Interleukin (IL)-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 human leukocyte antigen (HLA)-B27 transgenic rat model of spontaneous chronic inflammation. Experiments were performed using adult male HLA-B27 rats, whereas healthy nontransgenic 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 by 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 with placebo. After 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 cholinergic receptor sensitivity was normalized. Treatment with rhIL-11 had no significant effect on basal short circuit current 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.
Oral rhIL-11 Improves Colonic Transport in HLA-B27 Rats 207

Materials and Methods

Experimental Animals. Male Fischer 344 rats genetically engineered to express human HLA-B27 and β2-microglobulin were used as a model of chronic inflammatory disease in humans (IBD). Age-matched nontransgenic Fisher 344 rats were used as controls. HLA-B27 transgenic rats (n = 12) or Fisher 344 rats (n = 12) were purchased from Taconic Farms (Germantown, New York) at 10 to 12 weeks of age and were housed in the animal facility until the age of 36 to 40 weeks. All rats were housed in individual cages at room temperature of 21°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 Subcommittee, Oklahoma City, OK, 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 colitis. The dose and time course of rhIL-11 treatment were selected from a previous study (Greenwood-Van Meerveld 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 dissolves 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 8:00 to 9:00 AM 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 nontransgenic F344 rats (n = 6) treated with enteric-coated rhIL-11; and a placebo-treated group of nontransgenic F344 rats (n = 6) receiving enteric-coated sucrose multi-particles.

Evaluation of Stool Character. Stool samples were collected and scored daily during the 2 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.

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 middle colonic segments. The tissue samples were immediately frozen in liquid nitrogen and stored at −80°C. The samples were analyzed simultaneously as described previously (Venkova et al., 2000). Briefly, homogenization and MPO extraction was performed in hexadecyltrimethylammonium bromide phosphate buffer (pH 6). MPO activity was measured in 10-μl samples using 3,3′,5,5′-tetramethylbenzidine TMB micro-well peroxidase substrate system (Sigma-Aldrich, St. Louis, MO) and horseradish peroxidase as a relative standard. MPO activity was expressed as equivalent to the activity of the relative standard (nanograms of horseradish peroxidase) converting the same amount of TMB substrate for 10 min at room temperature. Mean values are given in ng normalized per gram wet weight of the tissue.

Ussing Chamber Experiments. The colon was harvested, and 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% O2 and 5% CO2. Isolated mucosal sheets were obtained after removal of the outer muscle layer from segments (15 mm in length) cut from the middle 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-cm2 window opening) and electrogenic mucosal transport was measured as described previously (Greenwood-Van Meerveld et al., 2000). The luminal and serosal side were washed with modified Krebs’ buffer maintained at 37°C and continuously aerated with 95% O2 and 5% CO2. PD and Isc were measured by two pairs of agar-salt bridge electrodes connected to an EVC 4000 voltage/current clamp apparatus (WPI, 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 (ADInstruments Pty Ltd., Castle Hill, Australia). Basal PD was lumen negative and measured in millivolts. Basal Isc was measured in micromolar, and normalized for the mucosal surface area (micromolar per square centimeter). 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. Neuromediated changes in Isc were induced by electrical field stimulation (EFS) applied via a pair of fine 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 (40–90 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 (Riegler 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/cm²) was calculated for each time point and was plotted against the Isc (microampere per square centimeter). 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/ cm²) = 1/conductance (mS/cm²) × 100]. Because the secretory response to SP involves changes in transepithelial resistance alone, the paracellular resistance has the same value for the ascending and descending phase of the response to SP.

**Solutions and Drugs.** The experiments were performed using modified Krebs bicarbonate solution of the following composition: 120 mM NaCl, 6 mM KCl, 1.2 mM MgCl₂, 1.2 mM Na₂HPO₄, 2.5 mM CaCl₂, 1.44 mM NaHCO₃, and 11.5 mM glucose. The solution was continuously gassed with 95% O₂ and 5% CO₂ (v/v), 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 and were dissolved in distilled water. All drugs were added to the baths in volumes less than 1% of the total bath volume. The enteric-coated formulation of rhIL-11 and the enteric-coated sucrose multi-particles (placebo) were provided by Wyeth Research (Cambridge, MA).

