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REVIEW ARTICLE

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Interleukin-6 and megakaryocytopoiesis: an update

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Abstract Interleukin-6 is a pleiotropic cytokine which shows a wide variety of biologic functions on various tissues and cells. Indeed, IL-6 acts not only on B cells but also on T cells, hepatocytes, hematopoietic progenitor cells, megakaryocytes, etc. In this review, I have attempted to summarize the new data concerning the effect of IL-6 on megakaryocytes and platelets.

Key words Megakaryocyte · Interleukin-6 · Thrombocytosis · Anemia · platelet function

Introduction

Interleukin-6 (IL-6) is a multifunctional cytokine which shows a wide variety of biologic functions on various tissues and cells. IL-6 acts not only on B cells but also on T cells, hepatocytes, hematopoietic progenitor cells, megakaryocytes, etc. [1, 6, 8, 50, 51, 63].

In vitro studies with IL-6

In in vitro serum-free cultures, IL-6 alone does not affect megakaryocyte colony formation but enhances the diameter, the ploidy, the number, the acetylcholinesterase activity, and the protein synthesis of megakaryocytes [11, 43, 44, 48, 49, 65]. In the presence of normal plasma (containing several growth factors) IL-6 is able to stimulate megakaryocyte proliferation, perhaps via complex interactions in a cytokine network [9, 10]. In addition, although IL-6 has little megakaryocyte progenitor proliferation capacity, it acts synergistically with IL-3 and IL-1 to augment megakaryocyte colony formation [9, 14, 15, 26, 72, 79, 86]. IL-6 stimulates megakaryocytes to form cytoplasmic processes in vitro which have the same morphology of collagen gel megakaryocyte processes and are considered to represent in vitro proplatelets [2, 55]. Proplatelets contain platelet-specific organelles and proteins. Ultrastructural analysis of these platelet-like particles reveals a little difference in the cytoskeleton organization of platelet in comparison to control conditions [55].

It has been shown that IL-6 receptors are present on immature but not mature megakaryocytes and that megakaryocytes also synthesize IL-6 [65] and express IL-6 and its receptor [64]. Immature megakaryocytes are identified by their size and morphology.

In vivo studies on animals

When given in vivo to rodents or primates, IL-6 induces an augmentation of size, number, ploidy, and protein synthesis of megakaryocytes and augments the platelet count [3, 13, 14, 26, 34, 35, 42, 43, 45, 59, 69, 78, 90]. However, important differences exist among species. In mice and primates, IL-6 has a major effect on the ploidy of megakaryocytes with a mode varying from 16N to 64N, whereas in dogs no effect on the ploidy is observed [13, 35, 78]. A synergistic effect between IL-3 and IL-6 is observed in vivo on the platelet production in primates [26]. More recently, Winton and colleagues showed that IL-6 prevents significant thrombocytopenia and shortens the neutropenic period in a nonhuman primate chemotherapy model. They showed that IL-3 used in combination with IL-6 permitted the correction of anemia observed when IL-6 is used alone [89].

Another study from Patchen and coworkers showed the efficacy of combined administration of IL-6 and granulocyte colony-stimulating factor on the recovery from radiation-induced hematopoietic aplasia on mice. The most notable effects were observed on recovery of bone marrow and splenic colony-forming units [67, 68].

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In a study by Asano and colleagues using monkeys, twice-daily subcutaneous injections of recombinant human IL-6 (5–8 μg/kg daily for 14 consecutive days) led to a dose-dependent increase in platelet count [3]. Elevations twofold or more above the baseline count were observed, and at doses of more than 20 μg/kg daily the increase became biphasic, with a second, higher peak noted 3 days after cessation of the injections. Morphological examination of marrow megakaryocytes after 7 days of injections of 80 μg/kg (per day) revealed a marked augmentation in megakaryocyte size. Notably, however, at the highest dose of IL-6 employed, signs of systemic toxicity were apparent in the animals, as indicated by a dose-dependent loss of body weight, anemia, neutrophilia, and monocytosis, elevation of serum C-reactive protein, and decrease of serum albumin. All of these abnormalities returned to normal within a week after cessation of the injections and were less of a problem at doses less than 10 μg/kg per day. In another study, Geissler et al. administered IL-6 to primates after priming the animals with IL-3 [26]. The rational for this study was that IL-3 increases the number of assayable megakaryocyte progenitor colonies, while IL-6 stimulates megakaryocyte maturation without consistent effect on the megakaryocyte progenitor colony proliferation. In this study, platelet counts doubled in treated animals, but, interestingly, the counts began to fall while the animals were still receiving IL-6.

