Molecular Pathways

Novel Insights into the Role of Interleukin-27 and Interleukin-23 in Human Malignant and Normal Plasma Cells

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Abstract

Multiple myeloma is a monoclonal postgerminal center tumor that has phenotypic features of plasmablasts and/or plasma cells and usually localizes at multiple sites in the bone marrow. The pathogenesis of multiple myeloma is complex and dependent on the interactions between tumor cells and their microenvironment. Different cytokines, chemokines, and proangiogenic factors released in the tumor microenvironment are known to promote multiple myeloma cell growth. Here, we report recent advances on the role of 2 strictly related immunomodulatory cytokines, interleukin-27 (IL-27) and IL-23, in human normal and neoplastic plasma cells, highlighting their ability to (i) act directly against multiple myeloma cells, (ii) influence the multiple myeloma microenvironment by targeting osteoclast and osteoblast cells, and (iii) modulate normal plasma cell function. Finally, the therapeutic implication of these studies is discussed.

Clin Cancer Res; 17(22); 6963–70. ©2011 AACR.

Background

Interleukin (IL)-27 and IL-23 are immunomodulatory cytokines that belong to the IL-12 superfamily (1), which comprises structurally and functionally related heterodimeric cytokines, including IL-12, IL-23, IL-27, and IL-35 (1). These cytokines are predominantly produced by antigen-presenting cells in response to microbial or host immune stimuli and are involved in the regulation of immune responses against infections and tumor development (2). Recent preclinical studies showed the direct antitumor activities of IL-12 family cytokines in different human hematologic malignancies, including pediatric acute leukemias and multiple myeloma, as well as in solid tumors (3–7). Here, we report and discuss recent advances in the role of IL-27 and IL-23 in multiple myeloma cells and their microenvironment and in human normal plasma cells.

Interleukin-27

IL-27 is composed of the EBI3 and p35 subunits and activates both STAT1 and STAT3 through a distinct IL-27 receptor, which consists of the unique receptor subunit WSX-1 paired with the gp130 chain (8). In T lymphocytes, the cytokine induces STAT1 and STAT3 activation, thus resulting in enhancement of the naïve cluster of differentiation (CD)4⁺ T-cell proliferation, promotion of the early T-helper (Th)1 differentiation, and suppression of the differentiation of Th2 and Th17 cells. In addition, IL-27 plays a key role in generating IL-10–producing regulatory T cells (1, 9).

In human tonsils, the different B-cell subsets show variable expression of IL-27 receptor (10). Investigators have found that naïve and memory B lymphocytes constitutively expressed both chains of IL-27 receptor, whereas germinal center B cells exhibited only barely detectable levels of the WSX-1 chain. However, the WSX-1 was found to be strongly upregulated during germinal center to memory B-cell transition (10). In terms of signal transduction, IL-27 induces a strong phosphorylation of both STAT1 and STAT3 in naïve B cells, whereas moderate STAT1 and low activation of STAT3 were observed in memory B lymphocytes (10). In addition, human plasma cells constitutively express WSX-1 and gp130, and IL-27 specifically induces STAT1 phosphorylation while not affecting STAT3 or STAT5 activation (5). Differences in the STAT activation driven by IL-27 may reflect the different ability of human B-cell subsets to respond to IL-27. For example, IL-27 enhances proliferation in naïve and activated germinal center B cells (10); induces surface expression of CD54, CD95, and CD86 in stimulated memory B cells (10); and exerts chemotactic activity on plasma cells (5).

