Orally Administered Recombinant Human Interleukin-11 Is Protective in Experimental Neutropenic Sepsis

Steven M. Opal, 1 James C. Keith, Jr, 2 Jhung Jhung, 1 John E. Palardy, 1 Nicolas Parejo, 1 Erik Marchese, 2 and Vasu Maganti 2

1 Infectious Disease Division and Department of Pathology, Memorial Hospital of Rhode Island and Brown University School of Medicine, Providence, Rhode Island; 2 Department of Inflammation Biology, Discovery Research, Wyeth Research, Cambridge, Massachusetts

Recombinant human interleukin (IL–11) is a multifunctional cytokine with hematopoietic, immunomodulatory, and epithelial cell protective activities. IL-11 receptors are expressed on the luminal surface of intestinal epithelial cells. It was hypothesized that orally administered IL-11 would prevent mucosal damage and protect against microbial invasion in a neutropenic rat model of gram-negative sepsis. IL-11 was administered daily by enteric, coated multiparticle pellets over the course of chemotherapy-induced neutropenia. Compared with the placebo group, IL-11–treated rats retained mucosal mass and had prolonged survival time, reduced pathologic changes, and reduced systemic levels of bacterial endotoxin and concentrations of Pseudomonas aeruginosa in target tissues. Enterocyte messenger RNA levels for tumor necrosis factor–α and interferon–γ revealed that oral IL-11 reduced but did not prevent increased expression of these cytokine genes. These results indicate that orally administered IL-11 may preserve epithelial cell integrity in the presence of cytoreductive chemotherapy. This may represent a new treatment strategy for the prevention of infection in neutropenic hosts.

Interleukin (IL–11) is a 178-aa, nonglycosylated, multifunctional cytokine that belongs to the IL-6 family of gp130 receptor ligands [1]. IL-11 binds to its own unique α receptor and then forms a multimeric complex with the gp130 signal-transducing unit [2]. The physiologic effects of IL-11 vary, depending on the cell type, microenvironment, and receptor density on the surface of each target cell. The multitude of effects of IL-11 include its hematopoietic growth factor activity, immunomodulatory properties, and epithelial growth factor and cytoprotective activities [3, 4]. IL-11 already has been demonstrated to be an effective platelet restorative agent after cytoreductive chemotherapy [5]. IL-11 also functions as an anti-inflammatory cytokine with activities that are similar to those of Th2-type cytokines [6–8].

Among the more interesting properties of IL-11 are its activities on epithelial cell integrity and physiology. IL-11 has been shown to be a epithelial growth factor [9–11] and an antiapoptotic agent after radiation- or chemotherapy-induced mucous-membrane injury [12–14] and modulates local levels of proinflammatory cytokines in the intestinal mucosa [7, 13].

In an attempt to isolate the local gastrointestinal effects of IL-11 on intestinal epithelia, an oral formulation of recombinant human (rh) IL–11 in microparticles has been developed [15]. This preparation has been shown to deliver IL-11 to the small-bowel intestinal epithelium via the expression of the IL-11α receptor on the luminal side of enterocytes. The oral for-
ulation has proven to be well tolerated in experimental animals and to be effective within the lumen of the gastrointestinal tract, without detectable systemic absorption [15]. Because IL-11 has been shown to provide a survival advantage in a number of experimental animal models against chemotherapy [11, 16], radiation [12, 13], sepsis [17, 18], and immunologic [13, 19] damage to the gastrointestinal epithelium, the present study was conducted to confirm the efficacy of rhIL-11 delivered by the oral route. This mode of administration has several advantages, including the limitation of systemic effects of IL-11, local delivery of high levels of IL-11 to enterocyte populations, and increased efficiency of targeted therapy to the small-bowel epithelium [15]. The activity of orally administered IL-11 was investigated in the neutropenic rat model of gram-negative sepsis by an invasive strain of Pseudomonas aeruginosa. This model was designed to mimic the series of pathophysiologic events that result in gram-negative sepsis in neutropenic patients after cytoreductive chemotherapy for cancer treatment [16, 18]. The activity of IL-11 in the prevention of bacteremia, maintenance of mucosal integrity, and improvement in survival was analyzed in the experiments described here.

