated intestinal diseases. In the present experiments treatment of HLA-B27 rats with chronic colitis with rhIL-11 resulted in a significant increase in basal transepithelial resistance, which reached a value not significantly different from that in placebo-treated healthy F344 rats. In contrast, rhIL-11 treatment had no effect on basal Isc implying that rhIL-11 is more likely influencing the passive transport of electrolytes rather than improving the active transport driven by ATP-dependent Na^+/K^+ exchange in the enterocytes. Such an assumption is supported by our observation that the increase in Isc induced by either SP or carbachol, caused by the active secretion of Cl^- ions (Kuwahara and Cooke, 1990), remained impaired in the colon of HLA-B27 rats following the treatment with rhIL-11. The passive transport of electrolytes and water is driven by

electrochemical concentration gradients and involves both a transcellular and a paracellular route. In our experiments, the low paracellular resistance defined in placebo-treated HLA-B27 rats was significantly increased in HLA-B27 rats treated with rhIL-11. The low paracellular resistance in the colon of HLA-B27 rats agrees with the findings of Schmitz et al. (1999) in colonic mucosa from patients with ulcerative colitis showing that alterations in the structure of tight junctions contribute to the barrier defect. It has also been found that increased colonic ion permeability in ulcerative colitis is associated with epithelial leaks defined as dramatic changes in epithelial conductance measured at sites of apoptotic activity (Gitter et al., 2001). In the context of these findings, our results suggest that a paracellular leak that contributes to diarrhea in colitis may be 'repaired' by rhIL-11. Moreover, the interaction between orally administrated rhIL-11 and the colonic epithelium in the HLA-B27 rats is likely to include a rhIL-11-induced decrease in the apoptosis of intestinal epithelium crypt cells as reported by Orazi and colleagues (1996). Most recently, it has been found that activation of IL-11 receptors expressed by rat intestinal epithelial cells involves up-regulation of inducible heat-shock protein protecting the enterocyte from local oxidant injury (Ropeleski et al., 2003). Finally, an unknown effect of rhIL-11 on mucosal permeability which is independent of the healing of inflammation is likely to contribute to the increased transepithelial resistance found in colonic mucosal sheets isolated from healthy F344 rats treated with rhIL-11 compared to placebo-treated F344 rats.

In a separate series of experiments we investigated the effect of oral treatment with rhIL-11 on neurally mediated secretion induced by EFS of enteric nerve terminals confined in the mucosa and submucosal plexus in muscle-stripped mucosal preparations. As described previously in guinea pig distal colon (Javed and Cooke, 1992), electrical stimulation of submucous neurons induces secretion of Cl^- ions that can be measured as an increase in Isc. Stimulation at 5 Hz has been associated with excitation of cholinergic pathways since it was found to induce the release of acetylcholine. In our experiments we investigated the effects of rhIL-11 treatment on maximal responses to EFS (5 Hz, 0.5 ms) induced in colonic mucosal sheets isolated from HLA-B27 or F344 rats. The Isc induced in colonic sheets from placebo-treated HLA-B27 rats was significantly lower compared to placebo-treated F344 rats. A similar impairment of the neurally mediated increase in Isc was present at NANC conditions, suggesting that the decrease in neurally mediated responses is not limited to an inflammatory damage of cholinergic neurons and may involve NANC nerves. Furthermore, we found that treatment with rhIL-11 caused an increase in EFS-induced Isc increasing both cholinergic and NANC components of the responses to levels that were not significantly different from those defined for F344 rats. A possible reason for this effect could be the ability of rhIL-11 to reduce the expression of proinflammatory cytokines which are closely related to enteric nerve dysfunction (Collins et al, 1989; Main et al., 1993). A recent study (Galeazzi et al., 2000) demonstrated that macrophages infiltrating the inflamed region are a major source of proinflammatory cytokines causing functional neural

