

Evaluation of Interleukin-17 serum levels in patients with chronic myeloproliferative diseases

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To the Editor: Interleukin 17 (IL-17) is a member of a recently described cytokine family, with unique sequences and functions in the immune system. It is produced primarily by T cells and appears to be involved in generation of autoimmunity, exhibits proinflammatory activities, and plays key roles in host defense¹⁻³. IL-17 has also been implicated in tumorigenesis. Ciree *et al.*⁴ reported that cutaneous T-cell lymphomas spontaneously secrete IL-17. It has been reported that a considerable proportion of ovarian carcinomas naturally express IL-17, and its expression is significantly correlated with increased vascularity⁵. In fact, IL-17 is a marker and a mediator of angiogenesis that stimulates vascular endothelial cell migration and regulates the production of a variety of proangiogenic factors, such as tumor necrosis factor-alpha and vascular endothelial growth factor from a number of cells, including keratinocytes, fibroblasts, epithelial cells, and tumor cells^{5,6}, thereby eliciting neovessel formation *in vivo*^{7,8}.

The aim of this study was to determine IL-17 serum levels in patients with chronic myeloproliferative disorders in order to investigate its role in the pathogenesis of such diseases.

The study included four groups of subjects. Serum levels of IL-17 were, in fact, investigated in 40 patients (26 males and 14 females; mean age, 61 ± 16 years) with chronic myeloproliferative diseases. Fifteen patients were affected by polycythemia vera, 15 by essential thrombocytemia, and 10 by primary myelofibrosis (PMF).

The diagnosis was made on the basis of clinical and biochemical data and with bone marrow biopsy, using the current World Health Organization diagnostic criteria for myeloproliferative disorders. According to the such criteria, PMF bone marrow grading was: stage I in 2 patients, stage II in 5 patients, and stage III in 3 patients. Ten normal subjects matched for sex and age were used as controls.

Patients with acute or chronic infections, diabetes, liver and kidney disease were excluded from the study.

None of the investigated patients had received chemotherapy at the time of blood collection. The study was performed according to the guidelines of the local ethics committee.

A venous blood sample was taken from all subjects in the morning after a 12-hour fasting period. The serum samples were stored at -70 °C until assay. Serum IL-17 levels were measured by standard quantitative sandwich ELISA kits (Quantikine; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The results represent the mean values of duplicate determinations.

Data are expressed as the mean value ± standard deviation. Comparison between two or more groups was performed by the unpaired Student's *t* test and analysis of variance. Correlations were assessed by Pearson's coefficient or with Spearman's coefficient. Statistical significance was considered at *P* < 0.05.

A significant difference was found between PMF patients and control subjects (39 ± 17 pg/ml *vs* 12 ± 4; *P* < 0.05), whereas no difference was found between polycythemia vera patients (17 ± 7 pg/ml) or essential thrombocytemia patients (21 ± 12 pg/ml) and control subjects (Figure 1). In the PMF group, there was no significant correlation between IL-17 and hemoglobin levels, erythrocyte, platelet or leukocyte count or splenomegaly.

The role played by IL-17 in tumorigenesis is complex. IL-17 was shown to promote growth and tumorigenicity of human cervical tumors in nude mice⁹. In contrast, IL-17 also inhibited the growth of other tumors in immunocompetent but not in nude mice¹⁰.

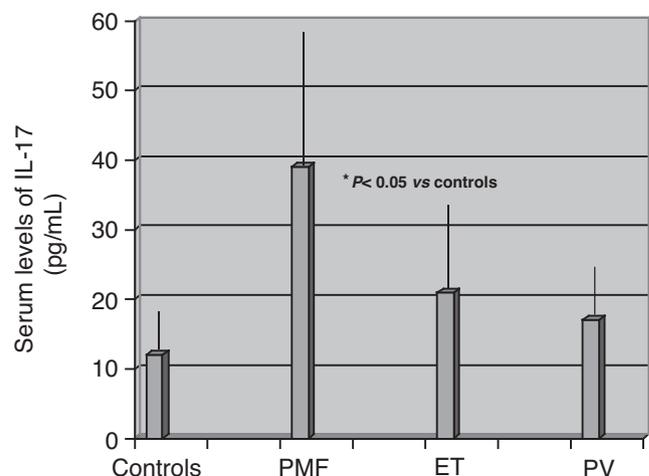


Figure 1 - Serum levels of IL-17 in patients with CMD. **P* < 0.05 vs control subjects. PMF, primary myelofibrosis; ET, essential thrombocytemia; PV, polycythemia vera.

As regards the relationship between IL-17 and hematological diseases, it was found that in multiple myeloma patients serum levels of the cytokine were higher than in normal subjects and associated with advanced stage and they were correlated with vascular endothelial growth factor levels and microvessel density⁸. Moreover, a human B-cell line cloned from the human myeloma cell line expresses IL-17¹¹, but serum levels of IL-17 are not elevated in acute myeloid leukemia patients¹².

The action of IL-17 on hemopoiesis is controversial and its role in patients with chronic myeloproliferative diseases is unknown. In fact, Numasaki *et al.*¹³ reported that IL-17 has an enhancing effect on macrophage-derived IL-1 beta- and tumor necrosis factor-alpha-induced granulocyte-macrophage colony-stimulating factor mRNA expression and production, whereas it is well known that polycythemia vera bone marrow colony-forming units show a marked hypersensitivity to human recombinant granulocyte-macrophage colony-stimulating factor¹⁴. In contrast, Jovicic *et al.*¹⁵ stated that IL-17 does not affect granulocyte-macrophage progenitors although the number of immature erythroid progenitor cells is increased after IL-17 administration. Finally, Starnes *et al.*¹⁶ affirmed that IL-17 suppresses the proliferation of myeloid progenitors in colony-formation assays.

