Cancer Chemother Pharmacol (1996) 38 (Suppl): S64–S68

ORIGINAL ARTICLE

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Role of glycoprotein 130 and c-Kit signaling in proliferation and differentiation of human hematopoietic progenitor cells

Abstract Glycoprotein (gp) 130, a receptor component for interleukin 6 (IL-6), can associate with a soluble IL-6 receptor (sIL-6R)–IL-6 complex. To examine the role of gp130 signaling in human hematopoietic progenitor-cell proliferation and differentiation, we studied the effects of the sIL-6R–IL-6 complex in combination with other cytokines on human $CD34^+$ cells in clonal and suspension cultures. The sIL-6R–IL-6 complex, but not sIL-6R or IL-6 alone, in the presence of stem-cell factor (SCF) produced dramatic increases in the populations of various cell lineages, including erythroid cells and various hematopoietic progenitors, in suspension culture. Significant numbers of colonies of (particularly) multilineage and blast cells were generated in methylcellulose culture supplemented with a combination of sIL-6R–IL-6 complex and SCF. Addition of anti-gp130 monoclonal antibodies (MAbs) and anti-IL-6R MAbs to the above-mentioned cultures dose-dependently inhibited the generation of cells of various lineages and of progenitor cells in suspension culture and completely blocked multilineage colony production in methylcellulose culture; an anti-erythropoietin antibody did not cause inhibition. These findings demonstrate that both proliferation and differentiation of hematopoietic progenitor cells can be induced through gp130 and c-Kit signaling, indicating that progenitor cells are responsive to the sIL-6R–IL-6 complex, even though they do not express IL-6R. Together with previous studies showing that detectable levels of sIL-6R, IL-6, and SCF are present in human serum, these results suggest that gp130 signaling may play an important role in human hematopoiesis in vivo.

Key words Hematopoietic stem cell · c-Kit · Gp130 · Soluble IL-6 receptor · Differentiation · Ex vivo expansion

Work presented at the 11th Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium "Cytokines and New Anticancer Agents on the Horizon of Oncology", 24–25 November 1995, Nagoya, Japan

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Introduction

Hematopoiesis requires a highly complex series of cellular events to maintain homeostasis in the hematopoietic system, in which a small population of stem cells has to generate continuously many different populations of blood cells. Intercellular communication in the hematopoietic system is mediated by soluble factors called interleukins (IL) or cytokines. These molecules exert their biological functions through specific receptors expressed on the surface of target cells. Cloning of most of the genes encoding receptors for cytokines regulating the hematopoietic system has revealed that the majority of cytokine receptors fall into a large hematopoietic cytokine-receptor superfamily. Most cytokine-receptor systems in this family, except erythopoietin receptor (EPOR), granulocyte colony-stimulating factor receptor (G-CSFR), and thrombopoietin receptor (TPOR; c-Mp1), consist of a multichain complex, a ligand-binding receptor chain (α-chain), and a signal-transducing chain (β-chain), the latter of which is often common to several receptor complexes [3, 7, 13].

Glycoprotein 130 (gp130), a 130-kDa transmembrane glycoprotein with a large intracytoplasmic domain originally identified as a signal-transducing receptor component for IL-6, is shared by IL-11, leukemia-inhibitory factor (LIF), oncostatin (OSM), ciliary neurotrophic factor (CNTF), and cardiotrophin 1 (CT-1; cytokines of the IL-6 family) [1, 9, 13]. Gp130 is ubiquitously expressed in all organs examined, whereas expression of receptors for IL-6 (IL-6R) and IL-6-related cytokines is more limited. In the IL-6–IL-6R interaction, IL-6 first binds to IL-6R, and this complex then associates with gp130, leading to its homodimerization. IL-6R has a very short cytoplasmic domain that has been demonstrated to be dispensable for signaling. In contrast, the cytoplasmic region of gp130 is required for signal transduction, and gp130 homodimerization results in the juxtaposition of the cytoplasmic regions of the two gp130 molecules, which appears to initiate a downstream signaling cascade such as RAS/MAPK and JAK/STAT, producing a cellular response (see [3, 13] and references