changes. In addition, the increase in EFS response may reflect a long-lasting post-inflammatory modulation of secretomotor responses characterized by a switch from predominantly cholinergic to NANC excitation (Venkova et al., 1999). Similar to EFS-induced responses, the maximal increase in Isc evoked by carbachol or exogenous SP was lower in mucosal sheets isolated from placebo-treated HLA-B27 rats with colonic inflammation compared to healthy F344 rats. In contrast to the significant improvement of EFS-induced responses, the treatment of HLA-B27 rats with rhIL-11 did not significantly change the carbachol or SP-induced increase in Isc. Similar hyporesponsiveness to carbachol and other secretagogues has been demonstrated in animal models of colitis induced by trinitrobenzenesulfonic acid and it has been found to extend for up to 12 weeks beyond the acute phase of the inflammatory response (Bell et al., 1995; MacNaughton et al., 1998). In the same model of colitis, prolong epithelial dysfunction persisted when the rats were treated with dexamethasone causing a normalization of Isc responses to EFS while the responses to carbachol remained significantly depressed (Asfaha et al., 1999). Although the mechanisms underlying rhIL-11 effect on EFS remain unclear, it is reasonable to suggest that a post-inflammatory remodeling of enteric neurons and a direct effect of rhIL-11 on post-synaptic receptors may partially compensate for the hyporesponsiveness of epithelial cells to secretagogues. The shift of the EC_{50} for carbachol to lower concentrations following treatment with rhIL-11 is one piece of evidence pointing in this direction, however specific experiments are required to elucidate the effect.

In conclusion, our findings are the first to indicate that oral rhIL-11 therapy is associated with suppression of mucosal inflammation and a concomitant improvement of epithelial resistance and neurally mediated secretion in a model of chronic HLA-B27 colitis. The results suggest that intestinal delivery of rhIL-11 targeting the mucosa while avoiding hepatic or systemic exposure to the cytokine may provide a new approach for the treatment of human IBD. Furthermore, when considered in view of previous findings (Booth and Potten, 1995) showing that IL-11 may regulate the normal growth of intestinal epithelium, while having no effect on tumor cell lines, our results support the assumption that rhIL-11 may be useful as an adjuvant to cytotoxic cancer therapies.

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FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1. Activity of MPO measured in colonic tissue isolated from HLA-B27 rats or F344 rats treated with placebo or rhIL-11 administered orally at a dose of 500 µg/kg given every other day during a 2-week treatment. Data are mean ± SEM from colonic tissue isolated from N=6 rats in each group. Differences between means were analyzed for statistical significance at $p<0.05$ using one-way ANOVA followed by Bonferroni post test. *a*- significantly different from placebo-treated HLA-B27 rats; *b*- significantly different from placebo-treated F344 rats.

Figure 2. Basal Isc and transepithelial resistance (R) in colonic mucosal sheets isolated from HLA-B27 rats or F344 rats treated with placebo or rhIL-11 administered orally at a dose of 500 µg/kg given every other day during a 2-week treatment. Data are mean ± SEM from colonic tissue isolated from N=6 rats in each group. Differences between means were analyzed for statistical significance at $p<0.05$ using one-way ANOVA followed by Bonferroni post test. *a*- significantly different from placebo-treated HLA-B27 rats; *b*- significantly different from placebo-treated F344 rats; *c*- significantly different from rhIL-11-treated F344 rats.

Figure 3. Effect of SP (1 µM) on Isc and transepithelial resistance (R) in colonic mucosal sheets isolated from HLA-B27 rats or F344 rats treated with placebo or rhIL-11 administered orally at a

dose of 500 µg/kg given every other day during a 2-week treatment. For the purpose of clarity, Isc (A,B) or transepithelial R (C,D) measured immediately before administration of SP, and during the upward and downward phase of the effect were plotted using different time scales. Data are mean ± SEM from colonic tissue isolated from N=6 rats in each group.

Figure 4. Effects of electrical field stimulation (EFS: 0.5 ms, 5 Hz, 40-90 V) in colonic mucosal sheets isolated from HLA-B27 or F344 rats treated with placebo or rhIL-11 administered orally at a dose of 500 µg/kg given every other day during a 2-week treatment. EFS-induced changes in Isc were measured in untreated colonic mucosal sheets (A) or in the presence of atropine (1 µM) and guanethidine (10 µM) to obtain NANC responses (B). Data are mean ± SEM for colonic preparations isolated from N=6 rats for each group. Differences between means were analyzed for statistical significance at $p<0.05$ using one-way ANOVA followed by Bonferroni post test. *a*- significantly different from placebo-treated HLA-B27 rats; *b*- significantly different from placebo-treated F344 rats; *c*- significantly different from rhIL-11-treated F344 rats.

