Cytokine Regulation of Stem Cells

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Abstract

Different types of stem cells are targeted by a number of cytokines that alter proliferation, differentiation, or other properties of stem cells. Stem cells are known to express various cytokine genes. As IL-12, IL-14, G-CSF, and GM-CSF expression is lost after the differentiation of MSCs, these factors might have major contribution to pluripotency. Several other cytokines that are produced by immune cells, frequently target stem cells. Modulation of stem cell functions by cytokines can be a cause of various diseases including cancer. Stem cells can show immunosuppressive properties by a number of mechanisms. MSC-induced immunosuppression is often mediated by IFN-γ, TNF-α, IL-1α, or IL-1β. In co-culture experiments, MSCs were able to control T cells IL-2 response, or, dendritic cells TNF-α and IL-10 secretion. MSCs are also known to cause decreased interferon γ (IFN-γ) and increased IL-4 production by immune cells. However, the outcome in most of the cases depends on the presence of various factors that might synergize or antagonize with each other.

Introduction

Cytokines are small glycoprotein messengers that facilitate efficient communication among immune cells. Immune responses depend on the orchestrated activities of various cytokines. Cytokines not only control innate and adaptive immune responses, but also affect non-immune organs and tissues [1]. Stem cells are probably the biggest breakthrough of applied medical research. These cells can not only self-renew, but differentiate into different types of cells under different conditions. Stem cell research has contributed to the understanding of fundamental
questions related to cell division, senescence, differentiation, and regeneration [2]. Potential benefits of stem cell technology are huge and new discoveries are eagerly awaited in this area. Cytokines regulate stem cell functions more than any other molecule. This review is a concise overview of all the cytokines that affect proliferation, differentiation, or other properties of stem cell(s), either alone or in combination of other cytokines. Cytokines that have not been reported to have any effect on stem cells are not included in this review.

**Stem cells understand the language of cytokines**

Stem cells are not only known to express various cytokine receptors, but also respond to various cytokines by activation of conventional cytokine signaling pathways [3]. Despite their pluripotent advantage, stem cells appear to be regulated by cytokines in the same manner as other immune cells [1]. The characterization of many cytokines that control hematopoiesis or neuroopoiesis has led to intense investigation to find out potential use of these cytokines for ex vivo expansion of various types of stem cells [2]. Effects of various 'colony stimulating factors' (CSFs) to stimulate growth of distinct colonies in bone marrow is the hallmark of stem cell biology. In this regard, Granulocyte colony-stimulating factor (G-CSF), Granulocyte macrophage colony-stimulating factor (GM-CSF), Macrophage colony-stimulating factor (M-CSF), Interleukin-3 (IL-3), Interleukin-6 (IL-6), erythropoietin (EPO), thrombopoietin (TPO), Stem cell factor (SCF), flt-3/flk-2 Ligand (FL), and leukemia inhibitory factor (LIF) are most important [4, Table 1].

