Interleukin-11: Review of Molecular, Cell Biology, and Clinical Use
By Xunxiang Du and David A. Williams

First isolated in 1990, interleukin-11 (IL-11) has proven to be a fascinating cytokine with pleiotropic effects on multiple tissues. Initially characterized as a hematopoietic cytokine with thrombopoietic activity, IL-11 has now been shown to be expressed and have activity in multiple other tissues, including brain, spinal cord neurons, gut, and testis. Yet to date, the physiologic role of this protein remains unknown. Our laboratory has recently generated a mutated allele of IL-11 in the mouse germline (X.D. and D.A.W., unpublished results, June 1997) and future studies of homozygous IL-11-deficient mice derived from these founder animals should illuminate the function(s) of this protein in vivo. In this article, we update current understanding of the biology of IL-11, concentrating on data published after the last comprehensive review published in 1994.1

Cloning and Genomic Characterization
Human IL-11 was cloned in 1990 and details of this cloning and early work on IL-11 have been summarized previously.1 More recently the murine IL-11 cDNA was cloned using an expression library generated from a lipopolysaccharide (LPS)-induced murine fetal thymic cell line (T2).2 The murine IL-11 CDNA shares 80% homology with human IL-11 at the nucleotide level.3 Both human and murine IL-11 genomic sequences consist of 5 exons and 4 introns and have been mapped to chromosome 19 at band 19q13.3-q13.4 Glu 16, Leu 17, Leu 22, Arg 25, Leu 28, Thr 31, Arg 32, Leu 34, and Lys 98) are located on the surface of the protein. Chemical modifications (alkylation or site-directed mutagenesis) of the Met8 residue results in a 25-fold decrease in vitro bioactivity of rhIL-11. Chemical modification of the Lys41 and Lys86 results in a 3-fold decrease in bioactivity. rhIL-11 lacking the four carboxyl-terminal residues has a 25-fold lower bioactivity and elimination of 8 or more carboxyl-terminal residues completely abolishes activity.9 The C-terminus of rhIL-11 is predicted to be helical and to be involved in the primary receptor binding site (site I). Important residues contributing to receptor binding in this site include Arg58, His153, Asp164, Trp165, and Arg168.8 Met12 is potentially involved in the receptor binding. In the region between Pro13 and Lys31, there are a number of residues (including Pro13, Glu16, Leu17, Leu22, Arg32, Leu38, Thr81, Arg52, Leu14, and Arg98) that are critical for the bioactivity of IL-11 and may constitute part of a gp130 binding site (site II).3 Lys41 and Lys86, as well as positively charged arginine residues, which

Protein Characterization
IL-11 precursor protein consists of 199 amino acids (aa), including a 21-aa leader sequence. The theoretical molecular weights of recombinant human (rh) and murine IL-11 are 19,144 daltons8 and 19,154 daltons,2 respectively. Mature human and primate IL-11 protein share 94% identity whereas human and murine proteins share 88% identity in the amino acid sequence.2,6,9 Although IL-11 is rich in proline residues (12%) and lacks cysteine residues (ie, lacks potential disulfide bonds), hIL-11 is highly helical (57% ± 1%) and is thermally stable (melting temperature [Tm] = 90°C).3 According to the structural model proposed by Czupryn et al,6 IL-11 contains a four-helix bundle topology (denoted A-D) whereby methionine residue 58 (Met58) and lysines (Lys41 and Lys86) are located on the surface of the protein. Chemical modifications (alkylation or site-directed mutagenesis) of the Met8 residue results in a 25-fold decrease in vitro bioactivity of rhIL-11. Chemical modification of the Lys41 and Lys86 results in a 3-fold decrease in bioactivity. rhIL-11 lacking the four carboxyl-terminal residues has a 25-fold lower bioactivity and elimination of 8 or more carboxyl-terminal residues completely abolishes activity.9 The C-terminus of rhIL-11 is predicted to be helical and to be involved in the primary receptor binding site (site I). Important residues contributing to receptor binding in this site include Arg58, His153, Asp164, Trp165, and Arg168.8 Met12 is potentially involved in the receptor binding. In the region between Pro13 and Lys31, there are a number of residues (including Pro13, Glu16, Leu17, Leu22, Arg32, Leu38, Thr81, Arg52, Leu14, and Arg98) that are critical for the bioactivity of IL-11 and may constitute part of a gp130 binding site (site II).3 Lys41 and Lys86, as well as positively charged arginine residues, which

