Interleukin-11: Review of Molecular, Cell Biology, and Clinical Use

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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 Our laboratory has recently generated a mutated allele of IL-11 in the mouse germline (X.D. and D.A.W., unpublished results, January 1997) and future studies of homozygous IL-11-deficient mice derived from these founder animals should illuminate the function(s) of this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.

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. 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). The murine IL-11 cDNA shares 80% homology with human IL-11 at the nucleotide level. Both human and murine IL-11 genomic sequences consist of 5 exons and 4 introns and have been mapped to chromosome 19 band 19q13.3-q13.4 and to the centromeric region of chromosome 7, respectively and (M. McAndrew-Hill and D.A.W., unpublished results, June 1996). The 5′-flanking region of the human IL-11 gene contains several DNA motifs postulated to be involved in transcriptional control. A “TATA” box-like sequence, TATATAA, is located 180 nucleotides upstream from the translation initiation codon ATG. A 10-bp promoter sequence (5′ GGGTAGTGCAG 3′) in this region contains an activator protein-1 (AP-1) site (underlined). JunD/ AP-1 complexes are responsible for the basal-level transcription of IL-11 gene in bone marrow (BM) fibroblast cells. There are two polyadenylation sites located in the 3′ untranslated region (UTR) at nucleotide positions 6762 and 5591 and these alternative sites give rise to the 2.5- and 1.5-kb IL-11 mRNA transcripts expressed in several IL-1α-induced cell lines.

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 daltons and 19,154 daltons, respectively. Mature human and primate IL-11 protein share 94% identity whereas human and murine proteins share 88% identity in the amino acid sequence. 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). According to the structural model proposed by Czupryn et al, IL-11 contains a four-helix bundle topology (denoted A-D) whereby methionine residue 58 (Met58) and lysines (Lys41 and Lys45) are located on the surface of the protein. Chemical modifications (alkylation or site-directed mutagenesis) of the Met58 residue results in a 25-fold decrease in in vitro bioactivity of rhIL-11. Chemical modification of the Lys41 and Lys46 residues 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. 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 Arg13, His135, Asp166, Trp165, and Arg166. Met58 is potentially involved in the receptor binding. In the region between Pro13 and Lys41, there are a number of residues (including Pro13, Glu16, Leu17, Leu22, Arg23, Leu28, Thr31, Arg32, Leu34, and Arg36) that are critical for the bioactivity of IL-11 and may constitute part of a gp130 binding site (site II). Lys41 and Lys46, as well as positively charged arginine residues, which
Table 1. Tissue/Cell Types Expressing IL-11

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell Type/Cell Lines</th>
<th>Inducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>Hippocampal neurons (H19-7)(^{10})</td>
<td>IL-1(^{2}), PMA, calcium inophore</td>
</tr>
<tr>
<td></td>
<td>Spinal motor and sympathetic neurons(^{18})</td>
<td>LPS</td>
</tr>
<tr>
<td></td>
<td>Astrocytic glioblastoma (U373, U87)(^{10})</td>
<td>IL-1(^{2}), PMA, calcium inophore</td>
</tr>
<tr>
<td></td>
<td>Myeloid? (T(^{2}))</td>
<td>LPS</td>
</tr>
<tr>
<td>Thymus</td>
<td>Fibroblast (MRC5, CCL202)(^{10})</td>
<td>IL-1(^{2}), TGF-(\beta_{1,2}), PMA, RSV, Ca(^{2+})/calmodulin</td>
</tr>
<tr>
<td></td>
<td>Epithelial (BHTE, A549)(^{10,17})</td>
<td>Rhinovirus, parainfluenza type 3, histamine (H(_{1}))</td>
</tr>
<tr>
<td>Lung</td>
<td>Muscle cells</td>
<td>IL-1(^{2}), TGF-(\beta_{1})</td>
</tr>
<tr>
<td></td>
<td>Fibroblast (P-34, KM102)(^{3,7,11})</td>
<td>IL-1(^{2}), PMA, PKC</td>
</tr>
<tr>
<td></td>
<td>Osteosarcoma cell lines(^{3,32})</td>
<td>IL-1(^{2}), TGF-(\beta_{1}), PTH, PTHrP, cAMP, PKC</td>
</tr>
<tr>
<td>Bone</td>
<td>Osteoblast(^{35})</td>
<td>IL-1(^{2}), TGF-(\beta_{1}), PMA, PKC</td>
</tr>
<tr>
<td>Connective tissues</td>
<td>Chondrocyte, synoviocyte(^{14})</td>
<td>IL-1(^{3}), TGF-(\beta_{1}), PMA, PKC</td>
</tr>
<tr>
<td></td>
<td>Vein endothelial cells(^{32})</td>
<td>IL-1(^{3}), TGF-(\beta_{1,2}), PMA</td>
</tr>
<tr>
<td>Uterus</td>
<td>Fibroblast</td>
<td>IL-1(^{2}), PTHrP</td>
</tr>
<tr>
<td></td>
<td>Trophoblast (TPA30-1)(^{5})</td>
<td>IL-1(^{2}), PMA</td>
</tr>
<tr>
<td>Skin</td>
<td>Keratinocyte</td>
<td>IL-1(^{2}), PTHrP</td>
</tr>
<tr>
<td></td>
<td>Melanoma cell lines(^{167})</td>
<td>IL-1(^{2}), PMA</td>
</tr>
<tr>
<td>Testis</td>
<td>Round spermatids(^{30})</td>
<td>IL-1(^{2}), PTHrP</td>
</tr>
</tbody>
</table>

are found on the exposed face of helix C, may also be involved in receptor binding site II.\(^{2}\)