Figure 5. Concentration-dependent increase in Isc induced by carbachol (CCh) in mucosal sheets isolated from the colon of HLA-B27 or F344 rats treated with placebo or rhIL-11 administered orally at a dose of 500 µg/kg given every other day during a 2-week treatment. Data points are the mean ± SEM measured from colonic preparations isolated from N=6 rats for each group.

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TABLE 1. Colonic mucosal sheets isolated from F344 and HLA-B27 rats receiving oral treatment with placebo or rhIL-11 administered a dose of 500 µg/kg every other day during 2 weeks. Paracellular resistance (R) was calculated for the ascending and descending phase of the response to SP (1 µM) as described in the methods.

Group	**Treatment**	**Ascending phase Paracellular R (Ω/cm2)**	**Descending Phase Paracellular R (Ω/cm2)**
HLA-B27 (N=6)	placebo	17 ± 2 [b,c,d] (12-22)	16 ± 2 [b,c,d] (11-20)
HLA-B27 (N=6)	rhIL-11	30 ± 2[a,d] (24-36)	31 ± 2[a] (27-35)
F344 (N=6)	placebo	40 ± 2[a] (34-45)	40 ± 3[a] (33-47)
F344 (N=6)	rhIL-11	45 ± 2[a,c] (39-50)	39 ± 2[a] (34-45)

Data are mean ± SEM from N=6 rats for each group and the 95% CI are given in parenthesis. There was no significant difference between the paracellular resistance estimated from the upward and downward phase of the response to SP for each group. One-way ANOVA of the data for each phase showed that the variation among group means is significant ($P<0.0001$). Differences between groups are indicated as follows: [a]-significantly different from placebo-treated HLA-B27 rats; [b]- significantly different from placebo-treated F344 rats; [c] – significantly different from rhIL-11 treated HLA-B27 rats; [d]- significantly different from rhIL-11 treated F344 rats.

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TABLE 2. Analysis of carbachol-induced concentration-dependent increase in Isc in colonic mucosal sheets isolated from HLA-B27 transgenic rats or F344 rats receiving oral treatment with placebo or rhIL-11 administered at a dose of 500 µg/kg every other day during 2 weeks.

Group	**Treatment**	**E_{max}**	**Linear regression analysis**			
		(µA/cm^2)	**R**	**slope**	**position**	**EC_{50} (µM)**
HLA-B27	placebo	33[b,c] (21-44)	0.97	50.5	301	10.5[b,c] (8.9-12.0)
HLA-B27	rhIL-11	28[b,c] (15-40)	0.98	67.0	449	1.1[a,b] (0.9-1.3)
F344	placebo	98[a] (65-106)	0.98	49.3	312	4.8[a,c] (3.3-6.3)
F344	rhIL-11	101[a] (84-123)	0.98	67.5	440	1.2[a,b] (1.0-1.3)

The data are derived by regression analysis of the linear portion of the concentration-response curves and 95% CI are given in parenthesis. E_{max} – maximal response to carbachol; R – linear regression coefficient; pD_2 – negative log of the concentration of carbachol producing 50% of the maximal response (EC_{50}); [a] –significantly different from placebo-treated HLA-B27 rats, [b] – significantly different from placebo-treated F344 rats; [c]-significantly different from rhIL-11-treated F344 rats.

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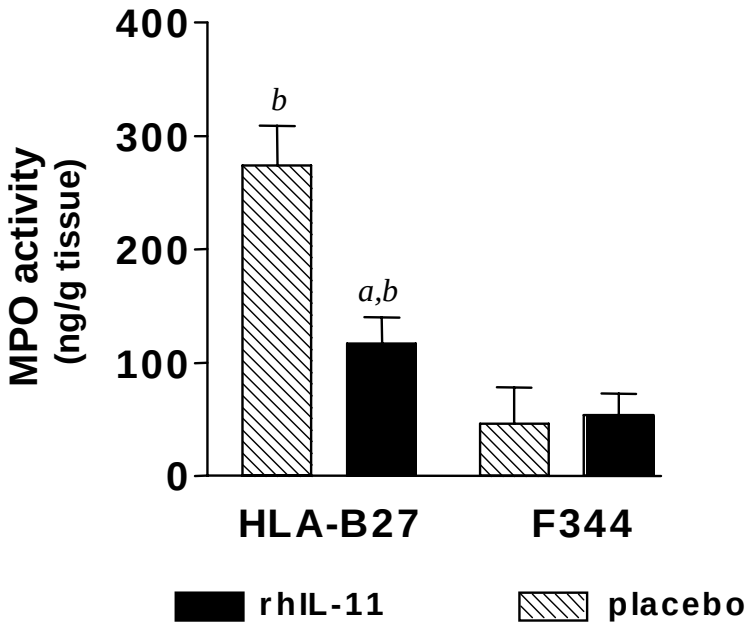