Antitumor activity of IL-27 against myeloma cells. IL-27, similar to IL-12, shows antitumor activity in different cancers through indirect mechanisms, such as induction of natural killer (NK) and CTL response or inhibition of angiogenesis, primarily due to induction of CXCL10 and CXCL9 (3, 11–14). In addition, it has been reported that IL-27 directly inhibits the growth of melanoma cell lines (7) and B–acute lymphoblastic leukemia (ALL) cells.
expressing complete IL-27 receptor (12). In multiple myeloma, it has been reported that both chains of IL-27 receptor are expressed in CD138⁺ multiple myeloma cells from patients (5, 11). Although IL-27 does not modulate multiple myeloma cell proliferation and apoptosis, a strong reduction of the angiogenic potential of multiple myeloma cells was clearly documented (5). It is of note that bone marrow neovascularization contributes to the biology of several hematologic malignancies (15), and multiple myeloma neoangiogenesis may be induced by angiogenic cytokines produced by tumor and microenvironmental cells (16). Cytokines, such as basic fibroblast growth factor, VEGF, and angiopoietins, play a leading role in new vessel formation as well as in tumor angiogenesis (17–19). However, other known and unknown factors may also be involved and are under investigation. We reported that IL-27 downregulates in vitro a wide panel of proangiogenic molecules, including VEGF-A, VEGF-C, and VEGF-D, angiopoietins, and matrix metalloproteinases (MMP), and it upregulates the antiangiogenic chemokines CXCL9 and CXCL10 (Fig. 1; ref. 11). Accordingly, preclinical studies using highly immunodeficient nonobese diabetic/severe combined immunodeficient mice injected with multiple myeloma cells revealed that IL-27 inhibits multiple myeloma cell growth through inhibition of angiogenesis, which caused ischemic necrosis in the tumors. In this model, the main proangiogenic molecules and multiple myeloma growth factors downregulated by IL-27 were IL-6, VEGF-D, and CCL2, which also serve as autocrine and/or paracrine growth factors (20, 21). Thus, we can envisage

Figure 1. IL-27 activity against multiple myeloma (MM) cells and the paracrine growth loop. IL-27 directly acts on multiple myeloma cells that express complete IL-27 receptor. IL-27 inhibits the angiogenic potential of multiple myeloma cells and downregulates different angiogenic factors that also serve as autocrine and/or paracrine growth factors. IL-27 may interrupt or reduce the growth loop in which cytokines and growth factors released by multiple myeloma cells induce paracrine stimulation of stromal cells, which, in turn, secrete additional growth factors that stimulate new vessel formation and support multiple myeloma cell proliferation, progression, and migration.
that IL-27 may interrupt or reduce the growth loop (Fig. 1), in which cytokines and growth factors released by multiple myeloma cells induce paracrine stimulation of stromal cells, which, in turn, secrete additional growth factors, such as IL-6 and VEGF, which induce new vessel formation and support multiple myeloma cell proliferation, progression, and migration. In addition, multiple myeloma cells and bone marrow stromal cells produce MMPs that degrade collagens and fibronectin, thus rendering multiple myeloma cells capable of invading both stroma and the subendothelial basement membrane (Fig. 1; refs. 22–25). The enhanced invasive ability of multiple myeloma cells and their increased angiogenic capacity may explain the frequent intramedullary and extramedullary dissemination observed in multiple myeloma pathogenesis. Preclinical studies showed that IL-27 downregulates MMP9, thus suggesting that IL-27 may also reduce multiple myeloma cell invasion (Fig. 1; ref. 11).

Interleukin-23

IL-23 is a heterodimeric cytokine composed of the promiscuous IL-12p40 and the exclusive p19 subunits (26). The IL-23 receptor is composed of the IL-12 receptor β1 chain and the unique IL-23 receptor chain, which is associated with Jak2 and STAT3 (26). In T lymphocytes and leukemic T cells, IL-23 induces activation of STAT family members STAT1, 3, 4, and 5 (26). IL-23 is produced predominantly by myeloid dendritic cells activated by Toll-like receptor 2, 4, and 8 ligands and by type 1 macrophages (27, 28). This cytokine is now considered the master switch in several T-cell–mediated inflammatory processes underlying regulation of miR-15a mediated by IL-23 (i) silencing of mir-15a in B-ALL cells abolished the effects driven by IL-23 and (ii) forced expression of miR15a had a similar antitumor activity to the treatment of IL-23. In vitro and in vivo experiments unambiguously showed that the antileukemic function of IL-23 was strictly associated with the upregulation of mir-15a and that enforced expression of mir-15a had antitumor activity that was similar to treatment with IL-23, causing a clear inhibition of leukemic cell growth in vivo (4). Although the molecular mechanisms underlying regulation of miR-15a mediated by IL-23 were not elucidated, it was hypothesized that, similar to other cytokines, components of the IL-23 signaling pathway may interfere with the biogenesis or transcription of miR-15a. This preclinical study provided the first evidence that cytokine-mediated regulation of the miRNA expression pattern plays a key role in the control of B-ALL cell growth.