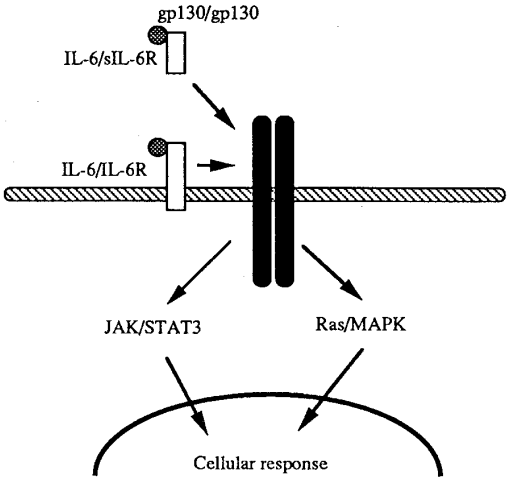

Fig. 1 IL-6 receptor complex. Both a membrane-anchored form (IL-6R) and a soluble form (sIL-6R) of the IL-6 receptor can induce gpl30–gp130 homodimerization on binding to IL-6. Gp130 homodimerization is believed to initiate a downstream signaling cascade such as JAK/STAT and Ras/MAPK, leading to a cellular response

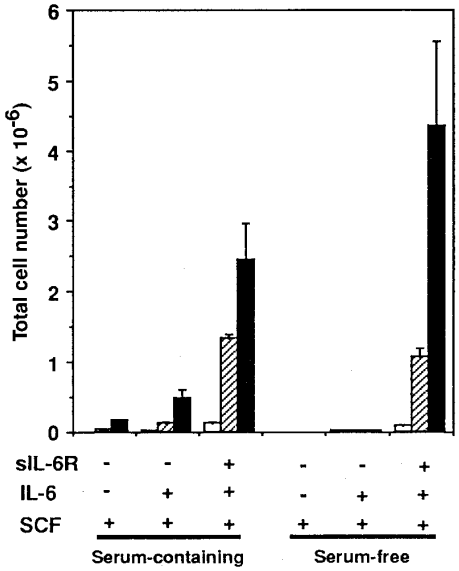

Fig. 2 Proliferative activity of sIL-6R on human CD34+ cells. CD34+ cells (2000) were initiated in all cultures and the progeny were examined at weekly intervals. The results represent data from 3 separate experiments; the standard deviation is shown by *error bars*. Day 7, day 14, and day 21 of culture

therein). Interestingly, a soluble form of IL-6R (sIL-6R) that lacks the transmembrane and cytoplasmic regions can also induce homodimerization of gp130 upon IL-6 binding. More importantly, the IL-6–sIL6R complex confers IL-6 responsiveness to cells on which gp130, but not the ligand-specific membrane receptor (IL-6R), is expressed (Fig. 1).

IL-6 has been shown to act on murine primitive hematopoietic progenitors and, together with IL-3, induces the proliferation of murine multipotential hematopoietic progenitor cells [4]. However, this is not the case with human hematopoietic progenitor cells, in which the effect of IL-6 in combination with other cytokines, including IL-3 and stem-cell factor (SCF), is barely detectable. Functional sIL-6R and the sIL-6R–IL-6 complex are present in human serum, suggesting that the sIL-6R–IL-6 complex may, by activating the signal transducer of gp130, mediate novel functions in the proliferation and differentiation of human hematopoietic stem/progenitor cells.

In the human hematopoietic system, information as to which cytokine receptors are normally expressed on stem cells remains incomplete. To investigate the potential role of gp130, we have examined the effect of gp130 activation by an sIL-6R–IL-6 complex on the proliferation and differentiation of normal human hematopoietic stem/progenitor cells. Our recent studies have revealed that gp130 signaling initiated by sIL-6R–IL-6 in association with c-Kit activation by SCF may play an important role in human hematopoiesis ([11, 12]; Tajima et al., unpublished data).

IL-6–sIL-6R complex in the presence of SCF stimulates growth of cells of various lineages from human CD34+ cells in suspension culture

When human CD34+ cells purified from umbilical cord blood were incubated with sIL-6R in the presence of SCF and IL-6, total cell numbers increased in an sIL-6R concentration-dependent manner. The addition of sIL-6R at the optimal concentration to IL-6 and SCF increased the total cell numbers by 5- to 10-fold in serum-containing suspension culture. However, in the absence of IL-6, sIL-6R failed to increase cell numbers. The increase in total cell numbers induced by IL-6–sIL-6R in the absence of SCF was also small, suggesting that the activity of IL-6–sIL-6R is synergistic with SCF.