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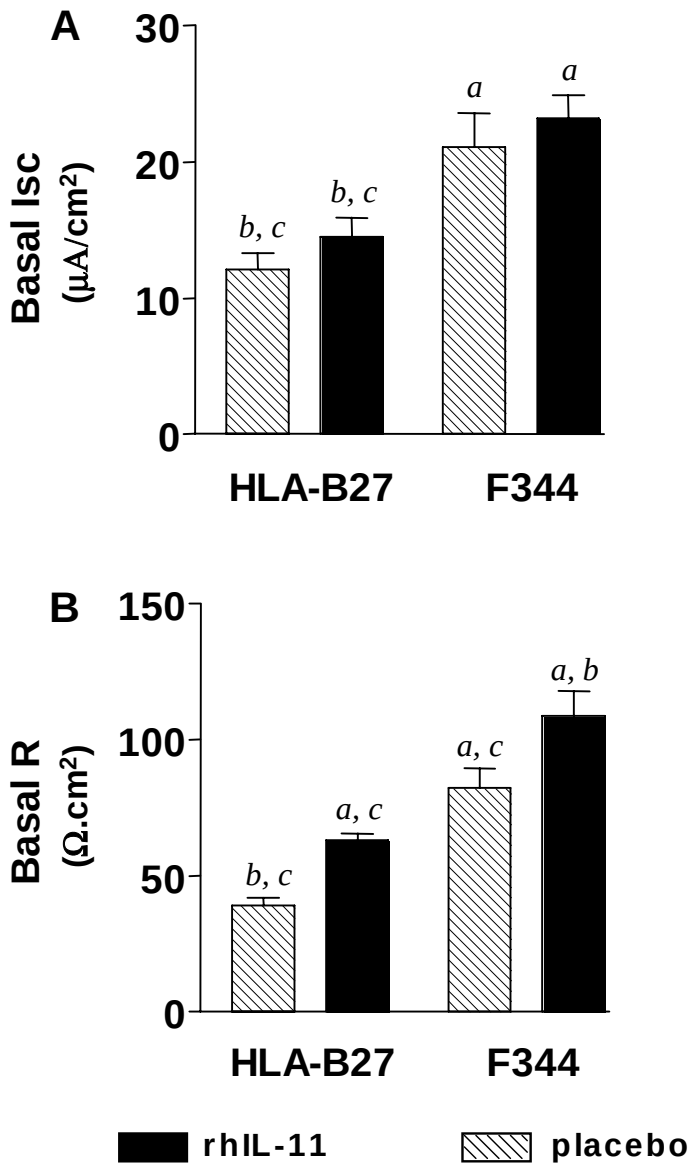