Finally, studies on the mechanisms underlying the proapoptotic effect of IL-23 showed that the cytokine not only downregulated the antiapoptotic BCL-2 but also induced PARP cleavage in the absence of caspase activation (4).

IL-23 and Th17 function in multiple myeloma. IL-23 is a well-known Th17-differentiating cytokine (39). Recent data highlighted that Th17 plays an important role in multiple myeloma pathobiology and may be an important therapeutic target for anti–multiple myeloma activity to improve immune function (40). In view of these data, a number of Th17–associated cytokines, including IL-23, IL-21, IL-22, and IL-17, were found to be significantly elevated in multiple myeloma compared with healthy donors, and elevated IL-17 serum levels have been correlated with stage 2 and stage 3 multiple myeloma (41). In addition, IL-23 was associated with reduced CD8+ T-cell infiltration in the tumor microenvironment (30), and a combination of Th17–associated proinflammatory cytokines may suppress T-cell responses. This recent evidence suggests a potential role of IL-23 in Th17-mediated promotion of multiple myeloma cell growth and inhibition of immune function. Finally, an imbalance between T-regulatory (Treg) and Th17, in favor of Th17, which is not
adequately suppressed by Treg cells, may be suggested, because it has been reported that Treg cells are decreased in number and are dysfunctional in multiple myeloma patients (42).

**Bone remodeling in multiple myeloma**

Bone destruction is a hallmark of multiple myeloma (43), and approximately 85% to 90% of multiple myeloma patients present with lytic bone lesions frequently associated with severe bone pains and fractures. Osteolytic lesions result from increased bone resorption due to stimulation of osteoclast formation and severely unbalanced bone remodeling or to decreased or absent bone formation activity that occurs close to multiple myeloma cells (44, 45).

The critical role of receptor activator of NF-kB ligand (RANKL) in multiple myeloma-induced osteoclastogenesis has been shown (44). RANKL is overexpressed in bone marrow in relation to multiple myeloma cell infiltration, whereas its decoy receptor osteoprotegerin is downregulated (44). Other cytokines produced by multiple myeloma cells, such as macrophage inhibitory protein (MIP)-1α (namely, CCL3), or induced in the microenvironment by cell contact are responsible for enhancement of osteoclast differentiation and activity and for inhibition of osteoblast functions (46). Interestingly, the growth factors released in the course of the increased bone resorptive process, as well as activated osteoclasts, in turn support multiple myeloma cell growth and survival, thus inducing a vicious cycle (47–49). It has been consistently reported that antosteoclastic agents may exert anti–multiple myeloma effects, both *in vitro* and *in vivo* (50).

**Effects of IL-27 and IL-23 on osteoblastic and osteoclastic cells and bone remodeling.** Recent evidence suggests that both IL-27 and IL-23 may affect the bone remodeling process in different ways. Osteoclast progenitors from multiple myeloma patients and controls (5) express functional IL-27 receptor, and IL-27 directly decreases osteoclast differentiation and activity (11). It has also been reported that IL-27 induced proliferation in osteoblastic cells while not affecting osteoblast formation (11, 51), indicating that IL-27 amplifies the mature osteoblastic compartment without modulating its functionality. Thus, IL-27 may counteract the unbalanced osteoclast and osteoblast activity induced by multiple myeloma cells, mainly exerting an inhibitory effect on osteoclastic cells.

