IL-6 promotes an increase in human mast cell numbers and reactivity through suppression of suppressor of cytokine signaling 3

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Background: IL-6, levels of which are reported to be increased in association with mastocytosis, asthma, and urticaria, is used in conjunction with stem cell factor to generate CD34$^+$ cell–derived primary human mast cell (HuMC) cultures. Despite these associations, the effects on and mechanisms by which prolonged exposure to IL-6 alters HuMC numbers and function are not well understood.

Objectives: We sought to study the effect of IL-6 on HuMC function, the mechanisms by which IL-6 exerts its effects, and the relationship of these findings to mastocytosis.

Methods: HuMCs were cultured in stem cell factor with or without IL-6. Responses to FcεRI aggregation and expression of proteases and receptors, including the soluble IL-6 receptor (sIL-6R), were then quantitated. Epigenetic changes in suppressor of cytokine signaling 3 (SOCS3) were determined by using methylation-specific PCR. Serum samples from healthy control subjects and patients with mastocytosis were assayed for IL-6, tryptase, and sIL-6R.

Results: IL-6 enhanced mast cell (MC) proliferation, maturation, and reactivity after FcεRI aggregation. IL-6 reduced expression of SOCS3, which correlated with methylation of the *SOCS3* promoter and increased expression and activation of signal transducer and activator of transcription 3. IL-6 also suppressed constitutive production of sIL-6R, and serum levels of sIL-6R were similarly reduced in patients with mastocytosis.

Conclusion: IL-6 increases MC proliferation and formation of a more reactive phenotype enabled by suppressing proteolytic cleavage of sIL-6R from IL-6R and downregulation of the SOCS3 autoinhibitory pathway. We suggest IL-6 blockade might ameliorate MC-related symptoms and pathology in patients with MC-related diseases associated with increased IL-6 levels, including mastocytosis. (J Allergy Clin Immunol 2016;137:1863-71.)

Key words: *Mast cells, signal transducer and activator of transcription 3, suppressor of cytokine signaling 3, stem cell factor, IL-6, mastocytosis*

The pleiotropic cytokine IL-6 is produced by T cells, macrophages, and other cells in response to infection and acute inflammation and has been associated with the pathogenesis of several CD34$^+$ cell–derived primary human mast cell (HuMC)–related diseases.[1,2] These include the clinical observations that IL-6 levels relate to the severity of disease in patients with systemic mastocytosis,[3,4] acute[5] and chronic urticaria,[6] and asthma.[7] *In vitro*, IL-6 promotes HuMC maturation,[8] adhesion to extracellular matrix,[9] chemokinesis,[10] and survival, the latter through IgE-dependent production of mast cell (MC)–derived IL-6.[11] IL-6 is also routinely used to supplement stem cell factor (SCF) to generate HuMCs from cord or peripheral blood progenitor (CD34$^+$) cells.[8,12,13] The consequences of long-term exposure to IL-6 on HuMC function and the mechanisms by which IL-6 alters MC behavior have not been investigated.

IL-6 receptor (IL-6R; IL-6Rα or CD126) is largely restricted to hematopoietic cells (reviewed by Mihara et al[2]), whereas its signaling coreceptor, glycoprotein 130 (gp130 [CD130]), is ubiquitously distributed. Even among the few types of cells that express IL-6R, gp130 is present in great excess.[14] Cells that express gp130 but not IL-6R can also respond to IL-6 through its binding to soluble IL-6 receptor (sIL-6R) generated by means of alternative splicing or proteolytic cleavage of the membrane form.[2,15] The IL-6/sIL-6R complex then interacts with spare gp130 on the cell surface, which is referred to as trans-signaling, in contrast to classic signaling initiated through IL-6R and gp130 at the cell surface. The formation of a dimeric IL-6/IL-6R/gp130 complex[16] is followed by mutual transactivation of gp130 and Janus kinase (JAK) 1,[17,18] activation of the Ras/Raf/mitogen-activated ERK kinase/extracellular signal-regulated kinase (ERK) pathway, and phosphorylation and dimerization of signal transducer and activator of transcription (STAT) 3, which then induces transcription of genes, including suppressor of cytokine signaling 3 *(SOCS3)*, which in turn modulates activation of these pathways.[19]

We have investigated the effect of constant IL-6 exposure *in vitro* and *in vivo*, and as reported here, such exposure promotes development of not only a more mature but also a more reactive HuMC phenotype with significantly enhanced FcεRI-mediated signaling, degranulation, and cytokine production. The prolonged effects of IL-6 on HuMC function occurred in association with loss of SOCS3 autoinhibition of the IL-6/JAK/STAT pathway and suppression of sIL-6R production. *In vivo* IL-6 levels in mastocytosis correlated with serum tryptase levels and inversely

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Supported by the Division of Intramural Research Programs of the National Institute of Allergy and Infectious Diseases and the National Heart, Lung, and Blood Institute, National Institutes of Health.

Disclosure of potential conflict of interest: C. Prussin receives support from the American Board of Allergy and Immunology. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication July 24, 2015; revised September 16, 2015; accepted for publication September 30, 2015.

Available online January 7, 2016.

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0091-6749

Published by Elsevier, Inc. on behalf of American Academy of Allergy, Asthma & Immunology

http://dx.doi.org/10.1016/j.jaci.2015.09.059

Abbreviations used
ADAM: A disintegrin and metalloproteinase
DNMT: DNA methyltransferase
ERK: Extracellular signal-regulated kinase
gp130: Glycoprotein 130
HuMC: Human mast cell
IL-6R: IL-6 receptor
JAK: Janus kinase
MC: Mast cell
PE: phycoerythrin
PLCγ: Phospholipase Cγ
SCF: Stem cell factor
sIL-6R: Soluble IL-6 receptor
SOCS3: Suppressor of cytokine signaling 3
STAT: Signal transducer and activator of transcription

correlated with serum sIL-6R levels. These data support the concept that decreasing IL-6 levels in patients with diseases, such as mastocytosis, might have a beneficial therapeutic effect.