**Cytokines regulate stem cell proliferation and self-renewal**

SCF, being a major regulator of stem cells, has been so important for stem cell research that most of the common cytokines have been tested along with it for potential synergistic or antagonistic effects [5]. SCF signaling, through its receptor c-Kit, is not only important for hematopoiesis, but also for pigment control, male fertility, and various aspects of nervous system development. LIF prevents apoptosis, and promotes cell proliferation during embryonic stem (ES) cell differentiation. IL-3 is crucial for proliferation and survival of Hematopoietic Stem Cells (HSCs), and in absence of IL-3, HSCs may remain quiescent [6]. Because of its crucial role, IL-3 is
routinely used during proliferation and differentiation of HSCs. Similarly, IL-3 withdrawal has been very useful for depletion of HSCs [7]. IL-3, SCF, and IL-6 have become the key cytokines for retroviral gene transfer into hematopoietic cells [8]. Most HSCs remain quiescent during homeostasis. However, in response to irradiation or other types of hematopoietic cell stress, HSCs get activated and generate all types of progenitor cells so that mature blood cells can be produced. Signals that regulate this transition in HSCs are a matter of intense research. One clearly identified factor that control this transition is interferon-alpha (IFN-α) [9]. Mice treated with IFN-α, quickly exit dormant phase and enter into active cell cycle. These events are associated with increased phosphorylation of STAT1 and AKT1, and, higher expression of stem cell antigens [9]. Interleukin-1 (IL-1) is associated with accelerated growth of HSCs specifically increasing the production of the cells of myeloid and the granulocyte–macrophage (GM) lineage [4]. These effects are mediated by the rapid activation of a transcription factor Pu.1, the master regulator of GM lineage commitment [4]. Furthermore, IL-1 receptor-deficient mice showed poor recovery of GM-lineage following fluorouracil-mediated myeloablation [10]. Although, HSCs from interleukin-2 (IL-2) knock-out mice were able to proliferate normally, they were defective for reconstitution after lethal irradiation [11]. Furthermore, IL-2 knock-out mice, despite having normal numbers of bone marrow cells (BMCs), had increased numbers of Lin (-) Kit(+)Sca1(+)CD34(-) and Lin(-)Kit(+)Sca1(+)CD34(+) cells [11]. IL-9 induces the proliferation of very primitive human erythroid cells, and synergize with SCF, c-kit, and other cytokines to promote growth of hematopoietic progenitors [12]. When tested in a hematopoietic stem cell line, both IL-4 and IL-9 stimulated growth promoting tyrosine phosphorylation of SHP-2, whereas IL-9 additionally phosphorylated STAT3 [13]. IL-10 disrupted mice show reduced primitive hematopoietic cell populations, and, HSCs from fluorouracil treated bone marrows showed enhanced repopulating ability in presence of IL-10 [14]. Stroma-free cultures of Lin–Sca-1+c-kit+ cells yield 3-4 fold more HSCs upon addition of IL-10, and this effect of IL-10 was direct, as, TNF-α or interferon-γ (IFN-γ) secretion was not changed in this case [15]. IL-11 or IL-6, when combined with SCF caused the expansion of murine hematopoietic progenitors that were derived from fluorouracil-treated bone marrow cells [16]. Furthermore, IL-6 and IL-11 were also found to be important for proliferation of megakaryocyte progenitor cells [16]. IL-13, like IL-4, can be very important during early myelopoiesis. In fact, IL-13 enhanced SCF-induced proliferation of Lin–Sca-1+ progenitor cells more efficiently than IL-4, although, IL-13 alone was not able to stimulate
colony formation in this case [17]. IL-13, but not IL-4, synergized with GM-CSF for enhanced colony formation of Lin-Sca-1+ progenitors, however, in case of granulocyte colony-stimulating factor (G-CSF)-induced colony formations, both IL-4 and IL-13 were able to synergize and promote colony formation [17]. Interleukin-32 (IL-32) induced the proliferation of hematopoietic progenitors, and decreased chemotherapy-induced bone marrow toxicities [18]. Interestingly, human cytomegalovirus has been reported to activate cellular IL-32 expression, which means that certain viruses can also directly or indirectly modulate stem cell functions [19]. Interleukin-33 is a cytokine that is secreted, when epithelial cells are exposed to pathogens, allergens, or injury. Other than targeting various immune cells during inflammation, IL-33 also targets hematopoietic stem and progenitor cells to promote myelopoiesis and myeloid cell migration [20].

While several cytokines promote growth of stem cells, some inhibit it. TNF-α is a potent suppressor of normal HSC activity, and its overexpression leads to bone marrow failure syndrome [21]. By downregulating c-kit receptor, TNF-α not only inhibits the SCF-stimulated proliferation of CD34+ hematopoietic progenitor cells, but also completely blocks the colony formations that is caused by combination of SCF and GM-CSF or SCF and IL-3 [22]. Most of these effects are through p55 TNF, not by p75 TNF, receptor. TNF-α also synergized with IFN-γ to abrogate NF-κB and SMAD7 mediated self-renewal and differentiation of mesenchymal stem cells (MSCs) [21]. TGF-β is known to regulate embryonic stem cell heterogeneity [4].