The effect of IL-23 on osteoclast and bone resorption has been evaluated, and results are conflicting. Yago and colleagues (52) showed that, in human peripheral blood mononuclear cells, IL-23 induced osteoclastogenesis in the absence of exogenous soluble RANKL. In their study, IL-17 induced by IL-23 from human-activated T cells was considered the pivotal cytokine for osteoclastogenesis through the involvement of the RANK–RANKL system and TNF-α. Thus, IL-17 acted on monocytes and induced osteoclast differentiation and TNF-α production by cooperating with RANKL. Similarly, it was reported that IL-23 stimulates RANKL expression on CD4⁺ T cells and contributes to osteoclastogenesis in an arthritis mouse model (53). In contrast, Quinn and colleagues reported that IL-23 inhibited osteoclastogenesis indirectly through CD4⁺ T cells and that IL-23p19⁻ reduced bone mass (54). Kamiya and colleagues (51) showed that IL-23 was ineffective on RANKL expression and that osteoclastogenesis induced by soluble RANKL was partially inhibited by IL-23, whereas proliferation of osteoclast progenitors was not affected. Any significant effect of IL-23 was reported on osteoblastic cells and on osteoblast formation (51).

Overall, this evidence suggests that under physiologic conditions, IL-23 favors high bone mass by limiting bone resorption, whereas in pathophysiologic conditions, IL-23 shares a stimulatory effect on osteoclast formation, mainly through the induction of RANKL by T cells and the differentiation of Th17 subset and IL-17 production. The role and the involvement of Th17 and IL-17 in osteoclast activation in multiple myeloma were recently shown (55). Because IL-23 is a well-known cytokine involved in Th17 differentiation and expansion, it is possible that the high bone marrow levels of IL-23 observed in multiple myeloma patients (40) contribute to the increased osteoclastogenesis through the expansion of Th17 and, consequently, the overproduction of IL-17.

**Human normal plasma cells**

In humans, 3 major subsets of plasma cells have been isolated and are thought to represent a gradual increase in plasma cell maturation: early plasma cells in tonsils, transitional plasma cells in peripheral blood, and mature plasma cells in the bone marrow (56). However, plasma cells are rare cells *in vivo*, representing only 1% to 2% of tonsil mononuclear cells and less than 0.5% of bone marrow cells in healthy individuals. Thus, investigators developed an *in vitro* reproducible model of peripheral blood B-cell differentiation into polyclonal plasmablastic cells (PPC), which have morphologic and phenotypic features of plasma cells (57). In addition, this model led to a homogenous population of normal plasmablastic cells that provided a powerful tool for identifying genes specifically involved in normal plasma cell differentiation to further understand the biology of multiple myeloma and to improve therapeutic developments.

**Expression and role of IL-27 and IL-23 receptor in normal plasma cells.** The expression of both chains of IL-27 receptor and IL-23 receptor was shown in tonsil and bone marrow plasma cells as well as in PPC generated *in vitro*, and the functional role of IL-27 and IL-23 was recently elucidated (5). Because production of immunoglobulin (Ig) represents the main plasma cell function and is a key step that occurs during antigen-specific immune response, we asked whether IL-27 and IL-23 might modulate Ig secretion in these cells. These experiments showed that stimulation of PPC with IL-2 in association with IL-27 or IL-23 caused significant upregulation of IgM secretion, paralleled by inhibition of IgG secretion and unaltered IgA production. However, no effects were reported in terms of modulation of IgG class switching (5, 10).
Thus, it has been hypothesized that these cytokines are effective on PPCs that have not yet undergone terminal differentiation into antibody-secreting cells, because such effects were reverted when IL-6 was present for the last days of PPC culture. This latter consideration was strongly supported by the finding that IL-23 and IL-27 had no effect on modulation of Ig secretion in CD138⁺ tonsil plasma cells.

Another novel finding was the first demonstration that IL-27 possesses chemotactic properties versus plasma cells and PPC (5), conceivably related to dependence on STAT1 

Finally, IL-27 upregulated in plasma cells and PPCs the expression and production of chemokines such as CXCL9, which induce migration of activated T cells and NK cells and possess potent antiangiogenic activity through IFN-γ-dependent and/or IFN-γ-independent pathways (11, 59, 60). However, in PPC and plasma cells, CXCL9—and CXCL10–modulated expression by IL-27 was not accompanied by induction of IFN-γ production.