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Table 1. Tissue/Cell Types Expressing IL-11

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell Type/Cell Lines</th>
<th>Inducers</th>
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<tbody>
<tr>
<td>CNS</td>
<td>Hippocampal neurons (H19-7)</td>
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<td>Spinal motor and sympathetic neurons</td>
<td>IL-1/β, PMA, calcium inophore</td>
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<td>Astrocytic glioblastoma (U373, U87)</td>
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<td>Thymus</td>
<td>Myeloid (T2)</td>
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<tr>
<td>Testis</td>
<td>Round spermatids</td>
<td>IL-1α, PMA</td>
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are found on the exposed face of helix C, may also be involved in receptor binding site II.\(^9\)

**REGULATION OF GENE EXPRESSION**

IL-11 is expressed in vivo in a wide range of normal adult murine tissues (including hematopoietic tissues) as detected by reverse transcriptase-polymerase chain reaction (RT-PCR).\(^10\) IL-11 is detected by in situ hybridization in neurons of the central nervous system (CNS) and in developing spermatogonia of testis, where expression is developmentally regulated.\(^10\) As summarized in Table 1, IL-11 gene expression is observed in a variety of cells of mesenchymal origin. Expression in these cells can be modulated by several inflammatory cytokines and agonists as well as hormones, either alone or synergistically. Signaling pathways involved in induction of IL-11 expression vary between different cell types. For instance, IL-11 gene expression induced by IL-1α and phorbol myristate acetate (PMA) in PU-34 cells is regulated mostly at the posttranscriptional level by increased IL-11 mRNA stabilization. IL-1α−induced IL-11 mRNA stabilization in these cells is effected through a tyrosine kinase pathway, whereas PMA-induced IL-11 mRNA stabilization is dependent on H7-sensitive serine/threonine kinases and protein kinase C (PKC) pathways. There are multiple regions (eg, 5′UTR, coding region, and 3′UTR) within the IL-11 mRNA involved in IL-1α− and PMA-induced IL-11 mRNA stabilization. In addition, the presence of ATTTA motifs in the 3′UTR of IL-11 mRNA may function as an RNA destabilizing sequence.\(^11\) Heparin, one of the extracellular matrix components that can trans-repress AP-1–mediated gene transcription, can also destabilize IL-11 mRNA after both IL-1α and PMA induction in PU-34 cells through competition for mRNA binding proteins.\(^12\) PKC-mediated signaling events may also be involved in the induction of IL-11 in connective tissues and osteosarcoma cell lines.\(^13,14\) Induction of IL-11 mRNA in these cells by the protein synthesis inhibitor, cyclohexamide, suggests that transcription of IL-11 is negatively regulated by protein(s) with short half-lives.\(^15\) In contrast to BM fibroblast cells, stimulation of IL-11 gene expression by IL-1α, transforming growth factor-β1 (TGF-β1) and TGF-β2 in respiratory epithelial and fibroblast cells is likely to be transcriptionally regulated\(^15,16\) via a pathway that is largely calmodulin-dependent and PKC-independent.\(^16\) In addition, increased intracellular calcium and inhibition of Na+/H+ pump activity can induce IL-11 mRNA accumulation in lung fibroblast cells. The synergistic effect of histamine and TGF-β1 in induction of IL-11 in human lung fibroblasts is, to a great extent, transcriptionally regulated and dependent on H1 receptors and a calcium/calmodulin-dependent activation pathway.\(^17\) Thus, regulation of IL-11 expression is complex and cell/tissue specific.