**Cytokines regulate stem cell differentiation**

IL-1α is known to enhance neurogenesis from human MSCs [23]. When cultured with IL-1β, human bone marrow-derived MSCs differentiated into osteoblast like cells that expressed bone sialoprotein, and deposited abundant mineral. IL-1β, in this case, phosphorylated multiple mitogen-activated protein kinases (MAPKs) and activated nuclear factor-κB (NF-κB) [24]. Interleukin-4 (IL-4) has been reported to cause differentiation of human embryonic stem cells into fibroblast-like cells [25]. In case of mesenchymal stem cells, IL-4 mediated signaling has been reported to activate the expression of genes that are characteristic of cardiac and skeletal muscle cells. However, differentiation was incomplete in this case, as, these cells lacked certain properties of functional muscle cells including lack of contractile activity [26]. Interleukin-6 (IL-6), the
principal proinflammatory cytokine, has recently gained attention as a neuromodulatory cytokine [27]. Maternal administration of IL-6 caused expanded adult forebrain neural precursors and altered olfactory neurogenesis in offsprings, months after fetal exposure [28]. This established the role of IL-6 as an important regulator of neural precursors. Both IL-6 and LIF has been reported to synergize with bone morphogenetic protein (BMP) 7 for neuroepithelial to astrocyte transition [29]. IL-6 inhibits the differentiation of bone marrow-derived MSCs into chondrocytes [B]. Interleukin-7 (IL-7) is indispensible for differentiation of T and B cells during haematopoesis, and, common lymphoid progenitors are known to upregulate IL-7 receptor before producing other type of lineages [30]. Interleukin-8 (IL-8) is a potent mobilization factor for hematopoietic progenitor cells, and is also known to be upregulated in the ischemic brain, henceforth, like IL-6, it is important for both hematopoiesis and neuropaioiesis [31]. IL-8 induced PI3K/Akt and MAPK/ERK-mediated VEGF production in human MSCs, and, decreased the infarction volume by enhancing angiogenesis in a rat stroke model [32]. Interestingly, SCF+G-CSF stimulation of Lin-Sca-1+ progenitors that normally results into the formation of 90% granulocytes, shifted towards the production of macrophages upon addition of IL-13 or IL-4 [17]. Interleukin-15 (IL-15) instructs the generation of human memory stem T cells from naive precursors [33]. Interleukin-16 (IL-16) also is reported to cause proliferation and differentiation of CD34 (+) hematopoietic cells into mature dendritic cells [4]. Interleukin-27 (IL-27) directly induces differentiation in hematopoietic stem cells. IL-27 transgenic mice exhibited greater myelopoiesis and defective B lymphopoiesis [35]. IL-27 has also been reported to synergize with SCF to induce differentiation of HSCs [35]. IL-27 can be of greater importance, as, significant proportion of HSCs are known to express IL-27 receptor. Interleukin-34 (IL-34) is an alternative ligand for colony stimulating factor 1 (Csf-1) receptor in both mice and humans. Mice lacking IL-34 displayed reduced Langerhans cells and diminished microglial cells. However, no effect was seen on monocytes, macrophages, and dendritic cells [36]. IL-34 appears to be indispensable for the development of Langerhans cells not only during embryogenesis but also during normal homeostasis in the adult skin [36]. Though, repopulation of Langerhans cells during inflammation appears to be dependent on Csf-1, post-inflammation survival of Langerhans cells is IL-34 dependent. IL-34 expression is generally high in brain tissues, and along with its receptor, it appears to be crucial for the development of microglial cells and their yolk sac precursors [37, 38]. Interestingly, a new receptor of IL-34 namely receptor-type protein-tyrosine phosphatase has been discovered that expresses on neural
progenitors, glial cells, and glioblastomas [39]. LIF promote differentiation of ES cells into neuronal cells, but inhibits the differentiation of ES cells to both mesodermal and extraembryonic endodermal lineages [40]. Interestingly, inhibition of LIF-induced STAT3 signaling abrogated the neuronal differentiation of ES cells, but inhibition of LIF-induced MEK signaling blocked the glial differentiation [40]. IL-6 and ciliary neurotrophic factor are also neuropoietic cytokines that control neural stem cell renewal, progenitor cell division, and differentiation. JAK/STAT pathway appears to be crucial for these effects [41]. However, the effects of cytokines on neural stem cells are more complex, and, these effects not only vary with the type of cytokine (s) but also with the concentration and tissue-specific localization of these molecules. TNF-α has also been shown to cause premature neural phenotype in human MSCs with the concurrent expression of LIF, BMP2, SRY box 2 protein, and glial fibrillary acidic protein [42]. Inhibition of ERK1/2 signaling abolished the TNF-α-mediated regulation of neural genes in this case. Furthermore, TNF-α enhanced the expression of CXCR4 on human MSCs that caused these cells to respond to stromal cell-derived factor 1 (SDF-1) [42].