Taken together, these findings support the concept that IL-27 and, more marginally, IL-23 play an important role in the course of immunologic response to pathogens by acting at different levels. First, IL-23 and IL-27 produced by antigen-presenting cells stimulated by Toll-like receptor signals may induce short-lived plasma cells to produce low-affinity IgM, providing a rapid initial response to pathogens. Thus, IL-27 may attract plasmablast and/or short-lived plasma cells that upregulate CXCL9, but not CCL chemokines, thus creating chemokine gradients that recruit to inflamed tissue-activated T cells, memory T cells, and NK cells but not to granulocytes and monocytes. Finally, CXCL9 and CXCL10 may limit tissue damage provoked by persistent activation of inflammatory cells in view of their well-known antiangiogenic activities. These findings may also be relevant in multiple myeloma pathogenesis because normal plasma cells take part in the multiple myeloma microenvironment. Thus, the antiangiogenic CXCL9 upregulated in normal plasma cells may amplify the inhibition of angiogenesis caused by the direct activity of IL-27 on multiple myeloma cells.

Clinical–Translational Advances

Cytokines of the IL-12 family: potential clinical implications

The potential translational implication of the new evidence on the role of IL-23 and/or IL-27 in the biology of normal and malignant plasma cells is related to the ability of these cytokines to stimulate immune responses, to directly target tumor cells expressing the corresponding receptors, and, at least for IL-27, to inhibit tumor angiogenesis. Either the modulation of IL-12 family cytokines, including IL-23 and IL-27, or the direct use of these cytokines, could be targeted in future clinical trials.

In the case of IL-12, preclinical studies showed that this cytokine can directly target multiple myeloma cells and cause a strong reduction of multiple myeloma proangiogenic potential, with marginal effects on multiple myeloma cell proliferation and survival (3). However, a particular note of caution about the potential therapeutic implications of IL-12 comes from the side effects that have been reported in clinical trials addressing the antitumor activity of IL-12 against different malignancies.

The initial clinical studies were done in phase I trials with patients affected by advanced malignancies (renal cancer, colon carcinoma, and melanoma) to determine the optimal treatment protocol. The parameters analyzed were toxicity, maximum tolerated dose (MTD), biologic and antitumor activity, and route of administration. Both i.v. (61–63) and s.c. (64, 65) injection schedules were tested in patients at different times and doses. The IL-12 MTD differed in i.v. and s.c. routes of injection (500 ng/kg and 1,000–1,500 ng/kg, respectively), and s.c. administration resulted in fewer and milder toxic effects than i.v. treatment. Although preliminary studies suggested that IL-12 might be efficacious, subsequent trials did not confirm the first encouraging results. Thus, despite an enhancement of immune response, the clinical efficacy was minimal. Moreover, escalating doses of IL-12 induced serious side effects in patients. Good therapeutic results have been obtained in patients affected by cutaneous T-cell lymphoma, AIDS-related Kaposi sarcoma, and non-Hodgkin lymphoma that showed a clear increase in circulating and tumor infiltrating T cells with objective responses of 56%, 50%, and 43%, respectively (66–68). The favorable results obtained in immunodeficient patients with AIDS suffering from Kaposi sarcoma were not surprising because this tumor is strongly angiogenic, and angiogenesis inhibition is one of the most prominent IL-12 activities. Thus, it was speculated that the antineoplastic activity of IL-12 was largely dependent on its ability to inhibit angiogenesis.

To overcome or reduce systemic toxicity, novel nonviral delivery forms of IL-12, such as alum, liposome, and polymer-based delivery, have shown promising effects in preclinical models (69). A further refinement of tumor-targeted IL-12 therapy would be the use of targeted formulations of the cytokine, such as that developed by Halin and colleagues (70), who generated a fusion protein containing an IL-12 moiety fused to a single-chain monoclonal antibody specific for tenascin-C, an isoform of this extracellular matrix protein expressed predominantly in the tumor stroma. This fusion protein, however, has not been tested in clinical trials. More recently, the effectiveness of electrogene therapy with IL-12 has been evaluated in preclinical studies (71), and the antitumor efficacy was tested in different types of primary tumors, distantly growing tumors, and induced metastases. Intratumoral IL-12 electrogene therapy proved to be very effective in local tumor control, and it also had a systemic effect; intramuscular and peritumoral IL-12 electrogene therapy had a pronounced systemic effect. The antitumor effectiveness of IL-12 electrogene therapy was due to the induction of adaptive immunity, innate resistance, and antiangiogenic action.