MATERIALS AND METHODS

Reagents and bacterial strains. The chemicals and reagents used in these experiments were obtained from Sigma, except for cefamandole, which was purchased from Eli Lilly. rhIL-11 was provided in an enteric-coated multiparticulate formulation from Wyeth Research. rhIL-11 was layered onto a sugar sphere, followed by layers of sealant and enteric coating, to generate multiparticulate particles containing ∼1.1 mg of active rhIL-11 per 100-mg pellet [15]. The P. aeruginosa strain used was strain 12.4.4, obtained as a gift from A. McManus (US Army Institute of Surgical Research, San Antonio, TX). This strain is a serum-resistant, human blood isolate belonging to Fisher-Devlin-Gnabasik immunotype 6.

Animal model. The neutropenic rat model of Pseudomonas aeruginosa sepsis has been described in detail elsewhere [16]. These experiments were conducted with specific pathogen–free female, albino Sprague-Dawley rats (Charles River Breeding Laboratories) weighing 150–200 g at the beginning of the experiment. Rats were kept in biosafety cabinets with 12-h day-night cycles and were allowed to feed and to drink sterile water ad libitum. Rats were given 7 days to acclimatize to the laboratory before the experiments began. Rats were treated with cefamandole (100 mg/kg intramuscularly daily), followed by oral colonization with P. aeruginosa strain 12.4.4 by orogastric feeding of 10^9 bacterial cfu at 0, 48, and 96 h. Cyclophosphamide (Bristol-Myers) was given intraperitoneally (150 mg/kg at 0 h and 50 mg/kg at 72 h). Rats were monitored daily with a clinical examination that included recording of body temperature by use of a noncontact, digital, infrared thermometer (Horiba Instruments–Marksen Sciences) and recording of body weight. Oral IL-11 or the identical multiparticulate pellets without IL-11 (control) were administered by orogastric feeding daily, beginning 1 day before the first dose of cyclophosphamide and continuing for a total of 12 days. For the survival study, 12 rats were randomly assigned to the oral IL-11 (0.5 mg/kg/day) group, and 11 rats received empty multiparticle pellets.

For the pathologic analysis, 16 rats were randomly assigned to receive oral IL-11 or empty multiparticle pellets. These rats were killed at day 8 and underwent immediate necropsy examination. The distal 10 cm of ileum were resected and used for histopathologic examination. The lumen was washed with PBS to remove residual particulate material before analysis and fixation. The mucosal surface epithelium was scraped off the bowel wall from a 10-cm length of ileum for measurement of mucosal mass.

Rats were assessed clinically and pathologically by daily assessments of body weight, body temperature, presence of bacteremia, circulating endotoxin levels, and pathologic evidence of damage to the gastrointestinal epithelium and liver by light microscopy and electron microscopy. The investigators who performed the animal experiments were not blinded to the treatment assignment; however, an independent pathologist who was unaware of the treatment assignment for each rat performed the light microscopic and electron microscopic interpretation. A pathologic injury scale of 0–4 was used to assess the degree of mucosal thinning, ulceration, and disruption. The height of the epithelial cells was measured by electron microscopy of sections from the basal-to-luminal surface of small-bowel enterocytes.

Quantitative mRNA determinations. Total RNA was extracted from liquid nitrogen snap-frozen tissues using Tri-Reagent (Molecular Research Center), according to the manufacturer’s specifications. In brief, 100 mg of tissue was homogenized in 1 mL of Tri-Reagent and was stored at room temperature for 5 minutes. The homogenate was centrifuged at 12,000 rpm for 5 minutes at 4°C to separate the supernatant from the aqueous–organic phase. The aqueous phase was collected and stored at −70°C. The RNA concentration was determined spectrophotometrically by absorbance at 260 nm. The mRNA expression of IL-11 was measured by reverse transcription–PCR using a one-tube, one-step kit (SuperScript II, Invitrogen). The specific primer and probe sets for IL-11 were designed using the PrimerExpress software (Applied Biosystems). The primers and probes were obtained from Integrated DNA Technologies. The 18S rRNA primer and probe sets were used as control. The cDNA samples were amplified in a 96-well plate in triplicate using a 7500 Real-Time PCR System (Applied Biosystems). The relative abundance of mRNA was calculated by the ΔΔCt method. Each sample was analyzed on three independent occasions.

