RAPID COMMUNICATION

A Bone Marrow Stromal-Derived Growth Factor, Interleukin-11, Stimulates Recovery of Small Intestinal Mucosal Cells After Cytoablative Therapy

By X.X. Du, C.M. Doerschuk, A. Orazi, and D.A. Williams

The proliferation of epithelial cells lining the small intestinal mucosa may be regulated by microenvironmental signals leading to differentiation of precursor cells in the small intestinal crypts. Proliferation of hematopoietic cells within the hematopoietic microenvironment is known to be regulated by a growing number of growth factors and cytokines. Interleukin-11 (IL-11), a growth factor isolated and cloned from an immortalized primate BM stromal cell line, has been shown to stimulate megakaryocyte, erythroid, and myeloid colonies and the number of immunoglobulin-secreting B lymphocytes in vitro and in vivo as well as accelerate the recovery of murine peripheral blood neutrophils and platelets, bone marrow cellularity, and myeloid/mixed progenitors in vivo after myeloablative therapy. In addition, IL-11 has been shown to increase peripheral platelet counts when administered to normal mice. Nonhematologic effects of IL-11 include inhibition of predipocyte differentiation in cell lines and in primary human long-term marrow cultures. These activities are distinct from the effects of several growth factors currently approved for use in the clinical setting or undergoing clinical trials.

Individuals receiving cytoablative therapy for cancer and leukemia as well as in preparation for BM transplantation are at risk for developing serious infections and bleeding. The infectious complications of such therapies are related to prolonged and severe neutropenia, as well as damage to the small intestinal mucosa barrier, leading to entry of gastrointestinal flora into the blood. Previous studies have documented that acute injury to rodent small intestinal mucosa from either chemotherapy or radiation leads to shortening of small intestinal mucosa villus length. This acute change is caused by cell death and continued migration of epithelial cells toward the apex of the villi in the absence of mitotic activity in the crypt base to replace differentiating cells.

To assess the functional significance of IL-11 administration in vivo, mice were given combination radiation/chemotherapy (combined modality, CM, therapy) with or without IL-11 administration. These animals are highly susceptible to sepsis from endogenous gastrointestinal bacteria caused by severe damage to the small intestinal mucosa. We found that IL-11 treatment results in a significant increase in survival of mice given combination radiation/chemotherapy. This increase in survival is associated with decreased bacterial foci in the liver, spleen, and mesentery, increased small intestinal villus length, increased villus/crypt ratio, and increased cycling of crypt cells. We conclude that IL-11 administration is associated with enhanced survival and evidence of rapid recovery of the small intestinal mucosa, which is severely damaged by these cytotoxic agents. This recovery was associated with an increase in the mitotic index of crypt cells and an increased frequency of staining of these cells with a monoclonal antibody to proliferating cell nuclear antigen, a member of the cyclin family of nuclear antigens.

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Fig 1. Survival of mice after combined chemotherapy/radiation. Results are from three independent experiments with a total of 13 mice in each group.

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recovery of small intestine mucosa after otherwise lethal doses of combined cytotoxic therapy. Therefore, IL-11 may prove useful in accelerating the recovery of both BM and small intestinal epithelial cells after cancer therapy.

MATERIALS AND METHODS

Mice and cytoablative therapies. Eight-week-old to 10-week-old C3H/HeJ mice (Jackson Laboratory, Bar Harbor, ME) were administered 5-fluorouracil (5-FU) (diluted in Hanks’ Balanced Salt Solution [HBSS] containing 0.025 mol/L HEPES) at 150 mg/kg body weight intraperitoneal (IP) 3 days before sublethal irradiation (6.0 Gy total body irradiation delivered from a Siemens 250 KVp x-ray therapy machine (Madison, WI), filtered with 1.0 mm Cu, giving a half value layer of 2.1 mm Cu at 50 cm source skin distance (SSD), and with a dose rate of 78.13 cGy/min). No BM infusions were given to these animals. Recombinant human IL-11 (rhIL-11) was administered beginning on the same day as irradiation. rhIL-11 (Genetics Institute, Cambridge, MA) was diluted in HBSS (GIBCO, Grand Island, NY) containing 0.1% bovine serum albumin (BSA) (wt/vol; Boehringer-Mannheim, Indianapolis, IN) and 0.025 mol/L HEPES (GIBCO). RhIL-11 (250 μg/kg body weight) was injected subcutaneously in 0.2 mL vol twice per day starting on the same day as irradiation. Control mice received the same volume of HBSS/0.1% BSA (vehicle injections). In some experiments, mice were irradiated with 7.0, 7.5, and 8.0 Gy γ-ray (using 137Cs source at 95.83 cGy/min) as above.

Fig 2. Histologic sections of small intestine. (A) Vehicle-treated control mice. (B) Mice treated with IL-11. The figure shows small intestine of mice killed 5 days after irradiation.