A more striking synergistic effect between IL-6–sIL6R and SCF was found in serum-free culture, in which an approximately 2000-fold increase in total cell numbers was observed at day 21 of culture (Fig. 2); IL-6 and SCF in the absence of sIL-6R induced only limited increases, indicating that sIL-6R can stimulate significant cell generation from human hematopoietic stem/progenitor cells in the presence of IL-6 and SCF.

We found it interesting to examine the characteristics of the cells developed from human CD34+ cells induced by IL-6–sIL6R in the presence of SCF. Cell-morphology and immunology studies using alkaline phosphatase-antialkaline phosphatase staining and fluorescence-activated cell-sorting (FACS) analysis revealed that the cells generated are heterogeneous and contain various cell lineages. Notably, whereas most of the cells were blast cells at day 7, a significant proportion of the cells generated were of erythroid or megakaryocytic origin or were blast and myeloid cells at days 14 and 21. Different developmental stages of various cell lineages, such as enucleated erythrocytes, were also detectable, indicating that a combination of IL-6–sIL6R and SCF may play a novel role in the development of various hematopoietic cell lineages ([11, 12]; Tajima et al., unpublished data).

sIL-6R, IL-6, and SCF stimulate colony formation by multiple cell lineages

It is likely that the development of large numbers of cells of various lineages in suspension culture in the presence of IL-6–sIL-6R and SCF is due to proliferation and differentiation of various lineage progenitors. This possibility has been confirmed by our progenitor cloning studies. In serum-containing methylcellulose cultures, IL-6 and sIL-6R either alone or in combination in the absence of SCF induce only limited myeloid colony formation [11]. However, in the presence of SCF, IL-6–sIL6R stimulates total colony formation with a plating efficiency as high as >50% [11]. Interestingly, colonies derived from various lineage progenitors such as colony-forming unit-granulocyte/macrophage (CFU-GM), burst-forming unit-erythroid (BFU-E), CFU-megakaryocyte (CFU-Mk), CFU-Mix, and CFU-blast can be observed. In serum-free methylcellulose culture, the addition of sIL-6R to SCF and IL-6 increased the total number of colonies by >15-fold, and colonies of erythroid and megakaryocytic as well as myeloid lineages were detectable [11, 12]. It was noteworthy that although colonies of various lineages were observed, almost half of the developed colonies were blast and mix colonies, which are believed to be derived from more primitive cells, suggesting that the IL-6–sIL6R complex in the presence of SCF acts on both primitive and committed progenitors. Thus, our studies indicate that the IL-6–sIL-6 complex in the presence of SCF is capable of stimulating the proliferation and differentiation of both early and late progenitors of multiple lineages.

Ex vivo expansion of hematopoietic progenitors by sIL-6R, IL-6, and SCF

Gp130 has been shown to be expressed in embryonic stem (ES) cells, and gp130 activation by LIF or the IL-6–sIL6R complex can sustain self-renewal of ES cells [14], suggesting that gp130 may play a role in the self-renewal process of stem cells. The marked increases in total cell numbers in suspension culture and efficient colony formation described above also suggest that the combination of SCF, IL-6, and sIL-6R may be useful for the expansion of human stem/progenitor cells. This was clearly demonstrated by our subsequent expansion studies [11]. In both serum-free and serum-containing suspension cultures supplemented with SCF, IL-6, and sIL-6R, significant expansion of all hematopoietic progenitors as well as $CD34^+$ cells was observed (Fig. 3). Importantly, whereas the various combinations of cytokines reported thus far have limited effects on the expansion of primitive progenitor cells such as CFU-Mix, a 60- to 80-fold expansion in CFU-Mix numbers was obtained with IL-6, sIL-6R, and SCF, indicating the role of IL-6–sIL6R in the expansion of primitive progenitor-cell numbers. In addition, we also tested the possible synergy of IL-6–sIL6R with various early- and late-acting