Figure 1. Enterocyte height by electron microscopy and mucosal mass of small-bowel intestinal epithelium in oral interleukin (IL)–11–treated or control rats.
Figure 2. Transmission electron microscopic views of small-intestinal epithelium in control group (A and B) and oral interleukin (IL)-11–treated rats (C and D). Note loss of tight junctions (TJs), subcellular degeneration, and bacterial invasion in panels A and B. In contrast, oral IL-11–treated rats retained TJs and microvilli (MV), and there was no evidence of translocation of luminal bacteria (B). L, lumen; M, mitochondria; N, nucleus.
Temperature for 5 min. One-tenth volume of 1-bromo-3-chloropropane was added and shaken vigorously for 15 s and then kept at room temperature for 10 min before centrifugation at 12,000 g for 15 min at 4°C. The aqueous phase was removed and placed into a new tube, where RNA was precipitated for 10 min at −20°C using 0.5 mL of isopropanol. RNA was collected by centrifugation at 12,000 g for 10 min at 4°C. The supernatant was removed, and RNA was washed with 1 mL of 75% ethanol, followed by subsequent centrifugation at 12,000 g for 10 min. Ethanol was removed, and RNA was allowed to dry for 5 min. RNA was resuspended in diethylpyrocarbonate-treated sterile water.

Total RNA was treated with RQ1 DNase 1 (Promega) and RNase inhibitor (Five Prime Three Prime) for 1 h at 37°C. RNA cleaning was performed using Qiagen RNeasy Minicolumns, according to the manufacturer’s specifications. Reverse-transcriptase (RT) DNA polymerase chain reaction (PCR) was used to reverse transcribe and amplify 125 ng of total RNA using the TaqMan EZ RT-PCR kit (Perkin Elmer Applied Biosystems) with gene-specific forward and reverse primers and fluorescently labeled probe at the 5′ end with 6-carboxy-fluorescein [20, 21]. Primer and probe sequences were generated as normalized TaqMan units, as determined by a standard curve [20, 21].

Measurement of serum samples for rhIL-11. After dosing with rhIL-11, a sensitive ELISA was used to determine the serum levels of rhIL-11, as described elsewhere [15].

Statistical analysis. Numeric values are presented as mean ± SD. Continuous variables were analyzed by 1-way analysis of variance (ANOVA) for multiple groups, and a Mann-Whitney U test was used for 2 groups for nonparametric data. Survival functions are presented as a Kaplan-Meier plot, and differences in survival time were determined by ANOVA. P < .05 was considered to be significant.

RESULTS

The activity of oral IL-11 was assessed by standard histopathologic examination of the small-bowel intestinal mucosa, mucosal mass, and epithelial height by transmission electron microscopy. The results of the electron microscopic measurements and mucosal mass per 10 cm of small intestine are displayed in figure 1. Rats were randomly assigned to receive oral IL-11 multiparticle pellets (n = 12) or empty multiparticle pellets (n = 11). The histologic score (based on a standardized measure of degree of mucosal thinning, apoptosis, necrosis, and ulcer formation) was significantly reduced from a mean score of 1 ± 0.5 for rats in the IL-11 group to 2.5 ± 0.8 for rats in the control group (P < .01). Similarly, the mucosal mass was highly significantly reduced (P < .001) in the control group, compared with the well-preserved mucosal mass in the small bowel in the oral IL-11–treated rats (figure 1).

![Kaplan-Meier survival plot for rats randomly assigned to receive oral interleukin (IL)–11 (n = 12) vs. placebo control group (n = 11) over the course of 12 days after chemotherapy.](image)

**Table 1.** mRNA levels for rat tumor necrosis factor (TNF)–α and interferon (IFN)–γ genes in enterocytes, by treatment group.

<table>
<thead>
<tr>
<th>mRNA gene type</th>
<th>Oral IL-11 group</th>
<th>Control group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>0.77 ± 0.2</td>
<td>2.46 ± 1.6</td>
<td>.07</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>3.4 ± 2.5</td>
<td>7.5 ± 6.5</td>
<td>.06</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SD normalized TaqMan units. Reference standard is glyceraldehyde 3-phosphate dehydrogenase mRNA levels. IL, interleukin.
Electron microscopic sections of epithelial membranes from the small-bowel intestinal mucosa confirmed the findings observed with light microscopy. A representative section of the electron microscopic views in the control group and the oral IL-11 treatment group is displayed in figure 2. The tight junctions, microvilli, and enterocyte architecture were well preserved in the oral IL-11–treated rats, whereas the control rats showed loss of membrane integrity, loss of tight junctions, and evidence of microbial entry into the intercellular space between degenerating enterocytes.