**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-1\(^{\alpha}\) and phorbol myristate acetate (PMA) in PU-34 cells is regulated mostly at the posttranscriptional level by increased IL-11 mRNA stabilization. IL-1\(^{\alpha}\)-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-1\(^{\alpha}\) and PMA-induced IL-11 mRNA stabilization. In addition, the presence of ATTATA motifs in the 3'UTR of IL-11 mRNA may function as an RNA destabilizing sequence.\(^{1,11}\) 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-1\(^{\alpha}\) 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.\(^{14}\) In contrast to BM fibroblast cells, stimulation of IL-11 gene expression by IL-1\(^{\alpha}\), transforming growth factor-\(\beta_{1}\) (TGF-\(\beta_{1}\)) and TGF-\(\beta_{2}\) 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.\(^{15}\) 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-\(\beta_{1}\) in induction of IL-11 in human lung fibroblasts is, to a great extent, transcriptionally regulated and dependent on H\(_{1}\) 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,\(^{20,22}\) IL-7,\(^{23,25}\) IL-13,\(^{26}\) stem cell factor (SCF),\(^{27}\) flt3 ligand,\(^{28}\) and granulocyte-macrophage colony-stimulating factor (GM-CSF),\(^{27}\) IL-11 stimulates the proliferation of primitive stem cells, multipotential and committed progenitor cells from various sources including cord blood,\(^{29,20}\) BM,\(^{2,30,33}\) and peripheral blood\(^{34}\) in different culture systems.\(^{16,20,25}\) This proliferation appears to be due to the entry of a quiescent (G\(_{0}\)) population of these cells into active cell cycle\(^{36}\) as well as shortening of the cell-cycle time in some cells.\(^{36}\) 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. 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. 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. 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, it has no effects on peripheral leukocyte counts when administered to normal rodents and nonhuman primates.

**Megakaryocytogenesis and thrombocytopenia.** IL-11 acts synergistically with IL-3, thrombopoietin (TPO) (also termed megakaryocyte growth and development factor [MGDF]), SCF, or IL-4 to stimulate various stages of megakaryocytogenesis and thrombopoiesis in both murine and human BM cells. In vivo treatment with IL-11 results in marked stimulation of megakaryocytogenesis in rodents, nonhuman primates, and humans (see also below), including the production, differentiation, and maturation of megakaryocytes. In the presence of soluble c-Mpl (the receptor for TPO), megakaryocyte colony formation and acetylcholinesterase (AChE) activities induced by IL-11 alone or in combination with IL-3 or SCF are reduced. Anti-TPO antiserum can also reduce IL-11–stimulated megakaryocyte colony formation by 90%, whereas anti–IL-3 antiserum effects a 28% reduction in colony formation. These studies suggest that IL-11 effects on megakaryocytogenesis and thrombopoiesis may be mediated in part via TPO. Recently, Weich et al have shown that IL-11α chain mRNA was detected in purified human CD41a(+) and CD14(–) megakaryocyte precursors. Further, incubation of purified cells with rhIL-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 G1, and this effect can be completely abrogated by addition of ACK2, a neutralizing antibody to c-kit, the receptor of SCF, 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. 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. 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.

**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− BM cells. These colonies are composed mostly of granulocytes and myeloid blasts. The combination of IL-11 with IL-3 or IL-4 can reduce the proportion of granulocytes and blasts in myeloid colonies, with a concomitant increase in macrophages. Combination treatment with IL-11, SCF, and G-CSF in the newborn rat has been shown to significantly increase peripheral neutrophil counts.

**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. Similar effects have been seen with flt3/flk-2 ligand using unfractionated murine fetal liver cells and with SCF and IL-7 in fractionated cells. IL-11 and IL-4 can also reverse the inhibitory effect of IL-3 on early B-lymphocyte development. The promotion of B-cell differentiation may be mediated by T cells.

**Effects on hematopoietic microenvironment.** IL-11 was originally isolated from cells derived from the hematopoietic microenvironment (HM) 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. Addition of IL-11 and SCF to bone marrow cultures derived from aplastic anemia patients significantly enhances the formation of an adherent stromal layer, 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 megakaryocytogenesis. IL-11 has been shown to modulate megakaryocyte-dependent BM fibroblast stimulation. IL-11 with other cytokines has been shown to mobilize primitive hematopoietic stem/progenitor cells both in vitro and in vivo. 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.

