Interleukin 10 Gene Polymorphism and its role in Pediatric Immune Thrombocytopenia

Ibrahim Oun[1], Mohamed Abdel-Salam[1], Tamer Hassan[1], Nermin Abdel Fattah[2]
[1]: Pediatrics Department, Zagazig University, Egypt
[2]: Medical Biochemistry Department, Zagazig University, Egypt

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Abstract: Interleukin 10 (IL-10) is an anti-inflammatory cytokine and suppresses immune responses. IL-10 is secreted by macrophages, Th2 cells, and mast cells. Cytotoxic T cells also release IL-10 to inhibit viral infection stimulated natural killer cell activity. IL-10 inhibits the synthesis of a number of cytokines involved in the inflammatory process including IL-2, IL-3, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) . It can promote the activity of mast cells, B cells, and certain T cells. The cytokine genes are polymorphic, which accounts for the different levels of cytokine production, and are related to regulation of the immune-mediated inflammatory process. Cytokine gene polymorphisms have recently attracted interest because distinct alleles of cytokine genes have been associated with various immunoinflammatory diseases (Audia et al., 2017).

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Introduction:

Cytokines are molecules responsible for controlling intracellular communication and directing the immunological reaction. This group of low-molecular glycoproteins forms a "cytokine network" in the body **(Poniatowski et al., 2014; Rochman et al., 2009)**.

Cytokines are a broad and loose category of small proteins (~5–20kDa) that are important in cell signaling. They are released by cells and affect the behavior of other cells, and sometimes the releasing cell itself. Cytokines include chemokines, interferons, interleukins, lymphokines, tumor necrosis factor but generally not hormones or growth factors **(Secombes et al., 2015.Sprague et al., 2010).**

Interleukin-10

IL-10 is an anti-inflammatory cytokine and suppresses immune responses **(Grayfer et al., 2010; Harun et al., 2011; Wei et al., 2013).** It inhibits the production of pro-inflammatory cytokines by T cells, NK cells and monocytes **(Yu2013; Ding et al., 1993).**

First identified as an inhibitor of IFN-synthesis in TH1 cells, IL-10 is an important immunoregulatory cytokine. It is an anti-inflammatory cytokine that was first called human cytokine synthesis inhibitory factor **(Fullerton et al., 2013).**

IL-10 is secreted by macrophages, TH2 cells and mast cells. Cytotoxic T cells also release IL-10 to inhibit viral infection stimulated NK cell activity **(Ouyang et al., 2011).**

IL-10 is a 36-kDa dimer composed of two 160-aminoacid- residue-long chains. Its gene is located on chromosome 1 in humans and consists of five exons. IL-10 inhibits the synthesis of a number of cytokines involved in the inflammatory process including IL-2, IL-3, GM-CSF, TNF-and IFN- **(Kuhn e al., 2006).** Based on its cytokine-suppressing profile, it also functions as an inhibitor of TH1 cells and by virtue of inhibiting macrophages, it functions as an inhibitor of antigen presentation. Interestingly, IL-10 can promote the activity of mast cells, B cells and certain T cells **(Iyer and Cheng, 2012; Karan et al., 2016).**

The expression of cytokines and transcription factors associated with Th1 and Th2 responses was increased by antigen but decreased by the presence of IL-10, with a concomitant increase in CD8_2 and CD8_1 expression. These data suggest that carp IL-10 has differential effects on memory CD4+ and CD8+ T cells, and promotes B cell differentiation and IgM antibody secretion in an antigen specific manner **(Piazzon et al., 2015a; Piazzon et al., 2015b).**

IL10 gene cluster locates in a 200 kb region on chromosome 1q31-32, and the three cytokine share a common receptor IL-20R1/IL-20R2 heterodimer **(Donnelly and Kotenko, 2010; Sabat, 2010).** These cytokines display many overlapping functions due to the similarity and shared receptor usage. Significant commonality exists also through conserved signaling cascades: the binding of IL-10 related cytokine to their receptors activates the JAK (Junus kinase), STAT (signal transducers and activator of transcription), and the MAPK (mitogen-activated protein kinase) pathways **(Lee et al., 2012).**

Though, the IL-10 family members mediate diverse activities, including enhanced antibacterial and antiviral immunity, immune and antitumor activities, and promotion of self-tolerance in autoimmune diseases **(O'Garra et al., 2014).**

IL-10 mediates its effects after binding to two receptor chains, IL-10R1 (α) and IL-10R2 (β). These receptors are members of the class II or IFN receptor family. The interaction of IL-10 with its receptors is highly complex and the IL- 10R2 (β) chain is essential for the production of its effects. Several hundred genes are activated after interaction of IL-10 with its receptors. The tyrosine kinases JAK1 and Ty k2 are activated by the interaction of IL-10 with its receptors, which results in the induction of transcription factors STAT1, STAT3 and STAT5, and eventual gene activation **(Johnson et al., 2013).**