METHODS

For detailed methods, including mice used, experimental protocols and procedures, clinical protocols, and statistical analysis, see the Methods section in this article's Online Repository www.jacionline.org.

RESULTS

Exposure to IL-6 enhances HuMC proliferation and maturation

HuMCs proliferated to a significantly greater extent ($P < .001$) when grown in the presence of IL-6 and SCF than when grown in SCF alone (Fig 1, *A*). However, SCF itself was obligatory because cells did not proliferate when cultured in IL-6 alone (Fig 1, *A*). This observation is similar to previous reports of the effect of IL-6 on cord blood–derived MCs[20,21] but differ from a single report that IL-6 decreases the growth of human MCs from cord blood in which the varied results were attributed to differing culture conditions.[8] Once cells had reached their most mature state at 6 weeks, those exposed to IL-6 exhibited greater cell size and granularity (Fig 1, *B*). Examination by means of flow cytometry of the major MC-specific granule proteases, namely tryptase, chymase, and carboxypeptidase,[22,23] indicated that all 3 were expressed regardless of growth conditions, although the chymase content was substantially increased in IL-6–conditioned cells (Fig 1, *C*). Thus these data are consistent with and extend previous reports that IL-6 increases the number[13,20,21,24] and maturity[8] of human MCs in culture.

HuMCs derived from cord blood $CD34^+$ cells express the SCF receptor (KIT, CD117), FcεRI,[25] gp130,[8] and IL-6R at the cell surface. Examination of HuMCs derived from $CD34^+$ cells from peripheral blood samples by means of flow cytometry indicated that IL-6 did not alter the surface expression of KIT, the FcεRI α-subunit, gp130, and IL-6R (see Fig E1, *A*, in this article's Online Repository www.jacionline.org). Western blots also indicated similar expression of IL-6R, as well as gp130, irrespective of whether cultures were grown in the presence or absence of IL-6 (see Fig E1, *B*). Therefore HuMCs grown under either condition express similar levels of the necessary receptors for responses to SCF, antigen/IgE, and IL-6.

Culture in IL-6 leads to more robust responses to FCεRI ligation

Stimulation of biotinylated IgE-sensitized HuMCs with graded concentrations of Streptavidin revealed significant enhancement of degranulation in response to concentrations of streptavidin greater than 0.1 ng/mL in SCF/IL-6–cultured HuMCs, with a maximal response approximately twice that of cells cultured in SCF alone ($P < .01$; Fig 2, *A*). The effect of IL-6 was concentration dependent, with significant enhancement with as little as 3 ng/mL and maximal enhancement at 30 ng/mL IL-6 (Fig 2, *B*). As described in more detail later, the onset of IL-6 action was time dependent, with significant increases in degranulation by 12 hours (data not shown).[25] Conditioning of HuMCs with IL-6 also augmented production of the cytokines GM-CSF and IL-8 (Fig 2, *C* and *D*), with optimal effects at 30 ng/mL IL-6 (Fig 2, *E*). In this experiment cytokine production was induced by costimulation with streptavidin and SCF because these stimulants individually elicit limited cytokine production.[26] SCF-induced chemotaxis of HuMCs[27] was not significantly affected by IL-6 (Fig 2, *F*).

Production of sIL-6R by HuMCs is inhibited by IL-6

We next investigated the production of sIL-6R and sgp130 by HuMCs to determine whether the effects of IL-6 were due to classical or trans-signaling and the potential effect in patients with inflammatory disease.[28] HuMCs produced sIL-6R and sgp130 spontaneously in approximately equimolar concentrations (Fig 3, *A* and *B*). The notable finding was that production of sIL-6R (Fig 3, *A*), but not sgp130 (Fig 3, *B*), was significantly suppressed by IL-6 in a concentration-dependent manner, including marked suppression at a concentration of 100 ng/mL IL-6, which is normally used for HuMCs. This action of IL-6 would minimize trans-signaling caused by a decrease in formation of IL-6/sIL6R complexes. Production of sIL-6R was also inhibited by IL-6 in the human LAD2 MC line, with an inhibitory concentration of 50% of approximately 1 ng/mL (see Fig E2, *A*, in this article's Online Repository www.jacionline.org). GI254023X and GW280264, inhibitors of a disintegrin and metalloproteinase (ADAM) 10 and ADAM17, the metalloproteases responsible for cleavage of IL-6R to form sIL-6R (see Scheller et al[28]), suppressed production of sIL-6R by LAD2 cells, which is consistent with the conclusion that sIL-6R resulted largely from proteolytic cleavage (see Fig E2, *B*).[29,30]

Evidence was obtained that IL-6 can similarly suppress sIL-6R production *in vivo* from studies of patients with systemic mastocytosis in whom serum levels of IL-6 correlate with serum tryptase[3] levels used as a measure of MC burden.[31] Extending this study, we found that although there was a positive correlation between serum IL-6 and tryptase levels, as we and others have reported (Fig 3, *C*),[3] there was a negative correlation between serum sIL-6R and IL-6 levels (Fig 3, *D*). No significant correlation was observed between serum sgp130 and serum tryptase levels or, as in MC cultures, with IL-6 levels (data not shown). These data support the possibility that IL-6 levels *in vivo* might also suppress the production of sIL-6R and thus limit trans-signaling *in vivo*.

Increased FcεRI-, KIT-, and IL-6R–mediated signaling in IL-6–conditioned HuMCs

To determine whether the enhanced responses in the presence of IL-6 could be attributed to stronger signaling through relevant