**Cytokines regulate stem cell-mediated immunosuppression**

MSCs and their immunosuppressive properties are subject of intense investigation, and MSC-mediated down-modulation of proinflammatory cytokines appears to be very important in this regard. MSCs produce multiple cytokines that not only affect their own functions, but also affect immune cells in the neighborhood. Phosphoinositide 3-kinase (PI3 kinase), serine/threonine protein kinases, and NF-κB are important for cytokine induced migration of mesenchymal stem cells [43]. MSC-derived matrix metalloproteinases (MMPs) caused downregulation of IL-2 signaling by cleavage of IL-2 receptor α (CD25) from the surface of activated T cells. During MSC co-culture experiment with PHA activated T cells; IFN-γ, TNF-α, IL-1β, IL-2, IL-12p70, and IL-17A were found to inhibit MSC-mediated immunosuppression of T cells [44]. Conditioned media from MSC culture that had elevated levels of VEGF, monocyte chemoattractant Protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1), and monokine induced by IFN-γ (MIG), induced angiogenesis in vascular endothelial cells [45]. In this case, MCP-1 and MIP-1α increased cell migration of MSCs while VEGF reduced it. Furthermore, this conditioned media lead to reduced caspase-3 activity in hypoxic H9c2 cells, and this effect was reversed by PI3-kinase inhibitors.
MSCs conditioned media inhibited phosphorylation at Ser112 of BAD and Ser473 of AKT, but increased AKT’s phosphorylation at Thr308 [45]. Long-term culture-initiating cells (LTC-IC) proliferated 30 fold more in combination of Flt3-ligand, Steel factor, and IL-3. Although, each one of the three factors were able to induce proliferation alone, the most potent single factor, in this case, was IL-3 [46].

Cytokines regulate cancer stem cells (CSCs)

CSCs are often resistant to chemotherapy and some reports suggest that IL-4 might be contributing to this phenomenon, as, abrogation of IL-4 signaling causes apoptosis in CSCs, and, IL-4 mediated STAT-6 expression enhances cancer-promoting survivin expression [47]. IL-8 is often found upregulated in certain types of cancers and has been suspected to promote CSC’s growth. Inhibition of IL-8 or SNAIL signaling appears to be beneficial for controlling colon and breast cancers [48]. Carcinopromoting role of M-CSF was confirmed, when disruption of M-CSF gene was found to cause diminished tumour growth and metastasis as a result of defective angiogenesis [49]. Defective c-Kit kinase activity has been found in a number of diseases including cancer and allergy [40]. Interestingly, sustained elevated levels of IFN-γ and TNF-α also potentiated NF-κB, c-Fos and c-Myc mediated malignant transformation in MSCs, and depletion of either IFN-γ or TNF-α in this case, abolished the MSCs impairment and malignant transformation [50]. Aspirin, which is known to reduce the levels of IFN-γ and TNF-α, also corrected MSC deficiency and tumorigenesis in this case. These effects were mediated by inhibition of NF-κB/SMAD7, NF-κB/c-FOS, and c-MYC pathways. Interestingly, MSCs can both suppress or promote cancer development under different conditions. Cancer cells can stimulate MSCs to produce RANTES (regulated on activation, normal T cell expressed and secreted) that in turn enhances cancer cell motility, invasion and metastasis [51]. On the other hand, TNF-α upregulates TRAIL from human bone marrow MSCs, which results into cancer cell apoptosis. MSCs that were preactivated with TNF-α, controlled the progression of lung and breast tumors, and induced apoptosis in various TRAIL-sensitive cancer cell lines [52]. IL34, like M-CSF, can increase vascular endothelial growth factor (VEGF) production and promote angiogenesis and vascularisation both in vitro and in vivo. Unfortunately, by promoting angiogenesis and extravasation of immune cells, IL34 can enhance tumour development too [53]. TNF-α has also
been reported to activate MSCs for IL-6 or IL-8 mediated angiogenesis, and, intramuscular injection of conditioned media derived from TNF-α-treated MSCs stimulated blood perfusion and angiogenesis in the ischemic limb [54]. Immunodepletion of IL-6 and IL-8, in this case, resulted into attenuated blood perfusion and angiogenesis. Furthermore, Intramuscular injection of TNF-α-treated MSCs into the ischemic limb caused increased homing of tail vein-injected endothelial progenitor cells (EPCs) in IL-6 and IL-8 dependent manner [54].

**Conclusion**

In summary, several cytokines target stem cells to alter pluripotency, differentiation, growth and proliferation, or other properties. However, the outcome in such cases depends on the presence of several other factors that might synergize or antagonize with each other. Presence of various soluble factors along with different types of cells in the proximity might reprogram stem cells. At the same time, stem cell can also produce different types of cytokines that can target various immune cells or the producer itself, in an autocrine manner.