**HEMATOPOIETIC EFFECTS OF IL-11**

**Progenitor cells.** IL-11 acts synergistically with other early and late acting growth factors to stimulate various stages and lineages of hematopoiesis. In synergy with IL-3,18-20 IL-4,19-22 IL-7, IL-12,23-25 IL-13,26 stem cell factor (SCF),27 flt3 ligand,28 and granulocyte-macrophage colony-stimulating factor (GM-CSF),29 IL-11 stimulates the proliferation of primitive stem cells, multipotent and committed progenitor cells from various sources including cord blood,29,30 BM,1,3-33 and peripheral blood\(^1\) in different culture systems.\(^16,20,25\) This proliferation appears to be due to the entry of a quiescent (G0) population of these cells into active cell cycle\(^29\) as well as shortening of the cell-cycle time in some cells.\(^26\) In combination with other cytokines present in hematopoietic microenvironment, IL-11 may increase commitment of primitive stem cells into the multilineage progenitor compartment and stimulate proliferation and differentiation of committed progenitor cells.37 This observation is consistent with published data showing that ex vivo expansion of murine BM cells with the cytokines IL-3, IL-6, IL-
11, and SCF is associated with impaired engraftment of expanded cells in both normal and irradiated hosts.\textsuperscript{38,39} However, ex vivo expansion of BM cells using IL-11 and SCF can enhance short-term engraftment potential and such expanded cells have been shown to sustain hematopoiesis during serial transplants in lethally irradiated mice.\textsuperscript{40} In addition, chronic expression of IL-11 in hematopoietic cells via retroviral-mediated gene transfer appears to be associated with maintenance of a primitive population of cells after serial transplantation.\textsuperscript{41} The contradictory results from these studies may be due to different cytokine combinations or concentrations used in expansion of BM cells in vitro. Although in vivo IL-11 increases the cycling rates and absolute number of myeloid progenitors in both BM and spleen of normal mice,\textsuperscript{42} it has no effects on peripheral leukocyte counts when administered to normal rodents\textsuperscript{43,44} and nonhuman primates.\textsuperscript{45}

**Megakaryocytopoiesis and thrombocytopoiesis.** IL-11 acts synergistically with IL-3, thrombopoietin (TPO) (also termed megakaryocyte growth and development factor [MGDF]),\textsuperscript{46,47} or SCF\textsuperscript{48} to stimulate various stages of megakaryocytopoiesis and thrombopoiesis in both murine\textsuperscript{29,50} and human\textsuperscript{51-53} BM cells. In vivo treatment with IL-11 results in markedly increased cycling rates and absolute number of myeloid progenitors in both BM and spleen of normal mice,\textsuperscript{42} it has no effects on peripheral leukocyte counts when administered to normal rodents\textsuperscript{43,44} and nonhuman primates.\textsuperscript{45} Antiserum can also reduce IL-11-stimulated megakaryocyte colony formation by 90%, whereas anti–IL-3 antisera effects a 28% reduction in colony formation.\textsuperscript{57} These studies suggest that IL-11 effects on megakaryocytopoiesis and thrombopoiesis may be mediated in part via TPO. Recently, Weich et al\textsuperscript{57a} have shown that IL-11α chain mRNA was detected in purified human CD41a (+), CD14 (-) megakaryocyte precursors. Further, incubation of purified cells with rHuIL-11 led to rapid phosphorylation of the gp130 subunit of the IL-11 receptor, indicating direct activation of the receptor signaling subunit by IL-11. IL-11 and TPO can also synergistically stimulate the proliferation of dormant multilineage progenitors by shortening G<sub>0</sub>, and this effect can be completely abrogated by addition of ACK2, a neutralizing antibody to c-kit, the receptor of SCF,\textsuperscript{56} suggesting that the synergistic effects of IL-11 and TPO on multilineage cells may be mediated in part by SCF/c-kit interactions.

**Erythropoiesis.** IL-11 alone or in combination with other cytokines (IL-3, SCF, or erythropoietin [Epo]) can stimulate multiple stages of erythropoiesis using murine and human BM cells and fetal liver cells as targets.\textsuperscript{1,32,59} The in vitro effect of IL-11 on burst-forming unit-erythroid (BFU-E) formation cannot be abrogated by antibodies against SCF, IL-3, or granulocyte-macrophage CSF (GM-CSF), suggesting a direct effect of IL-11 on human and murine erythroid progenitors.\textsuperscript{60} In vivo studies of cytokine administration indicate that IL-11 and SCF may increase the input from a multilineage cell compartment into the erythroid lineage, whereas IL-11 and Epo may stimulate further amplification of erythroid cells. Moreover, IL-11 and SCF may lead to a redistribution of erythroid cells from BM to spleen.\textsuperscript{61}

**Myelopoiesis.** IL-11 also modulates the differentiation and maturation of myeloid progenitor cells. IL-11 in combination with SCF stimulates myeloid colony formation from murine Lin /Sca 1<sup>+</sup> BM cells. These colonies are composed mostly of granulocytes and myeloid blasts. The combination of IL-11 with IL-13 or IL-4 can reduce the proportion of granulocytes and blasts in myeloid colonies, with a concomitant increase in macrophages.\textsuperscript{26} Combination treatment with IL-11, SCF, and G-CSF in the newborn rat has been shown to significantly increase peripheral neutrophil counts.\textsuperscript{62,63}

