INTRODUCTION

Primary immune thrombocytopenia, or idiopathic thrombocytopenic purpura (ITP), is an autoimmune disorder characterized by isolated thrombocytopenia due to accelerated platelet destruction and impaired platelet production. Autoantibodies against platelet surface glycoproteins (GP), such as GPIIb/IIIa and GPIb/IX complexes, play major roles in both platelet destruction and impaired platelet production ***(Provan et al., 2010)***

The diagnosis of ITP is a process of exclusion. First, it has to be determined that there are no blood abnormalities other than a low platelet count, and no physical signs other than bleeding. Then, secondary causes (5–10 percent of suspected ITP cases) should be excluded. Such secondary causes include leukemia, medications (e.g., quinine, heparin), lupus erythematosus, cirrhosis, HIV, hepatitis C, congenital causes, antiphospholipid syndrome, von Willebrand factor deficiency, onyalai and others ***(Najaoui et al., 2012).***

Although corticosteroids and splenectomy remain central to the treatment of ITP, a new class of drugs, i.e., thrombopoietin receptor agonists (TPO-RAs) and rituximab, have substantially broadened the therapeutic options for refractory ITP patients. Moreover, the success of TPO-RAs in ITP patients shows that reduced platelet production caused by impaired megakaryocytopoiesis plays a greater role in ITP than previously recognized ***(Liu et al., 2011).***

T cell-mediated cytotoxicity and T cell functional abnormalities may also be involved in its pathogenesis of ITP beside autoantibody-independent mechanisms by B cells ***(Hu et al., 2011).***

Regulatory T cells characterized by CD4, CD25, and transcription factor forkhead box P3, called Tregs, constitute approximately 5%-10% of T cells and play an important role in self-tolerance ***(Liu et al., 2007).***

Tregs contribute to maintenance of peripheral immune tolerance, and their defects are thought to play a role in the pathogenesis of various autoimmune diseases which one of them is ITP ***(Chow et al., 2010).***

ITP patients hadreduced frequency of Tregs in circulation, bone marrow, and spleen, and Treg function is impaired. Treg dysregulation is improved after platelet count is recovered by treatment with dexamethasone, rituximab, or thrombopoietin receptor agonists ***(Bao et al., 2010).***

The reasons for the Treg deficiency in ITP are unclear but thrombocytopenia observed in Treg-deficient mice is mediated through production of IgG anti-platelet autoantibodies, which is analogous to human ITP. Further studies evaluating mechanisms of Treg dysregulation in ITP patients are necessary to elucidate the pathogenesis of ITP and develop novel therapeutic strategies that suppress anti-platelet autoimmune response ***(Nishimoto et al., 2012).***

Aim of the Work

The aim of this study is to explore the profile and function of CD4+CD25+ regulatory T cells (Treg cells) in idiopathic thrombocytopenic purpura (ITP) patients and its correlation with corticosteroids therapy

Chapter 1

Immune Thrombcytopnic Purpura

Introduction

Platelets are 2-3 µm cellular elements, found in the blood of vertebrates, that are important for the initiation of blood clotting. Platelets form cytoplasmic fragments of bone marrow megakaryocytes which bud off into the circulation.On a stained blood smear, platelets appear as dark purple spots, about 20% the diameter of red blood cells. The smear is used to examine platelets for size, shape, qualitative number, and clumping (fig 1) ***(Machlus et al., 2014).***

Platelets

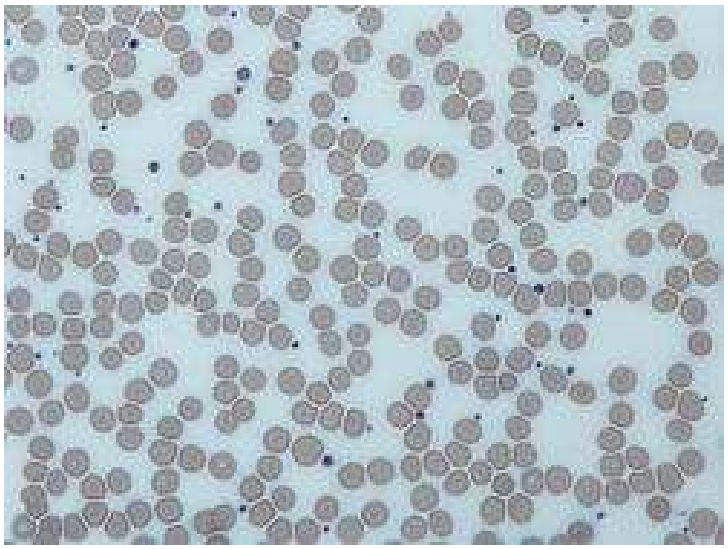

Figure (1): Image from blood smear Gimes stained showing platelets surrounded by red blood cells ***(Machlus et al., 2014).***