A further promising area of investigation, with potentially relevant clinical applications, stems from the emerg-
ing knowledge that both IL-23 and IL-27 have been used to effectively treat tumors in murine models. Systemic administration of IL-23 has been shown to be effective in the mouse tumor system, including melanoma and fibrosarcoma (32, 72). In addition, preclinical evidence suggests that IL-23 is a promising candidate as a tumor vaccine adjuvant (37). Interestingly, adverse toxic effects of systemic treatment with IL-23 seem to be much less than those observed for IL-12, although some authors reported weight loss and inflammation of the intestinal tract due to IL-23 schedule treatment (37). On the other hand, mice injected intraperitoneally with recombinant IL-23 did not show splenomegaly and liver inflammation compared with those treated with IL-12 (72). Therefore, from a clinical perspective, the IL-23 administration route should be designed to avoid its adverse effects and to exert efficient antitumor effect.

Despite the body of evidence describing the antitumoral effect of IL-23 in tumoral models, caution in its clinical application is justified because of the role of IL-23 in inflammatory diseases and its possible role in tumoral progression (30, 36, 73, 74). In fact, in mice lacking IL-23p19 or IL-23 receptor, IL-23 showed a tumoral-promoting effect in contrast to its potent antitumor effect. The reasons for this discrepancy are not known; however, the potential clinical use of this cytokine should be carefully evaluated. IL-23 seems to be ineffective against multiple myeloma cells, but its role in the control of the multiple myeloma microenvironment has not been clearly elucidated.

By contrast, we could envisage combinatorial approaches using IL-12 and IL-27 to promote T-cell and NK-cell responses against multiple myeloma, in addition to their direct antitumor activity against multiple myeloma and the ability of IL-27 to stimulate osteoblast proliferation and inhibit osteoclast differentiation and function. In light of this activity, exciting results have been reported against melanoma and breast cancer using IL-12 and IL-27 sequential gene therapy through intramuscular electroporation (75). More recently, the efficacy of IL-27 as an antitumor agent against prostate cancer using sonoporation, a new mode of gene delivery that involves low-frequency ultrasound irradiation, has been reported (76). Investigators used 3 models of immune-competent prostate adenocarcinoma, and detailed characterizations of tumor growth reduction, gene profile expression, and effector cellular profiles were reported. Results from this investigation suggest that IL-27 can be effective in reducing tumor growth and can help enhance accumulation of effector cells in prostate tumors in vivo (76).

In conclusion, preclinical studies support the concept that IL-27 may represent a novel, promising therapeutic agent for patients affected by different oncologic diseases, potentially including multiple myeloma, based on its documented multifunctional activity. Before this step, the development of future clinical trials to evaluate the toxicity and efficacy of IL-27 will be mandatory. An additional argument in favor of this proposal is the low toxicity shown by IL-27 in animal models, likely in relation to the low induction of IFN-γ in vivo (32). Clearly, dedicated studies are required to define the clinical utility of IL-27 as an antiangiogenic and antitumoral agent in patients with solid cancer and multiple myeloma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was supported by grants from A.I.R.C., Milan, Italy (numbers 4014 to I. Airoldi and 8530 to N. Giuliani), Italian Ministry of Health (BF, BC, 5/1000, Progetto Strategico Oncologico 2006 rib707010), International Myeloma Foundation (to N. Giuliani), Italian Ministry of Health-Progetti Regione Emilia Romagna, and the Special Program Molecular Clinical Oncology 5 per mille (number 9965).

Received July 5, 2011; revised August 3, 2011; accepted August 10, 2011; published OnlineFirst August 31, 2011.


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