**Lymphopoiesis.** IL-11 in combination with SCF or IL-4 effectively supports the generation of B cells in primary cultures of BM cells from 5-fluorouracil (5-FU)-treated mice.\textsuperscript{23,64,65} Similar effects have been seen with flt3/flk-2 ligand\textsuperscript{66} using unfractionated murine fetal liver cells and with SCF and IL-7 in fractionated cells.\textsuperscript{67} IL-11 and IL-4 can also reverse the inhibitory effect of IL-3 on early B-lymphocyte development.\textsuperscript{68} The promotion of B-cell differentiation may be mediated by T cells.\textsuperscript{58,69,70}

**Effects on hematopoietic microenvironment.** IL-11 was originally isolated from cells derived from the hematopoietic microenvironment (HM)\textsuperscript{5-7,71,72} and may act as a paracrine or autocrine growth factor in this environment. Addition of IL-11 to human long-term BM culture (LTBMC) significantly increases the cellularity of the adherent cells, inhibits adipose accumulation in adherent cells, and leads to enhanced hematopoiesis.\textsuperscript{72} Addition of IL-11 and SCF to bone marrow cultures derived from aplastic anemia patients significantly enhances the formation of an adherent stromal layer,\textsuperscript{72} suggesting that IL-11 may have therapeutic value in aplastic anemia patients with defects in the HM. BM fibroblast growth can also be stimulated by the presence of megakaryocytes and the evolution of myelofibrosis is often linked with abnormal megakaryocytopoiesis. IL-11 has been shown to modulate megakaryocyte-dependent BM fibroblast stimulation.\textsuperscript{73} IL-11 with other cytokines has been shown to mobilize primitive hematopoietic stem/progenitor cells both in vitro\textsuperscript{74} and in vivo.\textsuperscript{75} Treatment with IL-11 and SCF can enhance mobilization of long-term repopulating cells from the BM to the spleen and from the BM to the blood of splenectomized mice.\textsuperscript{76}

**NONHEMATOPOIETIC EFFECTS OF IL-11**

**Effects of IL-11 on epithelial cells.** As mentioned above, alveolar and bronchial epithelial cells produce large amounts of IL-11. The upregulation of IL-11 production by inflammatory cytokines, respiratory syncytial virus (RSV), and retinoic acid (RA) suggests that IL-11 may play an important role in pulmonary inflammation.\textsuperscript{77} IL-11 and IL-11Rα are also expressed in epithelial cells of the gastrointestinal (GI) tract.\textsuperscript{78,79} In vitro studies show that IL-11 can directly interact with GI epithelial cells and reversibly inhibit proliferation of the intestinal crypt stem cell lines (IEC-6 and IEC-18).\textsuperscript{80,81} Thus, IL-11 may be involved in the normal growth control of GI epithelial cells. IL-11—induced decrease in proliferation of these cells may due to prolongation of the G1-S phase transition which is also associated with accumulation of the
hypophosphorylated form of the retinoblastoma susceptibility gene product (pRB). In addition, IL-11 has been found to enhance GI absorption of iron in rats, which does not appear to be related to changes in erythropoiesis.

**Osteoclastogenesis.** IL-11 in combination with 1α,25-dihydroxyvitamin D3 [1α,25(OH)2D3] and parathyroid hormone (PTH) has been shown to stimulate osteoclast development and inhibit bone nodule formation in BM cultures and cocultures of BM with calvaria cells. Osteoblasts are important regulators of osteoclast-mediated bone resorption. The requirement of the presence of stromal/osteoblastic cells in IL-11-induced osteoclast development suggests that the effect of IL-11 may be mediated through the stimulation of stromal/osteoblastic cells.