**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. IL-11 and IL-11Rα are also expressed in epithelial cells of the gastrointestinal (GI) tract. 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). 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-di-hydroxyvitamin 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 other factors derived from stromal/osteoblastic cells. The osteoblast-dependent bone-resorptive activity of IL-11 can be inhibited by the calcitonin and cyclo-oxygenase 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). Primary osteoblasts express both IL-11Rα and gp130 mRNA, and gp130 mRNA can be upregulated by IL-1, PTH, and 1α,25(OH)2D3. Mature osteoects 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 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 further 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 extracellular 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 δ-chain (IL-11Rδ) by using a degenerate oligonucleotide probe corresponding to the conserved 5-aa motif Trp-Ser-Xaa-Trp-Ser (WSXWS) in the hematopoietin receptor family. The extracellular region of IL-11Rα shares sequence similarity with the α-chains of IL-6 and CNTF receptors (24% and 22% aa identity, respectively). The human IL-11Rα cDNA isolated by Nandurkar et al predicts a 422-aa protein and shares 85% and 84% nucleotide and aa identity with the murine IL-11Rα. The extracellular region of human IL-11Rα contains a hematopoietin domain with conserved cysteine residues and the WSXWS motif. The residue ‘X’ differs between the human and murine receptors. There are two isoforms of human IL-11 receptor δ-chain which differ in the cytoplasmic domain. One isoform of human IL-11 receptor, similar to the human IL-6 and murine IL-11 receptors, has a short cytoplasmic domain (IL-11Rα1). The other isoform, similar to the human CNTF receptor, lacks this domain (IL-11Rα2). The functional significance of differences between the two isoforms is not known yet. IL-6, CNTF, and IL-11 receptor δ-chains overall share 32% identity among extracellular domains and are also structurally related. There is 42% identity between the C-terminal cytokine-receptor–like domains of IL-11Rα1 and CNTFRα. The genomic structure of hIL-11Rα1 consists of 12 exons and 12 introns within a 9-kb genomic region. Human IL-11Rα gene is located on chromosome 9 band 9p13, where the CNTFRα gene is also located. Robb et al have recently reported the structure of the murine IL-11Rα gene, which contains 14 exons. Evidence suggests the use of alternative first exons in a developmentally regulated fashion. A second murine IL-11Rα–like locus (IL-11Rα2) has been reported with sequence homology to exons 2-13 of IL-11Rα1. This locus appears to be present in only some strains of mice.

Binding of IL-11 ligand to either human or murine IL-11Rα occurs at low affinity and, although necessary, is not sufficient for signal transduction. The generation of a high-affinity IL-11 receptor capable of generating a biologic signal requires coexpression of the IL-11Rα and gp130. IL-11 mRNA is detectable in several murine cell lines, including 3T3-L1 cells, BAE stromal cells, the embryonic carcinoma cell line PC13, and factor-dependent hematopoietic cell lines FDCP-1 and D35. A wide range of primary tissues express IL-11Rα mRNA, including hematopoietic tissues (BM, spleen, and thymus), liver, brain, heart, kidney, muscle, and salivary gland as well as cells of the GI tract. Human IL-11 receptor mRNA is expressed in myeloid (K562), megakaryocyte (Mo7E), and erythroid (TF1) leukemia cell lines as well as osteosarcoma cell lines (MG-63 and Saos-2).

As mentioned above, gp130 is the common subunit of the IL-6, OSM, LIF, and CNTF as well as IL-11 receptors. Binding of IL-11 to specific cell-surface IL-11Rα receptor induces heterodimerization, tyrosine phosphorylation, and activation of gp130. The activated IL-11 receptor gp130 complex probably activates tyrosine kinases of the Janus kinase (Jak) family (Fig 1). IL-11 has also been shown to promote the formation of the active GTP-
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 p60**.

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.

**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 rHL-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 leucocyte 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 primitive 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,129-133 and is associated with accelerated recovery of both hematopoiesis42,132 and the immune response42,70 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 fashion in mice treated with combined chemotherapy (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 vivo79 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 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 cytotoxicity/immunosuppression in different chemotherapy 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-B27 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 hypotension and decreases GI mucosal damage induced by lipopoly-
saccharide (LPS). 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.

IL-11 AND DISEASES

IL-11 acts as a synergistic factor with IL-3, GM-CSF, and SCF to stimulate proliferation of human primary leukemic cells, myeloid leukemia cell lines, megakaryoblastic cell lines, and erythroleukemic cell lines and to stimulate leukemic blast colony formation. 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. Although IL-11 stimulates the proliferation of murine plasmacytoma cells and murine hybridoma cells, 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. However, IL-11 can stimulate proliferation in two of eight human myeloma cell lines tested so far. As expected, anti-gp130 monoclonal antibodies can inhibit growth stimulation by IL-11 in human myeloma cell lines. 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.

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. 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. 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. However, IL-11 treatment was associated with increased BM cellularity, and increased numbers and cycling of immature erythroid and myeloid precursors.

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. 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.

In several phase II/III 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. 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. 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. 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. 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-mpl provides another, and potentially very useful, therapeutic approach to thrombocytopenic states. Early trials with TPO (also termed MGDF) 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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