In our study, we found a significant increase in IL-17 concentrations (a marker of angiogenetic activity) in patients with PMF, and the cytokine could have a role in the onset of the disease. Angiogenesis is critical in the pathogenesis of PMF. In fact, Boveri *et al.*¹⁷ demonstrated that patients with PMF at the prefibrotic stage had significantly higher microvessel density than those with polycythemia vera or essential thrombocytemia. Moreover, serum levels of angiogenetic proteins are significantly increased in PMF patients¹⁸.

Future studies should demonstrate whether IL-17 has any prognostic value in patients with PMF and whether anti-angiogenetic therapy could have an anticancer effect also in chronic myeloproliferative diseases.

References

- Gaffen SL, Kramer JM, Yu JJ, Shen F: The IL-17 cytokine family. *Vitam Horm*, 74: 255-282, 2006.
- Beerepoot LV, Mehra N, Vermmat JSP, Zonnbenberg BA, Gebbink MFG, Voest EE: Increased levels of viable circulating endothelial cells are an indicator of progressive disease in cancer patients. *Ann Oncol*, 15: 139-145, 2004.
- Pelosi E, Valtieri M, Coppola S, Botta R, Gabbianelli M, Lulli V: Identification of the hemangioblast in postnatal life. *Blood*, 100: 3203-3208, 2002.
- Ciree A, Michel L, Camilleri-Broet S, Jean Lous F, Oster M, Flageul B, Senet P, Fossiez F, Fridman WH, Bachelez H, Tartour E: Expression and activity of IL-17 in cutaneous T cell lymphomas (mycosis fungoides and Sezary syndrome). *Int J Cancer*, 112: 113-120, 2004.
- Starnes T, Robertson MJ, Sledge G, Kelich S, Nakshatri H, Broxmeyer HE, Hromas R: IL-17F, a novel cytokine selectively expressed in activated T cells and monocytes, regulates angiogenesis and endothelial cell cytokine production. *J Immunol*, 167: 4137-4140, 2001.
- Numasaki M, Tomioka Y, Takahashi H, Sasaki H: IL-17 and IL-17F modulate GM-CSF production by lung microvascular endothelial cells stimulated with IL-1 alpha and/or TNF-alpha. *Immunol Lett*, 95: 175-184, 2004.
- Numasaki M, Fukushi J, Ono M, Narula SK, Zavodny PJ, Kudo T, Robbins PD, Tahara H, Lotze MT: Interleukin-17 promotes angiogenesis and tumor growth. *Blood*, 101: 2620-2627, 2003.
- Aleksandrakis MG, Pappa CA, Miyakis S, Sfiridaki A, Kafousi M, Alegakis A, Stathopoulos EN: Serum interleukin-17 and its relationship to angiogenic factors in multiple myeloma. *Eur J Int Med*, 17: 412-416, 2006.
- Tartour E, Fossiez F, Joyeux I, Galinha A, Gey A, Claret E, Sastre-Garau X, Couturier J, Mosseri V, Vives V: Interleukin 17, a T-cell-derived cytokine, promotes tumorigenicity of human cervical tumors in nude mice. *Cancer Res*, 59: 3698-3704, 1999.
- Benchetrit F, Ciree A, Vives V, Warnier G, Gey A, Sautes-Fridman C, Fossiez F, Haicheur N, Fridman WH, Tartour E: Interleukin-17 inhibits tumor cell growth by means of a T-cell-dependent mechanism. *Blood*, 99: 2114-2121, 2002.
- Zhou L, Peng S, Duan J, Zhou J, Wang L, Wang J: A human B cell line AF10 expressing HIL-17. *Biochem Mol Biol Int*, 45: 1113-1119, 1998.
- Wrobel T, Mazur G, Jazwiec B, Kuliczowski K: Interleukin-17 in acute myeloid leukaemia. *J Cell Mol Med*, 7: 472-474, 2003.
- Numasaki M, Tomioka Y, Takahashi H, Sasaki H: IL-17 and IL-17F modulate GM-CSF production by lung microvascular endothelial cells stimulated with IL-1beta and/or TNF-alpha. *Immunol Lett*, 95: 175-184, 2004.
- Dai CH, Krantz SB, Dessypris EN, Meens RT Jr, Horn ST, Gilbert HJ: Polycythemia vera. II. Hypersensitivity of bone marrow erythroid, granulocyte-macrophage, and megakaryocyte progenitor cells to interleukin-3 and granulocyte-macrophage colony-stimulating factor. *Blood*, 80: 891-899, 2002.
- Jovicic G, Bugarski D, Patakov M, Krstic A, Vlaski M, Stojanovic N, Milenkovic P: In vivo effects of interleukin-17 on haematopoietic cells and cytokine release in normal mice. *Cell Prolif*, 37: 401-412, 2004.
- Starnes T, Broxmeyer HE, Robertson MJ, Hronas R: Cutting edge: IL-17 a novel member of the IL-17 family, stimulates cytokine production and inhibits hemopoiesis. *J Immunol*, 169: 642-646, 2002.
- Boveri E, Passamonti F, Umi E, Pietra D, Elena C, Arcaini L, Pascutto C, Castello A, Cazzola M, Magrini V, Lazzarino M: Bone marrow microvessel density in chronic myeloproliferative disorders. A study of 115 patients with clinicopathological and molecular correlations. *Br J Haematol*, 140: 162-168, 2008.
- Panteli K, Bai M, Hstzmichael E, Zagorianakov N, Agnantis NJ, Bourantes K: Serum levels and bone marrow immunohistochemical expression of vascular endothelial growth factor in patients with chronic myeloproliferative diseases. *Haematology*, 1: 155-158, 2007.