**Statistical Analysis.** The data presented in the study are mean ± S.E.M. of six animals for each experimental group. In the Ussing chamber experiments, four to six 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 (EC₅₀) and the 95% CI. Homogeneity of variances, i.e., no significant difference between S.D. among the groups was verified using Bartlett’s test. Comparison between multiple groups was made using ordinary one-way ANOVA followed by Bonferroni’s 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 to 28 weeks and persisted for 4 to 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 seemed to be healthy and had normal stools (stool consistency score 0). In HLA-B27 rats, oral treatment with rhIL-11 caused a significant improvement in stool consistency compared with 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 toward 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 (scores 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-11 Treatment 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 Fig. 1, MPO activity was significantly higher in the colon of HLA-B27 rats receiving placebo compared with placebo-treated healthy F344 rats. Treatment with rhIL-11 significantly reduced MPO activity in the colon of HLA-B27 rats, whereas 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 with the levels of MPO measured in the F344 groups.

**Effect of rhIL-11 Treatment on Basal Electrographic 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 tranacellular and paracellular permeability for water and electrolytes. The HLA-B27 rats receiving placebo showed significantly lower values of basal Isc (Fig. 2A) and transepithelial resistance (Fig. 2B) compared with the placebo-treated F344 group. Treatment of HLA-B27 rats with rhIL-11 resulted in a significant increase in transepithelial resistance compared with placebo-treated HLA-B27 rats (Fig. 2B), whereas rhIL-11 had no significant effect on basal Isc (Fig. 2A). A relatively small but statistically significant increase in resistance was found in mucosal sheets isolated from the colon of HLA-B27 transgenic rats or F344 rats receiving either rhIL-11 or placebo treatment. As shown in Fig. 1, MPO activity was significantly higher in the colon of HLA-B27 rats receiving placebo compared with placebo-treated healthy F344 rats. Treatment with rhIL-11 significantly reduced MPO activity in the colon of HLA-B27 rats, whereas 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 with the levels of MPO measured in the F344 groups.

![Fig. 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 ± S.E.M. 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’s post test. a, significantly different from placebo-treated HLA-B27 rats. b, significantly different from placebo-treated F344 rats.](image-url)
from the colon of F344 rats treated with rhIL-11 compared with placebo-treated F344 rats.

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 of the response. The increase in Isc induced by SP in mucosal preparations isolated from HLA-B27 rats was of lower amplitude compared with F344 rats, whereas there was no significant difference between the SP-induced increase in Isc in placebo-treated or rhIL-11-treated rats (Fig. 3, A and B). Specifically, the maximal Isc induced by SP in the colon of HLA-B27 rats was $61 \pm 9 \mu A/cm^2$ for the placebo-treated rats and $67 \pm 7 \mu A/cm^2$ for the rhIL-11-treated rats, whereas the maximal response measured in F344 rats was significantly higher ($p < 0.01$), reaching $166 \pm 16$ and $143 \pm 25 \mu A/cm^2$, respectively. Concurrent with the increase in Isc, SP induced a decrease in transepithelial resistence in the colon of F344 rats, whereas no substantial changes of resistance were observed after SP administration to the colonic mucosa from HLA-B27 rats (Fig. 3, C and 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 (Riegler 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 (0.5 ms, 5 Hz) was applied to the mucosal sheets to induce a maximal secretory response measured as an increase in Isc. The re-
response to EFS was completely inhibited by tetrodotoxin (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 Fig. 4A, the EFS-induced increase in Isc was significantly lower in mucosal sheets isolated from placebo-treated HLA-B27 rats compared with 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 compared with placebo-treated F344 rats. However, when HLA-B27 rats received rhIL-11 treatment, the EFS-induced changes in Isc were measured in untreated colonic mucosal sheets isolated from placebo-treated HLA-B27 rats compared with placebo-treated F344 rats. No significant changes in EFS-induced Isc were found for the group of F344 rats treated with rhIL-11. After 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 nonadrenergic noncholinergic (NANC) nerve stimulation were investigated (Fig. 4B). Whereas the NANC responses were of lower amplitude compared with 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 with 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 cholinoreceptors 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 (Fig. 5). The maximal carbachol-induced response in mucosal sheets isolated from placebo-treated HLA-B27 rats was of significantly lower amplitude compared with placebo-treated F344 rats (28 ± 6 versus 98 ± 10 μA/cm²; 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 EC50 values calculated from the concentration-response curves (Table 2) showed that mucosal sheets isolated from placebo-treated