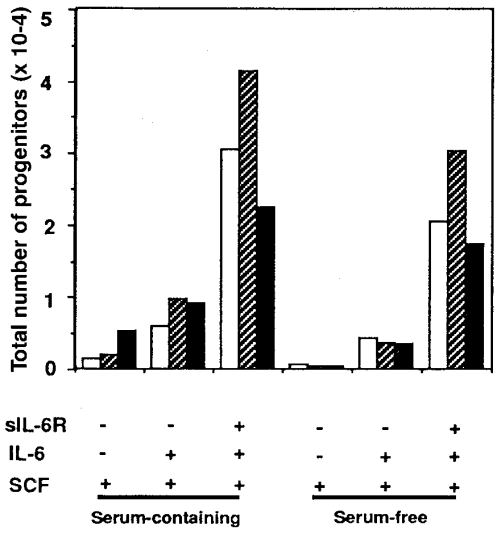

Fig. 3 Expansion of progenitor cells by SCF, IL-6, and sIL-6R. The addition of sIL-6R to SCF and IL-6 markedly enhanced the expansion of human hematopoietic progenitor-cell numbers. Results from 1 representative experiment in which 2000 $CD34^+$ cells containing 650 progenitors were initiated in the culture are presented. Day 7, day 14, and day 21 of culture

cytokines. The combination of sIL-6R and IL-6 with either IL-3, granulocyte colony-stimulating factor (G-CSF), erythropoietin (EPO), or granulocyte/macrophage-CSF (GM-CSF) failed to expand CFU-Mix numbers, suggesting that the IL-6–sIL6R complex specifically synergizes with SCF to expand human hematopoietic progenitor numbers, particularly primitive progenitors.

Ex vivo expansion of hematopoietic progenitor numbers is an attractive way to prepare suitable hematopoietic cells for potential clinical applications, including gene therapy. As shown in our study, coactivation of gp130 and c-Kit signaling pathways by IL-6, sIL-6R, and SCF, may provide a novel approach to the expansion of human hematopoietic progenitor numbers for potential clinical application. Since it is conceivable that human hematopoietic stem cells express both gp130 and c-Kit, our finding also raises the possibility that maintenance of human hematopoietic stem-cell self-renewal might be possible through coactivation of the two signal pathways. Such studies are currently hampered by the heterogeneity of the accessible normal progenitor/stem-cell population in vitro.

Gp130 is the signal transducer for IL-6–sIL6R and nonparallel expression of gp130 and IL-6R on human $CD34^+$ cells

To verify that membrane-anchored gpl30 is the signal transducer for IL-6–sIL6R, we used anti-gpl30 monoclonal antibodies (MAbs; GPX7, GPX22, and GPZ35). The three MAbs recognize different epitopes on gp130 and inhibit the IL-6-mediated biological response through inhibition of the IL-6-induced association of gp130 with IL-6 receptors [10]. The addition of anti-gp130 MAbs to cultures supple-

Table 1 Colony formation by CD34+ cells from human cord blood in serum-containing methylcellulose cultures[a] (*GM* Granulocyte-macrophage colonies, *Blast* blast colonies, *Meg* megakaryocyte colonies, *B* erythroid burst)

Factor	Number of colonies/500 cells					
	GM	Blast	Meg	B	Mix	Total
SCF	28±5	10±3	0	0	0	38±8
SCF + IL-6	35±3	10±6	0	0	0	45±6
SCF + IL-6 + sIL-6R	46±9	25±6	11±4	25±2	75±14	182±20
SCF + LIF	42±5	6±5	1±1	0	0	39±4
SCF + IL-6 + sIL-6R + LIF[b]	36±6	15±2	9±1	23±8	69±11	162±9
SCF + IL-11	30±2	17±6	0	0	0	47±5
SCF + IL-6 + sIL-6R + IL-11[b]	62±12	20±5	10±3	19±3	61±9	172±11
SCF + OSM	33±4	5±4	0	0	0	38±6
SCF + IL-6 + sIL-6R + OSM[b]	46±11	15±5	7±1	33±6	74±12	175±18
CNTF + SCF	32±5	4±1	0	0	0	32±3
SCF + IL-6 + sIL-6R + CNTF[b]	48±5	17±6	5±3	28±8	61±12	159±18

[a] Data represent mean values + SEM for triplicate cultures
[b] Results not significantly different from those recorded for SCF + IL-6 + sIL-6R ($P > 0.05$)

mented with IL-6, sIL-6R, and SCF completely blocked IL-6–sIL6R-mediated proliferation and differentiation of hematopoietic stem/progenitor cells. In contrast, anti-gp130 MAbs failed to affect blood-cell development in cultures supplemented with a combination of other cytokines, i.e., SCF, IL-3, G-CSF, GM-CSF, EPO, and TPO, confirming that gp130 functions as the signal transducer for IL-6–sIL-6R [11, 12].