The osteoblast-dependent bone-resorptive activity of IL-11 can be inhibited by the calcitonin and cyclooxygenase inhibitor, indomethacin. Neutralizing antibody to IL-11 can partially negate the bone resorptive effects of PTH and block IL-1, tumor necrosis factor (TNF), and 1α,25(OH)2D3-induced osteoclast development. IL-11 can be induced in both human and murine primary osteoblasts as well as osteoblast-like osteosarcoma cell lines (Table 1). IL-11 can be induced in both human and murine primary osteoblasts as well as osteoblast-like osteosarcoma cell lines (Table 1). Primary osteoblasts express both IL-11Rα and gp130 mRNA, and gp130 mRNA is upregulated by IL-1, PTH, and 1α,25(OH)2D3. Mature osteoclasts also express IL-11Rα mRNA. These studies suggest that IL-11 is an important osteoblast-derived paracrine regulator of bone metabolism and that both bone-forming and bone-resorbing cells are potential targets of IL-11 action.

**Neurogenesis.** Du et al recently showed that IL-11 mRNA is expressed in hippocampal neuronal cells and in motor and sympathetic neurons of the spinal cord. Exogenous IL-11 stimulates the proliferation of hippocampal neuronal progenitor cells (H19-7) in a dose-dependent fashion. In addition, it has been previously shown that IL-11 and several other hematopoietic growth factors are survival and/or differentiation factors for murine fetal hippocampal neuronal progenitors (MK31). The production of IL-11 by alveolar and bronchial epithelial cells may suggest that IL-11 is an important survival factor for sensory and motor neurons because the subepithelial space of lung is rich in nervous innervation and IL-11 stimulates production of substance P from sympathetic neurons. Previous investigators have speculated that mechanisms regulating the proliferation and differentiation of neural and hematopoietic cells may be similar.

**Other effects.** IL-11 has also been shown to have other nonhematopoietic activities such as stimulation of acute phase reactants both in vitro and in vivo, inhibition of adipogenesis, induction of a febrile response, and modulation of extra cellular matrix (ECM) metabolism, which may have a protective effect on connective tissues or could be involved in the pathogenesis of liver fibrosis and cirrhosis. In several in vitro cell culture systems, IL-11 appears to reduce pro-inflammatory cytokine expression, particularly the release of tumor necrosis factor-α (TNF-α) by monocytes/macrophages.

**RECEPTOR AND SIGNAL TRANSDUCTION**

IL-11, like IL-6, oncostatin M (OSM), leukemia inhibitory factor (LIF), and ciliary neurotrophic factor (CNTF), uses the gp130 receptor common subunit for receptor function (Fig 1). Hilton et al have cloned the murine IL-11 receptor particularly the release of tumor necrosis factor-α (TNF-α) by monocytes/macrophages.

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bound form of Ras and induce the tyrosine phosphorylation and activation of mitogen-activated protein kinase (MAPK), a key downstream signaling target of Ras. After activation of IL-11 receptor by IL-11 binding, Jak2 forms a complex with the adapter protein, growth factor receptor binding protein 2 (Grb2), and gp130, thus bringing SOS (Son Of Sevenless) to the plasma membrane where Ras is located, hence activating Ras and initiating the Ras signaling pathway. In addition to use of gp130 as a common subunit in signal transduction, the association of Jak and Ras signaling pathways on stimulation with IL-11 and similar cytokines is another unique feature of this family of cytokines. IL-11 and other cytokines using gp130 as a signal transducer can trigger the activation of MAPKs and the 85-92-kD ribosomal protein S6 kinase (pp90rsk.), which is followed by activation of a set of common primary response genes (Egr-1 or TIS 8, TTP or TIS 11, Jun B and 3CH134, which encodes a phosphatase which can inactivate MAPKs). Src-family protein tyrosine kinases, including Fyn, Yes, and Src, may also play an important role in IL-11 signaling. Jak2 and Fyn are transiently associated with Grb2 upon stimulation with IL-11, suggesting that IL-11-induced signaling in the Ras/MAPK pathway is partly through Fyn. Stimulation of 3T3-L1 cells with IL-11 results in a threefold increase in tyrosine phosphorylation of p62 and a 15-fold increase in phosphorylation of pp60src.

In addition to MAPK phosphatase (3CH134), the ubiquitous tyrosine phosphatase Syp also associates with gp130 and Jak2 in response to IL-11 stimulation. Herbimycin A, which is a tyrosine kinase inhibitor, can block the activation of MAPK and pp90rsk induced by IL-11. A serine/threonine kinase inhibitor H7, which may act on signaling pathways downstream of pp90rsk, can inhibit pp90rsk activity, suggesting H7-sensitive kinases are crucial in IL-11 signaling.