**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

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Paracellular R (Ω/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ascending Phase</td>
</tr>
<tr>
<td>HLA-B27 (n = 6)</td>
<td>Placebo</td>
<td>17 ± 28.4 (12–22)</td>
</tr>
<tr>
<td>HLA-B27 (n = 6)</td>
<td>rhIL-11</td>
<td>30 ± 24.2 (24–36)</td>
</tr>
<tr>
<td>F344 (n = 6)</td>
<td>Placebo</td>
<td>40 ± 27.4 (34–45)</td>
</tr>
<tr>
<td>F344 (n = 6)</td>
<td>rhIL-11</td>
<td>45 ± 29.1 (39–50)</td>
</tr>
</tbody>
</table>

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.

**Fig. 4.** Effects of 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 ± S.E.M. 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’s 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.

HLA-B27 rats yielded an EC50 value that was significantly higher compared with placebo-treated F344 rats. However, in both HLA-B27 or F344 rats treated with rhIL-11, the carbachol EC50 value was significantly lower compared with the respective group of placebo-treated rats.

**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 T helper 1 cytokines (Fiocchi, 1998). Therapies based on the antagonist activity of antibodies against tumor necrosis factor-α 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 a 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 *Clostridium 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, 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 after oral administration of the formulation. Experiments following the distribution of radio-labeled enteric-coated rhIL-11 confirmed intraluminal levels of rhIL-11 in the terminal ileum and proximal colon (Wyeth Research, unpublished data). Together, these results imply that the enteric-coated formulation designed for oral administration of rhIL-11 may provide topical action while preventing hematopoietic effects associated with systemic rhIL-11 administration. The use of HLA-B27 transgenic rats coexpressing the human major histocompatibility class I allele HLA-B27 and β2-microglobulin provided a model of T cell-dependent chronic multiorgan inflammation that is reminiscent of B27-associated spondyloarthritis and IBD in humans (Hammer et al., 1990). Our results confirmed that a 2-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 transmucosal 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, whereas 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 improve colonic active transport (basal Isc and induced Isc). However, the low basal transepithelial PD in the jejunum was restored after 2 weeks of 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 after 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 after 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 Pernue, 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 after 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,

![Graph](image-url)
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 administered 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 et al. (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 that 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 with 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 nerves 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 because 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 with 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 postinflammatory 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 with 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, whereas 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 postinflammatory remodeling of enteric neurons and a direct effect of rhIL-11 on postsynaptic receptors may partially compensate for the hyporesponsiveness of epithelial cells to secretagogues. The shift of the EC50 value for carbachol to lower concentrations after 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

### 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.

The data are derived by regression analysis of the linear portion of the concentration-response curves and 95% CI are given in parentheses.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>E_{max} (µA/cm²)</th>
<th>R</th>
<th>Slope</th>
<th>Position</th>
<th>EC_{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-B27</td>
<td>Placebo</td>
<td>33^{b,c} (21–44)</td>
<td>0.97</td>
<td>50.5</td>
<td>301</td>
<td>10.5^{b} (8.9–12.0)</td>
</tr>
<tr>
<td>HLA-B27</td>
<td>rhIL-11</td>
<td>28^{b,c} (15–40)</td>
<td>0.98</td>
<td>67.0</td>
<td>449</td>
<td>1.1^{b} (0.9–1.3)</td>
</tr>
<tr>
<td>F344</td>
<td>Placebo</td>
<td>98^{a} (65–106)</td>
<td>0.98</td>
<td>49.3</td>
<td>312</td>
<td>4.8^{a} (3.3–6.3)</td>
</tr>
<tr>
<td>F344</td>
<td>rhIL-11</td>
<td>101^{a} (84–123)</td>
<td>0.98</td>
<td>67.5</td>
<td>440</td>
<td>1.2^{a} (1.0–1.3)</td>
</tr>
</tbody>
</table>

a 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}).

b significantly different from placebo-treated HLA-B27 rats;

c significantly different from placebo-treated F344 rats; " significantly different from rhIL-11-treated F344 rats.

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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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References


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