Fig 3. PCNA staining of small intestinal crypts. (A) Vehicle-treated control mice. (B) IL-11 treated mice. The figure shows PCNA staining from mice killed 2 days after irradiation.
IL-11 STIMULATES INTESTINAL MUCOSAL CELLS

Table 1. Effect of IL-11 on Small Intestine Crypt Cell Recovery Post 5-FU and Irradiation

<table>
<thead>
<tr>
<th>Day</th>
<th>BSA</th>
<th>IL-11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crypt Depth</td>
<td>Villus Length</td>
</tr>
<tr>
<td>1</td>
<td>76.8 ± 9.2</td>
<td>71.1 ± 4.7</td>
</tr>
<tr>
<td>2</td>
<td>123.4 ± 14.3</td>
<td>129.0 ± 7.8</td>
</tr>
<tr>
<td>3</td>
<td>154.5 ± 10.2</td>
<td>141.8 ± 19.9</td>
</tr>
<tr>
<td>4</td>
<td>141.5 ± 11.9</td>
<td>118.5 ± 14.0</td>
</tr>
<tr>
<td>5</td>
<td>120.6 ± 11.7</td>
<td>117.8 ± 7.7</td>
</tr>
</tbody>
</table>

Each number represents data from 3 animals; 10 crypts/animal, 10 villi/animal.
* P < .01 versus BSA group.
† P < .05 versus BSA group.

Histologic and morphometric analysis. Mice dying or killed after combined radiation/chemotherapy were autopsied and tissues fixed in 10% buffered formalin overnight within 12 hours after death. Tissues from each organ (liver, spleen, kidney, small intestine and mesentery, abdominal wall, lung, heart, and femurs) were embedded in paraffin wax using standard techniques. Four-micron sections were cut and stained with hematoxylin/eosin. Ten independent measurements of villus height, crypt depth, and metaphases/crypt per specimen of small intestine were made from mice killed daily using an objective mounted micrometer at 200 × magnification. Hepatic bacterial foci were counted both macroscopically as surface colonies and microscopically on randomly chosen liver histologic sections.

Immunohistochemistry. Proliferating cell nuclear antigen (PCNA) immunohistochemical staining was performed on 2-μm jejunal sections obtained from CM mice killed daily from day 1 through day 5 postirradiation (day 4 through 8 post-5-FU). Tissue was fixed in 10% formalin for 6 to 12 hours, paraffin embedded, and sections were dried on polylysine-treated glass slides. Slides were then deparaffinized and rehydrated. Endogenous peroxidase was quenched by a 5-minute incubation in 3% hydrogen peroxide. The slides were covered with normal goat serum for 20 minutes, incubated overnight at 4°C with PC10 antibody against PCNA (1:80; Dako, Santa Barbara, CA), and then stained for 3 minutes with a biotin-conjugated goat-antisheep antibody, followed by peroxidase-conjugated streptavidin (both Kirkegaard and Perry Laboratories, Gaithersburg, MD) for 30 minutes. The enzyme was developed with 3,3'-diaminobenzidine (Sigma, St Louis, MO). PCNA+ nuclei stained brown. The percentage of PCNA+ crypt cells and absolute number of PCNA+ cells per crypt were measured by counting 20 randomly chosen crypts/section/mouse.

Table 2. Effect of IL-11 on Proliferation of Small Intestine Crypt Cells as Quantitated by PCNA Staining

<table>
<thead>
<tr>
<th>Day</th>
<th>BSA</th>
<th>IL-11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of +Nuclei</td>
<td>+Nuclei/Crypt</td>
</tr>
<tr>
<td>1</td>
<td>17.0 ± 6.8</td>
<td>3.1 ± 1.4</td>
</tr>
<tr>
<td>2</td>
<td>1.9 ± 1.3</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>2.4 ± 1.7</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>1.3 ± 0.4</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>1.7 ± 0.4</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>

Each number represents data from 3 animals; 20 crypts/animal, 400 to 600 nuclei.
* P < .01 versus BSA group.
† P < .05 versus BSA group.

RESULTS

After treatment with 5-FU,14 and sublethal doses of X-irradiation, damage to the small intestine of C3H/HeJ mice is extensive and the majority of mice die within 10 days (Fig 1). Dying animals show wasting, diarrhea, and tilting and rotating indicative of central nervous system infection. Treatment of mice with 250 μg/kg/d of rIL-11 was associated with a significant increase in survival after this cytoablative therapy, in spite of no increase in peripheral neutrophil counts or BM myeloid progenitors (Fig 1). Additional experiments in which increasing doses and an alternative source of irradiation (137Cs) were administered confirmed the increase in survival in IL-11-treated mice over several dose ranges. The experiments tested radiation doses of 6.0, 7.0, 7.5, and 8.0 Gy (all after 150 mg/kg 5-FU) and included a total of 215 mice. Survival in the control group in all experiments was 27% compared with 64% in the IL-11–treated mice (P < .0001). IL-11 treatment was associated with a marked reduction in the number of bacterial foci in multiple organs with none detectable in many mice after sacrifice (data not shown). Microbiologic analysis of foci dissected from the liver of control mice uniformly showed that the causative organism was Escherichia coli, a common enteric organism in mice.