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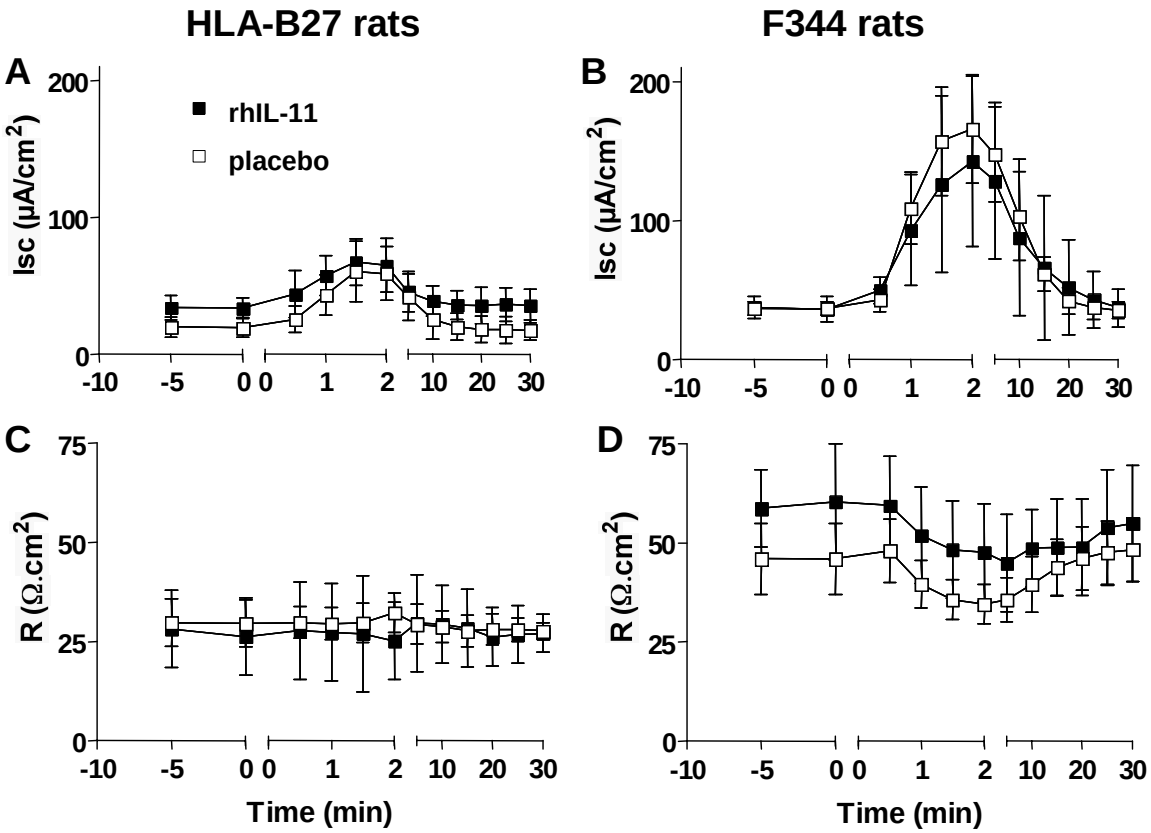

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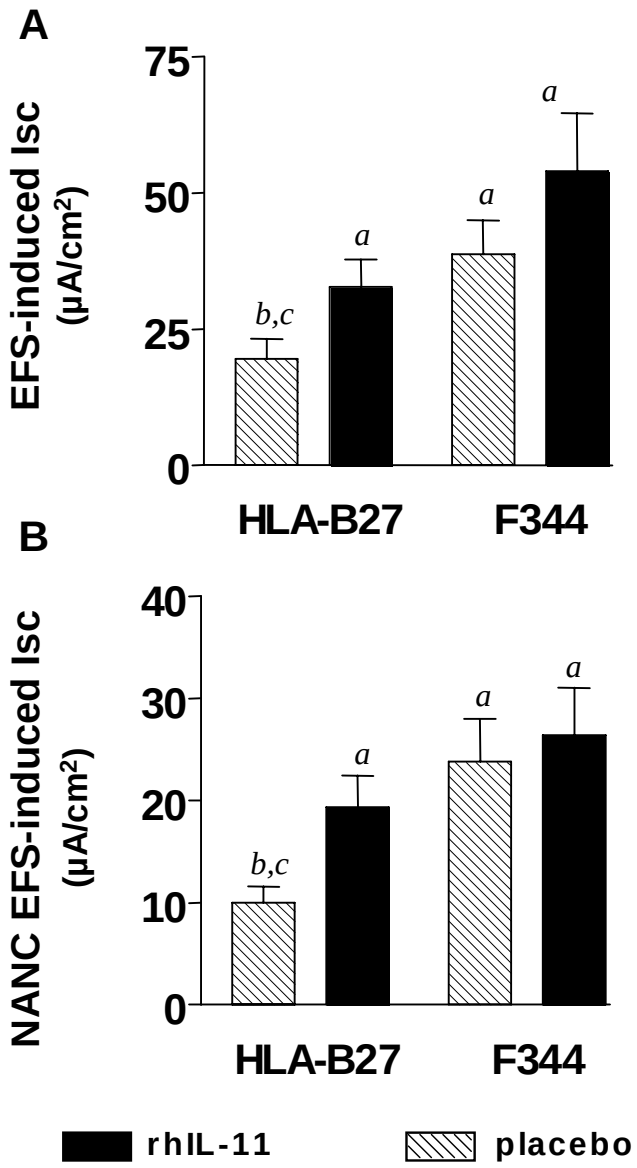

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