The main function of platelets is to contribute to hemostasis: the process of stopping bleeding at the site of interrupted endothelium. They gather at the site and physically plug the hole. First, platelets stick to substances outside the interrupted endothelium: *adhesion*. Second, they change shape, turn on receptors and secrete chemical messengers: *activation*. Third, they stick to each other: *aggregation.* Formation of this platelet plug (primary hemostasis) is followed by activation of the coagulation cascade with resultant fibrin deposition and linking (secondary hemostasis). These processes may overlap: the spectrum is from a predominantly platelet plug, or "white clot" to a predominantly fibrin clot, or "red clot" or the more typical mixture. The final result is the *clot* ***(Bouchard et al., 2010).***

Measurement:

Platelet concentration is measured either manually using a hemacytometer or by placing blood in an automated analyzer, the Coulter counter. The normal range (95% of population) for platelets is 150,000 to 400,000 per cubic millimeter ***(Bouchard et al., 2010).***

Kinetics

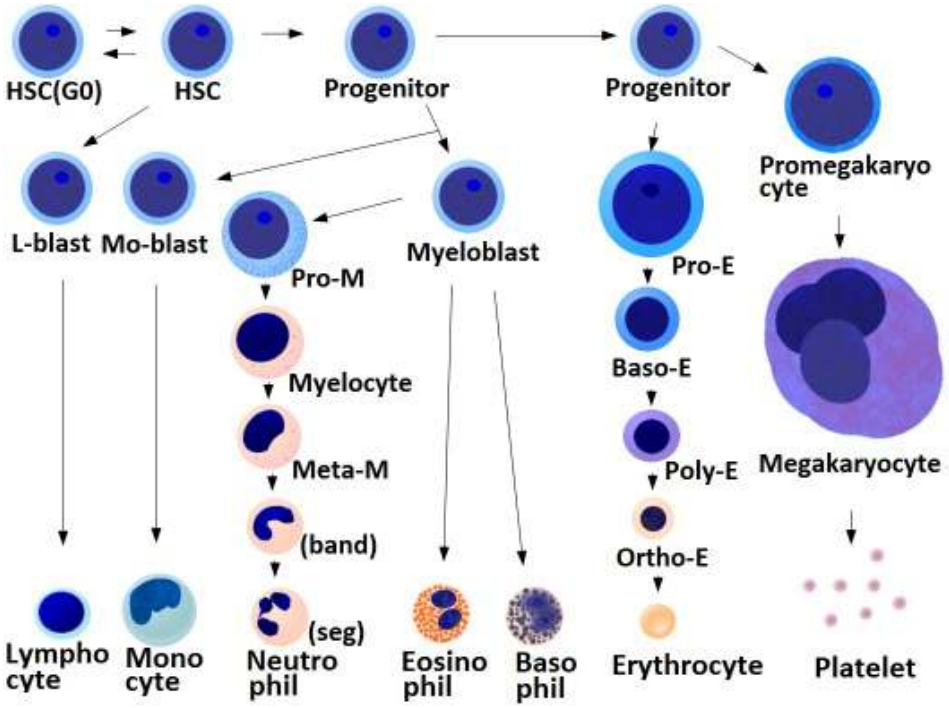

Figure (2): Platelets derive from blood stem cells ***(Harker et al., 2000).***

Megakaryocyte and platelet production is regulated by thrombopoietin, a hormone produced in the liver and kidneys. Each megakaryocyte produces between 1,000 and 3,000 platelets during its lifetime.An average of 10^{11} platelets are produced daily in a healthy adult.Reserve platelets are stored in the spleen, and are released when needed by splenic contraction induced by the sympathetic nervous system.The life span of circulating platelets is 8 to 9 days.Old platelets are destroyed by phagocytosis in the spleen and liver (fig. 2) ***(Harker et al., 2000).***

Dynamics:

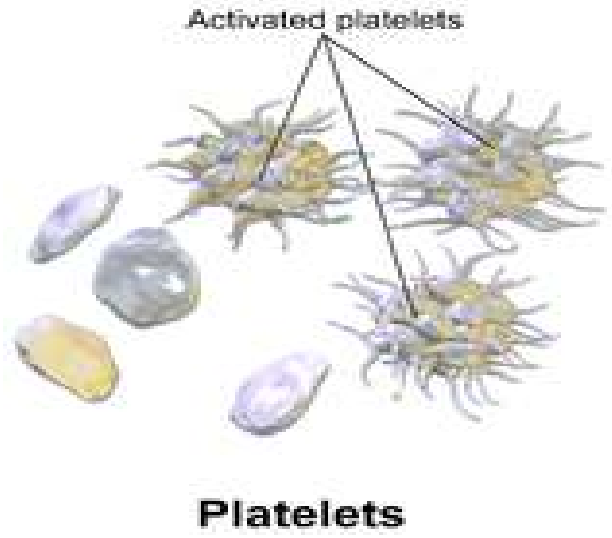

Figure (3): 3D Rendering of Platelets ***(Jones et al., 2012).***

The separation of platelet dynamics into three stages is useful there is overlap.Thrombus formation on an intact endothelium is prevented by nitric oxide, prostacyclin, and CD39 ***(Jones et al., 2012).***

Adhesion

When the endothelial layer is disrupted, collagen and vWF anchor platelets to the subendothelium. Platelet Glycoprotien (GP1b-IX-V) receptor binds with Von Willibrand Factor and GPVI receptor binds with collagen ***(Dubois et al., 2006).***

Trigger

Platelet activation begins seconds after adhesion occurs. It is triggered when *collagen* from the subendothilium, and/or

tissue factor from the media and adventitia bind with their respective receptors on the platelet (fig3) ***(Jones et al., 2012).***

Granule secretion

Platelets contain dense granules, lambda granules and alpha granules. Activated platelets secrete the contents of these granules through their canalicular systems to the exterior. Granule characteristics:dense (or delta) granules (containing ADP or ATP, calcium, and serotonin), lambda granules-similar to lysosomes and contain several hydrolytic enzymes. Alpha granules (containing P-selectin, platelet factor 4, transforming growth factor-β1(TGF_B), platelet-derived growth factor, fibronectin, B-thromboglobulin,Von willibrand facter(vWF), fibrinogen, and coagulation factors V and XIII) ***(Matarrese et al., 2009)***.

GPIIb/IIIa activation

Thromboxane A_2 synthesis increases during activation: it is secreted and acts on both its own thromboxane receptors (the so-called "out-in" mechanism), and those of other platelets. These receptors trigger intraplatelet signaling, which converts GPIIb/IIIa receptors to their active form to initiate aggregation (fig4) ***(Bouchard et al., 2010).***

Aggregation

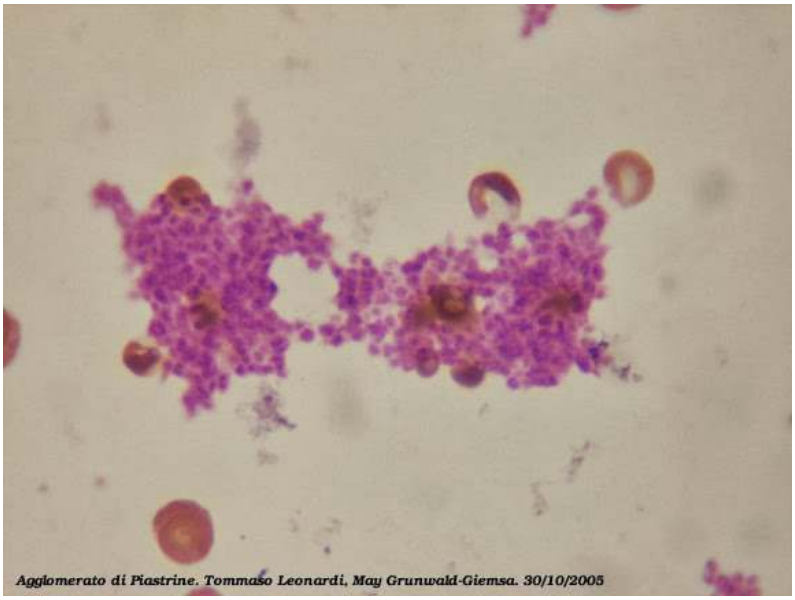

Figure (4): Platelet clumps in a blood smear ***(Yip et al., 2005).***

Aggregation begins minutes after activation, and occurs as a result of turning on the GPIIb/IIIa receptor, which allows these receptors to bind with vWF or fibrinogen, Classically it was thought that this was the only mechanism involved in aggregation) ***(Yip et al., 2005).***

Platelet membrane glycoprotein

Platelet membrane glycoproteins are surface glycoproteins found on platelets (thrombocytes) which play a key role in hemostasis. When the blood vessel wall is damaged, platelet membrane glycoproteins interact with the extracellular matrix ***(Nokes and Makris, 2010).***