The mRNA transcript quantities of enterocyte genes for TNF-α and IFN-γ are provided in table 1. The mean fluorescence intensity was calculated in relationship to a standardized curve normalized to murine GAPDH mRNA levels as a normal housekeeping gene function. The results are presented as gene-specific messages normalized to TaqMan units. All IL-11–treated rats showed a trend toward reduced levels of both TNF-α and IFN-γ gene expression, but these differences did not reach statistical significance (table 1).

A Kaplan-Meier survival plot for oral IL-11–treated neutropenic rats (n = 12) versus rats treated with the oral placebo (n = 11) is presented in figure 3. The oral IL-11–treated rats had a highly statistically significant improvement in survival over the 12-day course of the experiment (P < .001). Rats randomly assigned to receive oral IL-11 treatment had delayed and less pronounced body temperature elevation over the course of the neutropenic period and exposure to the oral P. aeruginosa microbial challenge (figure 4). Oral IL-11–treated rats also had significantly improved maintenance of total body weight over the course of the experiment, compared with the rats randomly assigned to the empty oral multiparticle pellet group (figure 5). No systemic rhIL-11 levels were detected after 7 days of daily oral administration of IL-11 at a dose of 0.5 mg/kg. Circulating endotoxin levels were significantly reduced 6 days after initiation of oral administration of IL-11, compared with endotoxin levels measured in rats given empty pellets. In addition, quantitative levels of the challenge strain of P. aeruginosa were markedly reduced in organ cultures from rats in the oral IL-11 group killed after 6 days of oral multiparticle treatment (figure 6).

**DISCUSSION**

The results of these experiments indicate that locally administered oral rhIL-11 provides a significant survival advantage against chemotherapy-induced cytotoxicity and bacterial sepsis in this experimental model of systemic infection due to P. aeruginosa. This model is designed to mimic the pathophysiologic sequence of events that frequently occurs in patients with febrile neutropenic cancer [16, 18]. In the presence of chemotherapy-induced neutropenia, microscopic ulcers in the gastrointestinal epithelium develop, which function as a portal of entry for endogenous gram-negative bacteria to translocate across the intestinal mucosal barrier and to gain access to the systemic circulation. Ways to provide mucosal protection in the face of chemotherapy have been sought for many years in an effort to prevent infections in this relatively common clinical situation [4, 10, 12, 16]. The results of the experiments described here suggest that such an approach may prove to be therapeutically useful in clinical medicine. A nontoxic [15], orally supplied mucosal defense strategy would be a useful adjunct in the care of compromised patients.

The precise mechanism of action of IL-11 at the level of the epithelial membrane is not completely understood. It is known that IL-11α receptors are expressed on the luminal surface of enterocytes, along with the ubiquitous gp130 receptor [4, 15, 22, 23]. Therefore, signal transduction of IL-11 activity should be feasible by the oral administration of IL-11. It has been shown that IL-11 defends against epithelial cell apoptosis, pro-
tects clonogenic stem cells from radiation- or chemotherapy-induced injury, and prevents increased intestinal permeability in a variety of experimental models of chemotherapy- or radiation-induced intestinal injury [4, 11, 18]. The present study verifies that oral IL-11 has its effects locally [24], because no systemic levels of IL-11 were measurable in this study’s experimental rats. IL-11 also is efficacious in a number of experimental models of gastrointestinal inflammation [25] and has shown promising clinical results in patients with inflammatory bowel disease [26].

IL-11 has modulating effects on cell cycling of epithelial cells in response to cellular injury. IL-11 has been shown to alter the phosphorylation of retinoblastoma protein kinase, a cell-cycling signal for entry of cells into the S cycle of replication [14]. IL-11 also has been shown to function as an epithelial growth factor in the presence of surgical excision of small-bowel mucosa and provides a survival advantage in animals with surgically created short-bowel syndrome.

The present study verifies the ability of IL-11 to preserve gastrointestinal membrane integrity and to limit the degree of translocation of bacterial endotoxin and viable bacteria into the systemic circulation of neutropenic rats. Maintenance of membrane integrity provides a significant survival advantage to rats receiving oral IL-11. Whether the benefits accrued from oral IL-11 in these experiments can be translated to a therapeutically viable treatment option for neutropenic patients remains to be demonstrated in future clinical trials.

References


Figure 6. Endotoxin levels in serum and organ colony-forming unit counts at day 6 of treatment with oral interleukin (IL)-11 (n = 16) or with empty multiparticle control (n = 16). LPS, lipopolysaccharide.