The significant proliferation and differentiation of human hematopoietic progenitor cells observed in cultures containing sIL-6R in the presence of IL-6 and SCF and the lack of this effect seen in cultures without sIL-6R suggest that sIL-6R confers responsiveness to IL-6 on human CD34+ cells on which gp130 but not IL-6R is expressed. The nonparallel expression patterns of gp130 and IL-6R on human CD34+ cells was first demonstrated by our recent flow-cytometry studies, in which all CD34+ cells were found to express gp130 but a majority of these cells did not express IL-6R. In cultures stimulated by a combination of SCF, IL-6, and sIL-6R or a combination of SCF, IL-3, EPO, and G-CSF, CD34+.IL-6R− cells generated a large number of cells and colonies of multiple hematopoietic lineages, whereas the progeny of CD34+.IL-6R+ cells were mainly myeloid under the same conditions (Tajima et al., unpublished data). Thus, CD34+.gp130+.IL-6R may be the phenotype of most human hematopoietic progenitors, including CFU-Mix, CFU-Blast, BFU-E, and CFU-Mk, and gp130 activation of these progenitors by the sIL-6R–IL-6 complex, but not by IL-6 alone, mediates a novel function in the proliferation and differentiation of human hematopoietic stem/progenitor cells.

Effects of gp130-stimulatory cytokines on CD34+ cells

Since gp130 is the signal-transducing receptor component utilized by IL-11, LIF, CNTF, and OSM in addition to IL-6, we found it interesting to examine their possible different roles and interactions in the development of hematopoietic cells. Of this family, IL-6, IL-11, and LIF have been well characterized and shown to have various effects on hematopoietic stem/progenitor cells; although they have strong effects on murine stem/progenitor cells, their effects on human stem/progenitor cells are much less pronounced. In our recent studies, the effect of IL-11, LIF, CNTF, and OSM on human CD34+ cells and their possible interactions with IL-6–sIL6R have been examined in both suspension and clonal cultures of CD34+ cells (Sui et al., unpublished data). IL-11 and LIF were shown to have effects comparable with those of IL-6 on colony formation in the presence of SCF, whereas no stimulatory effect on CD34+ cells was observed with OSM or CNTF (Table 1). The addition of IL-11, LIF, CNTF, or OSM to cultures supplemented with IL-6–sIL6R and SCF did not affect the colony formation mediated by IL-6–sIL6R. Similar effects were observed in suspension culture. Collectively, our studies suggest that the observed effects of IL-6–sIL6R on the proliferation and differentiation of human stem/progenitor cells are independent of the other members of the IL-6 family and that gp130 on CD34+ cells is activated specifically by the IL-6–sIL6R complex.

In vivo role and future prospects

The ubiquitously expressed gp130 and nonparallel expression of receptors for IL-6 and IL-6-related cytokines on CD34+ cells suggest that gp130 may play an important role in vivo and may function as a signal transducer for unknown cytokines or cytokine receptors. The essential role of gp130 in hematopoiesis in vivo has been confirmed in gp130 knockout mice [15]: mutant embryos have markedly reduced numbers of pluripotential and committed hematopoietic progenitors such as CFU-S, CFU-GM, BFU-E, and CFU-Mk in the liver, and some show severe anemia due to impaired maturation of erythroid cells. The in vivo role of c-Kit in hematopoiesis has been well documented in W mutation mice [8]. Taken together, our in vitro data suggest that gp130 and c-Kit signaling play a vital role in the proliferation and differentiation of human hematopoietic stem/progenitor cells in vivo. sIL-6R and

IL-6 as well as a functional IL-6–sIL6R complex and SCF are present in human serum [2, 5, 6]. In addition, the half-maximal effect of sIL-6R in serum-free culture was observed at a concentration within the physiological range of sIL-6R in human serum [Sui et al., unpublished data]. Thus, IL-6–sIL6R might be the physiological human stimulator for the ubiquitously expressed gp130 and may play a critical role in the development of human blood cells in vivo. However, we cannot exclude the possibility that a new member of the IL-6 family that signals via gp130 and plays a crucial role in human hematopoiesis may exist. The striking effects induced by the IL-6–sIL6R complex in human hematopoiesis may mimic the function of such a novel, unidentified cytokine.

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