Lipid second messengers are also involved in IL-11 signal transduction. IL-11 treatment in 3T3-L1 cells activates phospholipase D to produce phosphatidic acid (PA). Increased levels of PA enhance tyrosine phosphorylation of MAPKs and transduce some signals in this cell line. IL-11-induced phosphorylation of tyrosine kinases and H7-sensitive kinases are PKC-independent and cAMP-, cGMP-, and calcium/calmodulin-independent. IL-11 and other cytokines sharing the signal transducing subunit, gp130, can activate acute-phase response factor (APRF) by tyrosine phosphorylation in variety of cell types. This transcriptional factor is antigenically and functionally related to members of the signal transducer and activator of transcription (STAT) family, especially STAT91. STAT91 and related proteins were originally identified as interferon-activated transcription factors. This suggests a central role APRF in gp130-mediated signaling. IL-11 also stimulates tyrosine phosphorylation and nuclear translocation of STAT91 and a related 89-kD protein. The possible signaling pathways mediated by IL-11 are summarized in Fig 1.

**PRECLINICAL STUDIES**

*Syngeneic BM transplant (BMT) models.* Administration of IL-11 accelerates recovery of megakaryopoiesis and myelopoiesis in BMT mice (Table 2). Enhanced recovery of these lineages is associated with significantly decreased mortality and morbidity from lethal exogenous infection with *Pseudomonas aeruginosa* and decreased mouse-tail bleeding time. BMT recipient mice treated with the combination of IL-11 and SCF show shortened periods of cytopenia in all myeloid lineages. Lethally irradiated mice transplanted with syngeneic BM cells infected with a retrovirus expressing the human IL-11 cDNA demonstrate similar hematological changes as seen in BMT recipient mice treated with rhIL-11 until day 28 post BMT. However, in one such study, while elevated peripheral platelet counts were sustained chronically, no changes in peripheral erythrocyte or leukocyte counts were observed long term despite a greater than 20-fold increase in splenic myeloid progenitor content. Two of 20 secondary recipients of BM cells transduced with a retrovirus expressing hIL-11 cDNA developed myeloid leukemia. All mice showed systemic effects of chronic IL-11 exposure (Table 2). A recent study has shown that ectopic expression of murine IL-11 via a retrovirus vector...
accelerated recovery of platelets and leukocytes (neutrophils) in secondary and tertiary BMT mice. This study also suggests that IL-11 expression in vivo may enhance maintenance of hematopoietic stem cells.41

Sublethal radiation (non-BMT) models. In contrast to the effects in BMT models, IL-11 treatment has little effect on progenitor compartments in sublethally (600 cGy) irradiated mice.122 IL-11 treatment was shown to restore thymus and spleen cell numbers as well as T- and B-cell mitogen responsiveness in mice exposed to 200 cGy irradiation (Table 2). Sublethal irradiated dogs (200 cGy) treated with IL-11 show a modest trend toward faster platelet recovery. Some of the dogs in this study demonstrated pneumonitis, the etiology of which is unclear.128

Chemotherapy models. Chemotherapy is often associated with blood cytopenias and immunosuppression as well as GI mucosal damage. IL-11 treatment significantly reduces chemotherapy related morbidity and mortality,129,132 and is associated with accelerated recovery of both hematopoiesis42,132 and the immune response,133,134 in different chemotherapy preclinical models (Table 2). Mortality associated with repeated doses of 5-FU is abrogated by pretreatment with IL-11 and SCF, but not by infusion with BM cells, suggesting that in this model IL-11 and SCF pretreatment may protect tissues other than hematopoietic tissues adversely affected by chemotherapy.129 In a hamster model of oral mucositis, IL-11 decreases the frequency, severity, and duration of oral mucositis in a dose-dependent fashion131,133 with little changes on BM cellularity, strengthening the suggestion that the protective mechanism of IL-11 on mucositis is due, at least in part, to effects on epithelial and/or connective tissues.133