Glycoprotein Ib-IX-V complex (GPIb-IX-V)

The binding site for von Willebrand factor (vWF), α-thrombin, leukocyte integrin αMβ2 and P-selectin. The deficiency in glycoprotein Ib-IX-V complex synthesis leads to Bernard-Soulier syndrome ***(Lepage et al., 2012).***

Glycoprotein VI (GPVI)

Glycoprotein VI is one of the immunoglobulin superfamily type I transmembrane glycoproteins. It is an important collagen receptor involved in collagen-induced platelet activation and adhesion ***(Jandrot et al., 2000).***

Glycoprotein Ia/IIa complex (GPIa/IIa=integrin α2β1)

This is a receptor for collagen type I and IV.. The interaction with collagen leads to stabilization of the platelets ***(Lepage et al., 2012).***

Glycoprotein IIb/IIIa complex (GPIIb/IIIa=integrin αIIbβ3).

This complex interacts with fibrinogen and thus plays an important role in platelet aggregation and adhesion to endothelial surfaces. Activation of this complex initiates the platelet aggregation and the formation of primary platelet plug, a fibrin clot. The IIb/IIIa complex is a major platelet membrane component ***(Matarrese et al., 2009)***.

This complex also binds vWF, fibronectin and vitronectin. Deficiency in the IIb/IIIa complex is described as Glanzmann's thrombasthenia. Patients completely lack the ability to aggregate platelets ***(Jandrot et al., 2000)***.

GPV/IIIa (GPV/IIIa=integrin α5β3)

This complex is located mainly on endothelial cells but also on smooth muscle cells, macrophages and platelets. Its main function is in the adhesion of cells to the extracellular matrix components ***(Lepage et al., 2012).***

Immune Thrombocytopenic Purpura

Defenition

Primary immune thrombocytopenia (also known as idiopathic thrombocytopenic purpura; ITP) is an autoimmune disorder characterized by isolated thrombocytopenia without abnormalities in the erythroid and myeloid/lymphoid lineages ***(Kurata et al., 2011).***

The incidence:

The incidence in adults is estimated at approximately 1.6-3.9 per 100,000 person-years ***(Provan et al., 2010).***

Phases of the disease:

One of the major new concepts provided by the International Working Group (IWG) focused on ITP was to

split the disease into three different phases based on what is known of the natural history of ITP especially in adults. While ITP has formerly been defined as "chronic" if it lasted for more than 6 months and "acute" if it was a self-limited and transient disease, the IWG members agreed on three distinct phases of the disease, namely (1) the initial phase, "newly diagnosed ITP," from the time of diagnosis up to 3 months; (2) "persistent," between 3 and 12 months from diagnosis; and (3) "chronic phase," now defined as a disease duration of more than 12 months, a period of time beyond which the occurrence of a spontaneous remission becomes less likely although still possible ***(Sailer et al., 2006).***

This distinction of three different phases is actually very important in term of potential consequences in that it suggests that different therapeutic options should be considered for every phase and at least theoretically implied that both the time of splenectomy, the recognized "gold standard" of treatment for adult chronic severe ITP as well as the use of thrombopoitien receptoragonists (Tpo-R) agonists licensed in Europe only for chronic ITP should be postponed (Fig. 5) ***(Bussel et al., 2007).***

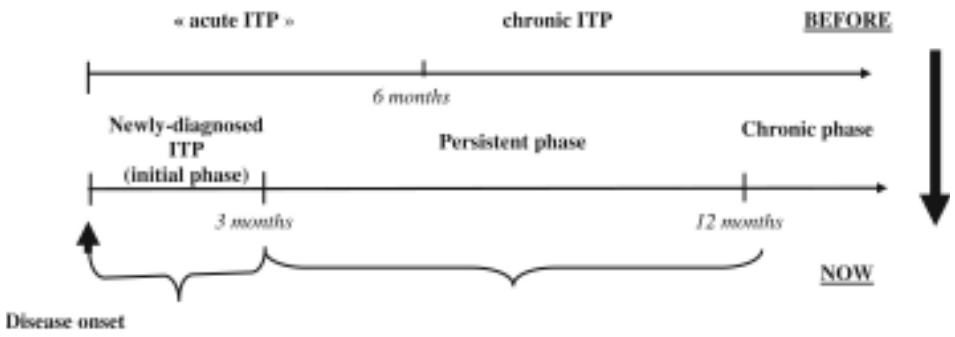

Figure (5): New definitions for the phases of the disease ***(Bussel et al., 2007).***