Ryffel et al. showed that administration of IL-6 to marmosets caused a selective and sustained stimulation of thrombopoiesis that was ablated only by the appearance of neutralizing antibodies [73].

In general, in vivo infusions of recombinant human IL-6 augment platelet production two- to eightfold, depending on the individual animal and dose of IL-6 given. The effect of IL-6 on platelet count appears to be biphasic, with an initial decline in numbers followed by a rebound increase [78]. However, the biphasic pattern has not been confirmed by all studies [21].

Furthermore, IL-6 was shown to enhance the recovery of megakaryocytopoiesis and thrombopoiesis after bone marrow damage [13, 33].

The only argument for a role of IL-6 in the regulation of megakaryocytopoiesis is provided by Cox and colleagues, who demonstrated that IL-6 mRNA (in spleen and bone marrow) and IL-6 protein increase in response to acute thrombocytopenia induced in mice [16]. However, the kinetic of the IL-6 increase does not follow the evolution of the platelet number. Furthermore, mice in which the gene coding for IL-6 has been inactivated by homologous recombination do not have thrombocytopenia [71]. Finally, the activities of the most pure fractions of (plasmatic) thrombopoietic activity are not neutralized by antibodies neutralizing IL-6 [36].

Clinical studies

Clinical studies of IL-6 have shown its stimulatory effects on thrombopoiesis in patients with advanced malignancies [21, 27, 53, 74, 85, 87]. The recent study by Gordon et al. showed that patients with myelodysplastic syndromes who received IL-6 (1.0 to 5.0 μg/kg/day) had a persistent increase in platelet counts and that IL-6 increased the frequency of higher ploidy megakaryocytes but did not significantly increase the number of assayable megakaryocytic progenitor cells [27]. Another important clinical study by van Gameren and coworkers has revealed that in cancer patients, IL-6 increased leukocyte and platelet counts with a decrease in the mean platelet volume [84]. Another study of the effect of IL-6 was done following autologous bone marrow transplantation in patients with advanced breast cancer [53]. The authors concluded that the maximal tolerated dose was 1 μg/kg/day, a dose lower than the maximal tolerated dose after conventional cytotoxic therapy [53]. These reports gave positive results on thrombocytopoiesis of IL-6, in contrast to the study of Schrezenmeier and colleagues, who studied the effect of IL-6 in patients with aplastic anemia and revealed that IL-6 given alone at low doses does not increase platelet counts and can precipitate a sudden worsening of pre-existing anemia and thrombocytopenia [74].

IL-6 and mobilization of primitive hematopoietic cells into the circulation

Pettengell and co-workers have shown that after treatment with IL-6, a significant increase in the level of circulating mononuclear cells and granulocytic and macrophagic colony-forming cells was observed in cancer patients. However, no change in the number of circulating megakaryocyte colony-forming cells was shown. Pettengell and colleagues concluded by suggesting that IL-6 alone is not clinically sufficient to mobilize progenitor cells in cancer patients [70].

IL-6 and anemia

Clinical investigations using IL-6 have shown a dose-dependent decrease in hematocrit levels [4] which may be reversible [66]. Atkins and colleagues have shown that anemia is largely due to hemodilution secondary to a significant increase in plasma volume. Anemia has been postulated to be caused by red blood cell sequestration, hemolysis, blood loss, or diminished erythropoiesis [4]. Hypotheses concerning the mechanism by which IL-6 caused an increase in the plasma level could be an increase in the serum cortisol levels, a decline in myocardial contractibility, or a peripheral vascular dilatation related to a decrease in peripheral vascular resistance [4].

IL-6 implications in thrombocytosis and in the inflammatory response

Thrombocytosis (platelet count $>450000/\mu l$) can be classified into primary and secondary forms. Primary thrombocytosis seen in myeloproliferative syndromes is believed to result from an overproduction of platelet production, which could be due to independence from growth factor control [12, 77]. On the other hand, secondary or reactive thrombocytosis is noted in numerous clinical situations [12, 46]. Its cause has been proposed to be a persistent overproduction of a thrombocytopoietic factor acting on megakaryocytes and their progenitors, which are capable of responding to growth factors [12, 37, 46, 77].