Combined chemo-radiation therapy models. IL-11 administration markedly decreases morbidity and mortality due to sepsis by endogenous gut organisms79 and accelerates recovery of spermatogenesis10 in mice treated with combined chemo-radiation therapy (5-FU and sublethal irradiation). The increased survival is associated with increased proliferation of crypt cells and decreased apoptosis of villous/crypt cells.134 The seemingly contradictory effects of IL-11 on GI crypt cell proliferation seen in vitro78,80 and in vivo studies may be due to distinctly different effects on damaged versus undamaged cell populations; inhibition of proliferation before damage (seen in in vitro cell lines) and stimulation of proliferation post damage (seen in vivo models of gut cell damage). This explanation is supported by the finding that pretreatment of mice with IL-11 followed by irradiation is associated with significant increases in the survival of intestinal crypt stem cells.135 In addition, recent studies show pretreatment of mice with IL-11 significantly reduces ischemia/reperfusion-induced small-bowel injury.136 The effect of IL-11 on combined chemo-radiation therapy–induced gut mucosal damage may prove to be important in clinical use in cancer chemotherapy and BM transplant protocols in the future. The effects of IL-11 on cytoablative preclinical models are summarized in Table 2.

Other GI disease models. Acute colitis caused by chemical damage and chronic inflammatory bowel disease in transgenic animals expressing human HLA-A2-B7 and β2-microglobulin are improved at both the gross and microscopic level by administration of IL-11.131 IL-11 treatment has proliferative effects on intestinal mucosa in mice after ischemic bowel necrosis,136 in a murine burn model,137 and in a rat short-bowel model.138 In all of these models, significantly increased survival rates are seen in mice treated with IL-11. IL-11 treatment also increases peripheral lymphocyte counts and decreases enteric bacterial translocation in both bowel ischemia and systemic burn models.

Sepsis models. Pretreatment with IL-11 significantly reduces mortality in a murine model of toxic shock syndrome139 and in experimental group B streptococcal (GBS) sepsis in neonatal rats.140 Endogenous IL-11 may play a role in the pathophysiologic response of neonatal animals to bacterial sepsis and associated thrombocytopenia.140 In a rabbit model of endotoxemia, IL-11 treatment prevents hypothermia and decreases GI mucosal damage induced by lipopoly-

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### Table 2. Effects of IL-11 on Cytoablative Preclinical Models

<table>
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<tr>
<th>Models</th>
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<td>Cellularity?</td>
<td>CFU-mix†</td>
<td>T- &amp; B-cell function?</td>
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<td>Pancytopenia</td>
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<td>Chemoradiation therapy</td>
<td>Late recovery of</td>
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<td>Pit &amp; Hct</td>
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<td>Mild *Pit at early</td>
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Abbreviations: CFU-mix, colony-forming unit-mix; CFU-GM, colony-forming unit-granulocyte/macrophage; CFU-MK, colony-forming unit-megakaryocyte; WBC, white blood cells; N, neutrophils; Pit, platelets; Hct, hematocrit; SRBC, sheep red blood cell; PCNA, proliferating cell nuclear antigen.
The anti-inflammatory effects of IL-11 on both murine and rabbit models of endotoxemia appear to be due to inhibition of the production of proinflammatory mediators through effects on macrophages.142

IL-11 AND DISEASES

IL-11 acts as a synergistic factor with IL-3, GM-CSF, and SCF to stimulate proliferation of human primary leukemia cells, myeloid leukemia cell lines,143,144 megakaryoblastic cell lines,145 and erythroleukemic cell lines188 and to stimulate leukemic blast colony formation.143,144 IL-11 mRNA expression in leukemic cells and inhibition of leukemic cell growth by IL-11 antisense oligonucleotides suggest that IL-11 may function as an autocrine growth factor in leukemic cell lines.144,145 Although IL-11 stimulates the proliferation of murine plasmacytoma cells146 and murine hybridoma cells,147-149 the effect of IL-11 on the growth of human myeloma/plasmacytoma cells is controversial. IL-11 has no effect on the growth of freshly isolated human plasmacytoma cells.146,148 However, IL-11 can stimulate proliferation in two of eight human myeloma cell lines tested so far.146,151 As expected, anti-gp130 monoclonal antibodies can inhibit growth stimulation by IL-11 in human myeloma cell lines.152 The plasmacytoma growth inhibitor restrictin-P (also called activin A, follicle-stimulating hormone releasing protein, or erythroid differentiation factor), another growth regulatory protein derived from BM stromal, can inhibit the growth of IL-11-stimulated murine hybridoma cells.153