The major immunobiological effect of IL-10 is the regulation of the TH1/TH2 balance. TH1 cells are involved in cytotoxic T-cell responses whereas TH2 cells regulate B-cell activity and function. IL-10 is a promoter of TH2 response by inhibiting IFN-production from TH1 cells. This effect is mediated via the suppression of IL-12 synthesis in accessory cells **(Galimova et al., 2016).**

IL-10 is involved in assisting against intestinal parasitic infection, local mucosal infection by costimulating the proliferation and differentiation of B cells. Its indirect effects also include the neutralization of bacterial toxins. IL-10 is a potent inhibitor of IL-1, IL-6, IL-10 itself, IL-12, IL-18, CSF and TNF **(Khan et al., 2016).**

It not only inhibits the production of proinflammatory mediators but also augments the production of anti-inflammatory factors including soluble TNF-receptors and IL-1RA. IL-10 downregulates the expression of MHC class II molecules (both constitutive and IFN--induced), as well as that of costimulatory molecule, CD86, and adhesion molecule, CD58. It is an inhibitor of IL-12 production from monocytes, which is required for the production of specific cellular defense response. IL-10 enhances the expression of CD16, CD32 and CD64 and augments the phagocytic

activity of macrophages. The scavenger receptors, CD14 and CD163, are also upregulated on macrophages by IL-10 **(Galimova et al., 2016).**

It is a stimulator of NK cells, enhances their cytotoxic activity, and also augments the ability of IL-18 to stimulate NK cells. Based on its immunoregulatory function, IL-10 and ligands for its receptors are tempting candidates for therapeutic intervention in a wide variety of disease states, including autoimmune disorders, acute and chronic inflammatory diseases, cancer, infectious disease, psoriasis and allergic disease **(Mocellin et al., 2003).** Modest but significant improvement has been observed in patients with chronic hepatitis C, Crohn's disease, psoriasis and rheumatoid arthritis after subcutaneous administration of IL-10 in human clinical trials **(Jandl et al., 2016).**

The systemic administration of IL-10 produces general immune suppression, inhibition of macrophage and T-cell infiltration, less secretion of IL-12 and TNF-by monocytes and suppression of nuclear factor (NF)- B induction. In patients with acute myelogenous leukemia, IL-10 increases the serum levels of TNF-and IL-1. The use of IL-10 for human cancer therapy is under investigation and despite its immunosuppressive effects it may serve a role as a facilitator in preconditioning tumors to be recognized by immune effector cells **(Asadullah et al., 2003; Togashi et al., 2011).**

Relationship between IL10 and ITP

IL10 and ITP:

Immune thrombocytopenic purpura (ITP) is an acquired hemorrhagic disease characterized by an immune-mediated platelet destruction by the activated reticulo-endothelial system, following their sensitization by antiplatelet glycoprotein autoantibodies **(Cooper and Bussel, 2006; Nugent et al., 2009)**. ITP is usually a self-limiting disease in children **(Provan et al., 2010).** However, newly diagnosed ITP might progress to the chronic form, defined according to standardized criteria, with a heterogeneous clinical expression **(Rodeghiero et al., 2009).**

The clinical differences between newly diagnosed and chronic ITP suggest the existence of different pathophysiological mechanisms in the two forms **(Del Vecchio et al., 2012).**

Some authors have investigated the role of genetic and immunologic factors in the development of this disorder **(Foster et al., 2001; McMillan, 2000; Semple et al., 1996; Wang et al., 2005; Wu et al., 2005)**. They failed in identifying specific characteristics of children with ITP who will likely develop the chronic form of the disorder, mainly due to differences in the study design and patient's management.

Platelet destruction is mediated by T cells play roles in the pathogenesis of ITP **(Rodeghiero et al., 2009).** CD4+ T cells can orchestrate host responses with proper cytokine profiling, which is achieved from the appropriate balancing between regulatory T (Treg) and T effector (Teff) cells. It is widely known that Th1 cells, a subset of Teffs, are abnormally activated in ITP patients **(Panitsas et al., 2004).**

Particularly change interleukin (IL)-10 serum levels or altered function of T-regulatory (Treg) cells have been described, suggesting that a defective immune regulation might play a part in the pathogenesis of the disease **(Guo et al., 2007; Stasi et al., 2008).** We have recently showed in a preliminary analysis that IL-10 expression was significantly increased in patient presenting with acute course of ITP with respect another who had a chronic progression of the disease, even if it was not possible to define a clear cut-off value of IL-10 with a prognostic significance **(Del Vecchio et al., 2011).**