Pathophysiology of primary ITP

Total platelet mass in the body is regulated by the balance between production and clearance of platelets. In hypoplastic thrombocytopenias, such as aplastic anemia or chemotherapy-induced thrombocytopenia, platelet counts are decreased due to reduced platelet production. In ITP, platelet mass shrinks as a result of accelerated platelet clearance, which is mainly due to autoantibody-mediated destruction by macrophages in spleen, and moderately impaired platelet production due to antibody- and/or cytotoxic T cell-mediated megakaryocytic damage (Fig. 6) ***(Hirokazu and Yoshiaki, 2013).***

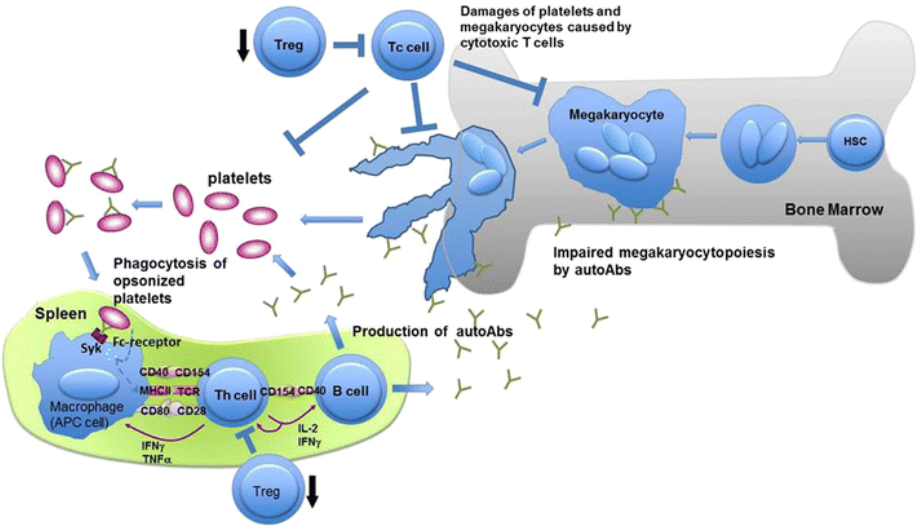

Figure (6): Schematic representation of pathophysiology of cITP. Opsonized platelets by autoantibodies are destroyed by macrophages in spleen and peptide fragments expressed with MHC class II stimulate helper T cells, following activation of autoreactive B cells. Impaired Tregs fail to suppress this vicious cycle. Autoantibodies also suppress megakaryocytopoiesis. Autoreactive cytotoxic T cells may play a role in the destruction of platelets and megakaryocytes. Thrombopoietin receptor (TPO-R) agonists stimulate megakaryocyte proliferation and maturation. Rituximab targets CD20-positive B cells ***(Hirokazu and Yoshiaki, 2013).***

1- Abnormalities in B cells: production of anti-platelet autoantibodies:

Historical experiments in 1950 and subsequent studies, which demonstrated that the passive transfer of plasma [including immunoglobulin (Ig) G-rich fractions] from ITP patients induced the development of transient thrombocytopenia in healthy recipients, confirmed the immunologic etiology of this disease, Subsequent studies in 1970s and 80s revealed the plasma factors in question to be IgG

antibodies against platelet surface glycoproteins, mainly GPIIb/IIIa and/or GPIb/IX ***(McMillan, 2007).***

Although plasma autoantibodies are clinically relevant, anti-platelet autoantibodies are more frequently detected in the platelet-associated (PA) form than in plasma form. The levels of PA autoantibodies, but not those of plasma autoantibodies, are correlated with the clinical course of ITP, and some studies shown that sera in ITP may contain antibodies against the cytoplasmic domain of GPIIIa and/or cytoplasmic proteins, such as vinculin, likely as a secondary effect of platelet destruction. Thus, in many cases, pathophysiologically important autoantibodies appear to be already bound to platelets. PA anti-GPIIb/IIIa and anti-GPIb/IX antibodies are detected in 43-57 % and 18-50 % in chronic ITP (cITP) patients, respectively ***(Tomiyama and Kosugi, 2005).***

For more than two decades, efforts have been focused on identifying target epitopes for PA autoantibodies. Studies reported that PA anti-GPIIb/IIIa antibodies frequently bind to cation-dependent conformational antigens ***(McMillan, 2007)***.

Studies also demonstrated that in most of ITP patients are positive for PA anti-GPIIb/IIIa antibodies, reactivity of PA anti-GPIIb/IIIa antibodies is markedly impaired with KO-variant GPIIb/IIIa (a loss-of-function mutation in the β-propeller domain of GPIIb) as compared with wild-type GPIIb/IIIa.These data