To determine the potential source of E. coli organisms shown in mice after CM treatment, histologic sections of the small intestine were examined in control and IL-11–treated mice sacrificed daily after irradiation. The small intestinal mucosa of control mice showed marked destruction of villus structure with shortening of the villus length, vacuolization and pyknotic nuclear structures (Fig 2A). In contrast, IL-11–treated mice showed mild changes in morphology of the SIV (Fig 2B). Morphometric analysis of crypt and villi length showed a significant increase in the ratio of the crypt depth/villi length in IL-11–treated mice compared with control mice (Table 1). Villus shortening was most prominent 24 hours after irradiation in both groups of mice. Villus length recovered quickly in IL-11–treated mice, while remaining abnormal in surviving control mice through day 9 (data not shown). The changes shown in control mice after CM treatment were qualitatively similar to changes noted previously after treatment with either 5-FU16 or radiation18 alone, although the magnitude of the damage and the length of time to recovery in the few control animals that survive...
over the first week are larger and longer than previous studies. These differences may be caused by the combination of high-dose chemotherapy followed by high-dose radiation during the postchemotherapy recovery phase.

Because the villus length is dependent on proliferation and differentiation of crypt stem and progenitor cells, the mitotic index of crypt cells was determined in control and IL-11-treated mice killed daily after irradiation. Significant increases in the number of mitoses/crypt (2.0 ± 0.5 vs 0.9 ± 0.4, IL-11 vs control, \( P < .001 \)) as well as in the number of mitoses/100 μm of epithelial basement membrane (0.22 ± 0.06 vs 0.10 ± 0.04, \( P < .01 \)) were seen on day 5 after irradiation in the IL-11–treated mice. Control mice surviving to day 9 postirradiation showed slightly increased numbers of mitotic crypt cells, but the numbers were still depressed compared with normal or IL-11–treated mice.

The increase in cell cycle activity after CM treatment and IL-11 administration was further characterized by staining with PC-10,20-22 a monoclonal antibody directed against PCNA, a member of the cyclin family of nuclear proteins (Table 2 and Fig 3). IL-11 administration was associated with a twofold to fivefold increase in the number of crypt cell nuclei staining with PC-10 on days 2 through 4 after irradiation. Taken together, these data show that IL-11 administration to mice after severe damage to the small intestinal crypt cells hastens recovery of the villus structure because of increased proliferation of presumably the crypt progenitor cell.

**DISCUSSION**

Cytotoxic agents used in BM transplantation and cancer therapy affect rapidly proliferating cells in both the BM and small intestine, leading to severe and often dose-limiting toxicities. IL-11 has been shown to have pleiotropic effects on reconstitution after ablative therapy depending on the model examined.6-10,23 The accelerated recovery of peripheral blood counts noted in the above studies are likely caused by effects of IL-11 on hematopoietic progenitor compartments. Girasole et al have also shown induction of osteoclast formation and stimulation of bone resorption in mice treated with IL-11 (G. Girasole, personal communication, October, 1993).

IL-11 has also been reported to have effects on several nonhematopoietic tissues. Kawashima et al13 cloned an identical protein, adipogenesis inhibitory factor, based on inhibition of fat accumulation in 3T3-L1 cells. Such inhibition of fat accumulation has also been shown in primary human long-term marrow cultures and was associated in these cultures with the apparent inhibition of differentiation of preadipocytes in the hematopoietic microenvironment.11 Mehler et al24 have shown that IL-11 has effects on neuronal differentiation. From these studies, it is clear that IL-11 has effects on a wide variety of tissues, particularly mesenchymal cells.

Several growth factors, including IL-125 and Steel factor,26 have been shown to be radioprotective in murine studies. Radioprotection by these growth factors has been reported to be the result of changes in the progeny of BM stem and progenitor cells. In contrast, in the study reported here, analysis of small intestinal mucosa showed rapid recovery of villi length and increased proliferative activity within the crypt cells of IL-11–treated mice compared with control mice, although it remains unclear whether this is a direct effect or one mediated through accessory cells. IL-11 may be a unique protein that has regulatory roles in a variety of complex environments containing stromal cells. The positive effects on the recovery of several tissues exhibiting dose-limiting toxicities after cytotoxic therapies may prove clinically useful in the future.

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