IL-10, a cytokine with broad immunoregulatory function, was originally described as a unique product of T helper (Th) type 2 cells, but it was later shown to be expressed in a variety of lymphocytic as well as myeloid cell populations **(Mosser and Zhang, 2008).**

It inhibits the formation of proinflammatory cytokines such as TNFα in T cells and monocytes **(Fiorentino DF, Z, et al., 1991),** and down-regulates MHC class II expression in these latter cells24. In contrast to its inhibitory function on T cells and macrophages, IL10 stimulates the production of immunoglobulins and the expression of MHC class II antigens in B cells **(Roncarolo et al., 2006).** Moreover, IL10 is the main effector of IL10-secreting type I regulatory T cells in humans. These cells are able to suppress antigen-specific effector T-cell responses via a cytokine-dependent mechanism and do, therefore, have a role in immuno-tolerance. Interestingly, **Saitoh et al. (2011)** have recently suggested in Japanese adults with chronic ITP that IL-10 polymorphisms may reflect the severity of this form of ITP. These polymorphisms in the promoter region of the IL-10 gene can affect the production of this cytokine in several inflammatory disorders **(Crawley et al., 1999; Edwards-Smith et al., 1999; Turner et al., 1997).**

Interleukin (IL)-10 family cytokines IL-10, IL-19, IL-20, and IL-24 have been implicated in autoimmune diseases and previously reported that genetic variants in IL10 gene cluster were associated with ITP **(Del Vecchio et al., 2012).**

Many researchers have investigated the role of genetic factors humoral and cellular immunity and inadequate platelet production in the development of this condition, but failed to identify specific characteristics of ITP who will probably develop the chronic form of the disorder, mainly because of the study design and differences in patients' immunomodulating therapy (**Provan D, Stasi R, 2010.)** Serum levels of T helper (Th) type 1, Th2 and T-regulatory associated cytokine (IL10), was assessed in different phases of ITP in patients and in healthy controls. We aimed to investigate whether this biomarker might be considered predictors of ITP progression or not **(Del Vecchio, et al., 2012, Riccardina Tesse et al., 2012).**

Giovanni Carlo Del Vecchio, et al. (2012) were worked on a series of newly diagnosed ITP cases at the onset of the disease, before starting any treatment, and a group of patients already affected by chronic ITP who received treatment (intravenous immunoglobulin or steroids) for their condition. In all these patients searching for significant variations in immunological markers during the clinical progression (newly diagnosed or chronic) of their disease to determine the potential diagnostic and prognostic role of serum biomarkers in ITP **(Zeller et al., 2005).** In these studies the patients with chronic ITP showed a higher prevalence of asymptomatic-paucisymptomatic forms of ITP compared to patient with newly diagnosed ITP.

Studies observed that the concentrations of Th1 andTh2 cytokines were not significantly different within patients' groups and between cases and controls. However, IL10 serum concentrations measured inpatients at the onset of their disease were higher than in healthy person and patients with ITP lasting for at least 2 years. Moreover, IL10 expression was significantly higher in the group of patient with an acute course of the disease than in patient who had chronically progressive ITP even if it was not possible to define a clear cut-off value of IL10 with prognostic relevance. Further prospective studies on larger population are needed to establish such a specific cut-off level and validate our results. Some authors have previously described that serum levels of IL2, IFNγ and IL10 in patient with ITP were increased in some cases of chronic ITP, however in this study subjects were not grouped based on the clinical phases of the disease **(Semple et al., 1996).** Moreover, high IL10-producing polymorphisms were found less frequently in patients with chronic ITP than in controls **(Saitoh et al., 2011).**

Liet al. (2014) reported that excessive activated CD4+ T effector cells (Teffs) and compromised regulatory T cells (Tregs) were reported in ITP patients, yet little is known about the mechanisms. Interleukin-10 (IL-10) is an important regulatory cytokine of Tregs in inflammatory condition.