In the studies of Hollen et al., serum IL-6 levels of patients with reactive thrombocytosis were shown to be significantly greater than those of controls [38, 39]. In contrast to patients with reactive thrombocytosis, they observed a normal level of serum IL-6 in patients with myeloproliferative disorders, which is consistent with the hypothesis that the thrombocytosis of myeloproliferative disorders is autonomous, and not attributable simply to a positive feedback mechanism [12, 38, 39, 46, 77]. However, they did not observe a correlation between the platelet number and the plasmatic IL-6 level in patients with reactive thrombocytosis.

IL-6 is produced by a variety of cells that are involved in the inflammatory response [31, 50, 75]. It has been shown that IL-6 is involved in the acute-phase response by induction of C-reactive protein, but also that elevated serum IL-6 levels reflect the severity of acute inflammation [54]. Inflammatory disorders are commonly associated with thrombocytosis, and an increase in the levels of serum and plasma IL-6 has been observed in patients with these disorders [24, 30, 32, 40, 41, 83]. Surgery and trauma have been associated with both thrombocytosis and elevations in IL-6 [17, 22, 28, 76]. The thrombocytosis of malignancy may also be related to increased serum IL-6 levels associated with inflammation or cell damage, or to IL-6 produced by tumor cells [7, 25, 50, 58, 80, 82]. It is of interest that patients with multiple myeloma given anti-IL-6 antibody have developed thrombocytopenia, which might be due to a decrease in endogenous IL-6 levels [60].

Alteration of platelet function by IL-6

Peng et al. showed that a quantitative change in the platelet number is not the only effect of IL-6, as they also observed the appearance of large platelets in the circulation [13, 69]. However, changes in platelet size in mice have not been observed by other investigators [35, 57]. Stahl et al. reported that megakaryocyte ultrastructural abnormalities are observed after IL-6 administration, suggesting the possibility that platelets produced in response to IL-6 administration might be dysfunctional [78]. Peng et al. showed that IL-6 administration to dogs results in augmentation of the capacity of platelets to be activated in response to thrombin and to platelet-activating factor [69].

Comparison between IL-6 and other cytokines acting on megakaryocytopoiesis

Like IL-6, other hematopoietic growth factors, including leukemia inhibitory factor (LIF), IL-11, and most importantly, thrombopoietin, have been shown to stimulate the megakaryocytic lineage [20, 27, 62].

LIF has effects rather similar to those of IL-6 on megakaryocytopoiesis. In the mouse, LIF potentiates the effects of IL-3 on the formation of colonies, and its injection in vivo induces thrombocytosis [61, 62]. However, the inactivation of the LIF gene by homologous recombination does not lead to thrombocytopenia [23]. In man, the action of LIF on megakaryocyte maturation is less marked than that of IL-6 [18].

IL-11 also has an in vitro action close to that of IL-6 on megakaryocytopoiesis. IL-11 alone is not a megakaryocyte colony-stimulating factor. However, it can act by synergy with IL-3 to stimulate the formation of megakaryocyte colonies in man or in mice [10, 81]. Its action, like that of IL-6, is potentiated on the late megakaryocyte differentiation, increasing size and ploidy of megakaryocytes. Like IL-6, IL-11 can be an autocrine stimulus of some megakaryoblastic cell lines [52]. Injected in vivo in mice, IL-11 increases the number of platelets and the megakaryocyte ploidy without any modification in the megakaryocyte number. In man, IL-11 is well tolerated. However, IL-11 augments the acute-phase proteins.

When given in vivo, IL-6, IL-11 and LIF act very progressively and have the potential disadvantage of causing unwanted effects on a variety of other lineages. In contrast, the recently described thrombopoietin is a novel cytokine whose biologic effects implicate it as the regulator of megakaryocyte growth and platelet production [5, 19, 47, 56, 88]. The thrombopoietin activity seems to be specific to the megakaryocytic lineage and is regulated in the serum by the number of platelets [29, 88]. Thrombopoietin supports early megakaryocyte-progenitor colony formation and induces expression of megakaryocyte differentiation markers, polyploidization, and maturation into platelets [47]. In vivo, thrombopoietin significantly expands bone marrow and spleen megakaryocytes and the $CD34^+$ precursors, resulting in increased platelet production [19, 47, 56, 88].

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