HUMAN STUDIES AND CLINICAL TRIALS

IL-11 has now been evaluated in several human clinical trials. In the initial phase I trial, women with advanced-stage breast cancer undergoing high-dose chemotherapy were treated with increasing doses of IL-11 (up to 100 μg/kg/d) both before therapy and after each of four cycles of combined chemotherapy. IL-11 administration was associated with a dose-dependent trend toward increased platelet counts, and patients receiving rhIL-11 at doses ≥25 μg/kg/d showed attenuated postchemotherapy thrombocytopenia after the first and second cycles.55 Increased peripheral platelet counts were associated with both stimulation of platelet production and megakaryocyte maturation, as evidenced by increased numbers of BM colony-forming unit-megakaryocyte (CFU-MK), increased megakaryocyte numbers, and higher megakaryocyte ploidy.56 In contrast to effects seen in various preclinical studies, IL-11 treatment in this trial had no significant effect on leukopenia or neutropenia due to chemotherapy.55 However, IL-11 treatment was associated with increased BM cellularity, and increased numbers and cycling of immature erythroid and myeloid precursors.56

IL-11 treatment in these patients was well tolerated at doses of 10 to 50 μg/kg/d. The most common side effect noted was a reversible anemia. The anemia was non–dose-related and decreases of ≈20% in hematocrits, possibly due to increased plasma volume, were seen.55,154 Other reversible side effects included arthralgias, myalgias, fatigue, nausea, headache, and edema. Unlike many other cytokines, IL-11 treatment was not associated with an increased incidence of fever. IL-11 administration increased the plasma concentrations of acute-phase reactants, including C-reactive protein, fibrinogen, and haptoglobin at all doses.55

In several phase I/II trials, IL-11 has also been well tolerated in doses up to 50 μg/kg/d and appears to be a promising agent for accelerating hematopoietic recovery after multiple cancer therapies. The combined administration of IL-11 with G-CSF (5 μg/kg/d) in breast cancer patients receiving high-dose cyclophosphamide, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), and thiopeta followed by autologous BMT effectively accelerates both peripheral neutrophil and platelet recoveries.55 In a phase I/II trial in children with solid tumors or lymphoma, IL-11 (50 μg/kg/d) and G-CSF (10 μg/kg/d) administration after ICE (ifosfamide, carboplatin, and etoposide) chemotherapy appears to decrease the median number of platelet transfusions required (12 v 2), and reduces the days to recovery of both neutrophils (21 v 17.5 days) and platelets (27 v 22 days) when compared to ICE + G-CSF alone.56 Preliminary results from both trials cited above have not been reported in full at this point and it is not clear whether these differences are significant.

A multicenter, randomized, placebo-controlled IL-11 phase II clinical trial has been conducted in 93 cancer patients who had received at least one platelet transfusion during a prior chemotherapy cycle (secondary prophylaxis design). These patients were given an additional cycle of the same chemotherapy without dose reduction and were randomized to receive either rhIL-11 (at a dose of 25 or 50 μg/kg) or placebo. The patients treated with rhIL-11 in this phase II study were less likely to require platelet transfusions than the patients receiving placebo. For the patients treated with IL-11 at 25 μg/kg and 50 μg/kg, 30% (8 of 27) required no platelet transfusions compared to 1 of 27 patients treated with placebo. This difference was statistically significant (P < .05). The median number of platelet transfusions required among the groups treated with 50 μg/kg, 25 μg/kg, and placebo was 1, 2, and 3, respectively. The profile of side effects was similar to that seen in phase I studies. Most side effects were mild to moderate in severity and were reversible after IL-11 treatment was discontinued.157,158 Based on observations of the potent effects of IL-11 in models of gut damage, a major advantage of IL-11 may be the simultaneous effects of the cytokine on both BM and GI toxicities of chemotherapy and irradiation. A dose-escalating phase II randomized placebo-controlled human study examining the effects of IL-11 in patients with Crohn’s disease has recently been completed.168 Based on the results of this trial, additional trials in Crohn’s disease and in chemotherapy-induced mucositis are anticipated (Genetics Institute, personal communication, James Kaye, October 1996).

The recent cloning of the ligand for c-mpl159,162 provides another, and potentially very useful, therapeutic approach to thrombocytopenic states. Early trials with TPO (also termed MGDGF) appear promising and it will require multiple trials in various pathologic conditions to determine optimal cytokine combinations to enhance recovery of hematopoietic lineages with the least side effects. At the present time it would appear that IL-11 will be a useful thrombopoietin and may be uniquely useful in stimulating the recovery of the BM and the GI tract simultaneously after therapy-induced damage.
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