**Table 1.**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Main Effect(s) on Stem Cells</th>
<th>Type of Stem Cell Affected</th>
<th>Mechanism of Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF</td>
<td>Proliferation and self-renewal.</td>
<td>Most types</td>
<td>c-Kit Signaling</td>
</tr>
<tr>
<td>LIF</td>
<td>Promotes cell proliferation, promotes differentiation to neuronal cells.</td>
<td>Most types</td>
<td>Activation of STAT3 and MEK</td>
</tr>
<tr>
<td>IL-1</td>
<td>Accelerated growth.</td>
<td>HSCs</td>
<td>Activation of transcription factor Pu.1</td>
</tr>
<tr>
<td>IL-1α</td>
<td>Enhanced neurogenesis.</td>
<td>Neural stem cells (NSC), MSCs</td>
<td>Not reported</td>
</tr>
<tr>
<td>IL-1β</td>
<td>MSCs differentiate into osteoblast.</td>
<td>MSCs</td>
<td>Activation of MAPKs and NF-κB</td>
</tr>
<tr>
<td>IL-2</td>
<td>Might be important for reconstitution ability.</td>
<td></td>
<td>Not reported</td>
</tr>
<tr>
<td>IL-3</td>
<td>Proliferation and survival.</td>
<td>Most types</td>
<td>IL-3 Signaling</td>
</tr>
<tr>
<td>IL-4</td>
<td>Very important during early myelopoiesis, Promote differentiation to cardiac and skeletal muscle cells.</td>
<td>Most types</td>
<td>Activation of STAT3</td>
</tr>
<tr>
<td>IL-6</td>
<td>Regulator of neural precursors. When combined with SCF, caused the</td>
<td>Most types</td>
<td>JAK/STAT pathway</td>
</tr>
<tr>
<td>IL-7</td>
<td>Differentiation of T and B cells.</td>
<td>HSCs, ESCs</td>
<td>IL-7 Signaling</td>
</tr>
<tr>
<td>IL-8</td>
<td>Important for both hematopoiesis and neuropoiesis.</td>
<td>HSCs, NSCs</td>
<td>Induced PI3K/Akt and MAPK/ERK-mediated VEGF production.</td>
</tr>
<tr>
<td>IL-9</td>
<td>Proliferation of very primitive human erythroid cells.</td>
<td>HSCs</td>
<td>Phophorylation of STAT3</td>
</tr>
<tr>
<td>IL-10</td>
<td>Enhanced repopulating ability.</td>
<td>HSCs</td>
<td>Not reported</td>
</tr>
<tr>
<td>IL-11</td>
<td>When combined with SCF caused the expansion of murine hematopoietic progenitors.</td>
<td>HSCs</td>
<td>Not reported</td>
</tr>
<tr>
<td>IL-13</td>
<td>Very important during early myelopoiesis.</td>
<td>HSCs</td>
<td>IL-13 Signaling</td>
</tr>
<tr>
<td>IL-15</td>
<td>Generation of human memory stem T cells from naive precursors.</td>
<td>HSCs</td>
<td>IL-15 Signaling</td>
</tr>
<tr>
<td>IL-16</td>
<td>Proliferation and differentiation of CD34 (+) hematopoietic cells into mature dendritic cells.</td>
<td>HSCs</td>
<td>IL-16 Signaling</td>
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<tr>
<td>IL-27</td>
<td>Induces differentiation in hematopoietic stem cells.</td>
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<td>IL-32</td>
<td>Proliferation of hematopoietic progenitors.</td>
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<td>IL-34</td>
<td>Indispensible for the development of Langerhans cells.</td>
<td>HSCs</td>
<td>IL-34 Signaling</td>
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<tr>
<td>TNF-α</td>
<td>Potent suppressor of normal HSC activity.</td>
<td>HSCs</td>
<td>Signaling through p55 TNF receptor</td>
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<tr>
<td>IFN-α</td>
<td>Activation of HSCs, NSCs.</td>
<td>HSCs, NSCs</td>
<td>Increased phosphorylation of STAT1 and AKT1</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Regulates embryonic stem cell heterogeneity</td>
<td>ESCs</td>
<td>Nodal signaling</td>
</tr>
</tbody>
</table>

**HSCs:** Hematopoietic Stem Cells, **MSCs:** Mesenchymal Stem Cells, **ESCs:** Embryonic Stem Cells

**Competing interests**

No competing interests were disclosed.

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