It has been recently highlighted that IL-10-producing Tregs contribute to the effective controls of several autoimmune diseases. Hence this study was aimed to examine the role of IL-10 produced by Tregs in newly diagnosed ITP patients. Newly diagnosed ITP patients and healthy subjects were enrolled to assess the numbers of peripheral Th1, Th17 cells and Tregs and IL-10 concentration is assessed. The role of IL-10 in Tregs' inhibitory function was also determined. In ITP patients, Teffs were excessively activated, while the Tregs were numerically and functionally impaired **(Harringtonet al., 2005).**

The percentages of IL-10+Tregs in Tregs' population were found elevated dramatically in ITP patients but decreased in the remitted patients. The IL-10 concentrations in the cultured supernatant were decreased in ITP patients but elevated in the remitted patients **(Sollazzo et al., 2011).**

Furthermore, the IL-10 secretion by Tregs was dramatically decreased in ITP patients. IL-10 treatment enhanced the suppression effect of Tregs toward Teffs, whereas anti-IL-10 treatment boosted the proliferation of Teffs and Th17 cells. Excessive activated Teffs and impaired Tregs play major roles in the exuberant CD4+T cells immune responses of ITP. The inhibitory effect of Tregs toward Teffs is largely exerted by IL-10. Insufficient secretion of IL-10 compromises the inhibitory capability of Tregs against Teffs in newly diagnosed ITP patients **(Liet al., 2014).**

SO, the immune imbalance of CD4+ T cells in newly diagnosed adult ITP is a combined consequence of excessive activated Teffs accumulation and numerical, as well as functional compromise of Tregs. IL-10 could promote Tregs' suppression against Teffs, while Tregs of ITP patients could not secret enough IL-10 to sufficiently inhibit Teffs.

Effective corticosteroids treatment could restore the IL-10 secretion of Tregs. Future studies into the conveyance of IL-10 in Tregs, and even in B10 cells or other immune cells, may enlighten novel treatment in ITP. Nevertheless, the possible IL-10 signalling turbulence in Teffs makes the problem much complicated, which needed to be further explored **(Li et al., 2014).**

Makhlouf and Abd Elhamid (2014) reported that The Th2-cell secretion interleukin 10 (IL10) leads to the suppression of Th1 responses by down regulating the production of macrophage-derived IL10 and inhibiting the differentiation of Th1-type cells **(Cooper and Bussel, 2006).**

IL10 is the most important anti-inflammatory cytokine in the human immune response. IL10 is a potent inhibitor of Th1 cytokines, including both IL2 and interferon (IFN)-γ. This activity accounts for its initial designation as cytokine synthesis inhibition factor; it is mainly produced by macrophages, monocytes, T cells, B cells, dendritic cells, mast cells, and eosinophils. IL10 also limits the inflammatory response and regulates the differentiation and proliferation of T cells, B cells, natural killer cells, antigen-presenting cells, and mast cells. The gene encoding IL10 has been identified on chromosome 1q31–32.6

Wu et al (2005) reported that IL10 was associated with the pathogenesis of ITP and contributed to the susceptibility of developing ITP**,** and that this might be the basis for immunomodulatory therapies for ITP and provide a tool for early diagnosis of susceptibility to ITP.

They aims to study the expression IL10 gene polymorphisms by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) among patient with ITP, and to define their role in modulating susceptibility to development of ITP and their correlation with the clinical presentation and laboratory data.

The comparison between the chronic ITP, acute ITP, and control groups with regard to demographic, clinical, and laboratory parameters revealed statistically significant differences between the 3 groups. In chronic ITP patient, a significant difference was observed between IL10 genotypes. In acute ITP patients, significant differences in these parameters occurred between

IL10 genotypes. Most people have B cells directed to make autoantibodies as well as detectable peripheral blood T cells reactive to glycoprotein IIb/IIIa. Therefore, the immunological machinery does not need to be created de novo but merely turned on. The limited reports illustrating genomic associations suggest that patients with ITP may have a more general genetic susceptibility towards antiplatelet antibody production. The associations with other diseases.

Wu et al. (2005) reported that occurrence of the A allele of IL10 was significantly different in ITP compared to control patients. However, this study revealed a statistically significant difference in the occurrence of this allele between acute and chronic ITP.

In this study, there was a statistically significant correlation between chronic ITP patients demonstrating IL10 gene polymorphism and low platelet count, suggesting that IL10 gene polymorphism reflects the severity of chronic ITP. This finding is in concordance with **Satoh et al. (2011)** who showed that the immune response in ITP patients is towards Th1 polarization and that IL10 is an important factor regulating Th1 and Th2 cytokine synthesis, has a significant role in autoimmunity and tumorigenesis, and that patients with chronic ITP and the IL10 gene polymorphism have lower platelet counts compared to patients without polymorphism. According to **Mouzaki et al**, raised IL10 levels occur in children with chronic ITP, further suggesting Th1 involvement in the pathology of ITP illustrated by an increase in the Th1 cytokines; these findings may be related to ongoing immune activation related to autoimmunity.

Finally the study reported that IL10 was detected more frequently among ITP patients compared to controls. A statistically significant difference was observed between acute and chronic ITP patients, with higher level among chronic ITP patients versus acute ITP patients. So IL10 gene polymorphisms may contribute to susceptibility for ITP patient.

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