INTRODUCTION

Idiopathic thrombocytopenic purpura [ITP], is a bleeding disorder caused by an abnormally low level of platelets in the blood resulting in both accelerated destruction of platelets and reduced platelet production ***(Linker and Charles, 1997).***

In reviewing the current literature, it is clear that the pattern of presentation in acute ITP of childhood has changed very little over the years. For decades, physicians and researchers have commented on the rapid onset of bruising and mucosal bleeding in a severely thrombocytopenic child with minimal or no trauma.There is an equal incidence of ITP in both males and females in early childhood ***(Chandra et al., 2006).***

Forkhead box protein 3 (Foxp3) is a key transcription factor believed to be restricted to a subset of regulatory T (Treg) cells and is required for their development ***(Fontenot Rudensky, 2007).***

Mutations of the human gene FOXP3 are the cause of the genetic disease IPEX (immune dysregulation, polyendocrino-pathy, enteropathy, X-linked syndrome), which is the human counterpart of Scurfy ***(Ochs et al., 2005).***

The specificity and restriction of Foxp3 expression to a subset of T lymphocytes has provoked controversy in the scientific literature. However, more recent studies indicate that

Foxp3 is expressed in some epithelial cells and some tumor cells ***(Fontenot et al., 2005).***

Foxp3 also functions as a passive transcriptional repressor by physically interacting with proteins, Foxp3 may be playing a similar role in megakaryocytes by suppressing or activating transcription factors. Genetic lesions in megakaryocytes that cause thrombocytopenia ***(Li and Greene, 2007).***

Regulatory T cells (Treg) play important roles in many different immune responses. Foxp3 is the major transcription factor that determines the fate and identity of regulatory T cells. Signals important for induction of Foxp3 include IL-2 and TCR ***(Wang et al., 2006).***

AIM OF THE WORK

To evaluate Foxp3 mRNA level in mononuclear cells in children with acute thrombocytopenic purpura and the relationship between foxp3mRNA level of expression and disease characteristics [age at onset, sex, presentation, platelet count and outcome] will be studied.

REGULATORY T LYMPHOCYTE

Regulatory T ($CD4^+$ $CD25^{high}$nTreg) cells play central role in regulation of immune responses to self antigens, allergens, and commensal microbiota as well as immune responses to infectious agents and tumors. Transcriptional factor Foxp3 serves as a lineage specification factor of $CD4^+$ $CD25^{high}$nTreg cells. Paucity of naturally occurring T cell (nTregs) due to loss-of-function mutations of the Foxp3 gene is responsible for highly aggressive, fatal systemic immune mediated inflammatory lesions in mice and men ***(Rudensky, 2011).***

Some studies showed that the Foxp3 was largely expressed in CD4+CD25+T cells; and retroviral transduction of conventional CD4+ T cells with Foxp3 converted them to regulatory T cells with suppressive ability ***(Hori et al., 2003; Bennett et al., 2001; Fontenot et al., 2004).***

Thus, the current understanding is that the natural T regulatory cells are cells that possess the CD4+CD25+Foxp3+ phenotype ***(Nandakumar et al., 2009).***

$CD4^+$ regulatory T-cells can be further divided into induced regulatory T-cells such as: Interleukin-10 (IL-10) producing regulatory T-cells type 1(Tr1) and transforming growth factor-β (TGF- β) secreting T-helper 3 (Th3) cells and

the so-called naturally occurring $CD4^+$ $CD25^{high}$ regulatory T-cells ***(Levings et al., 2002).***

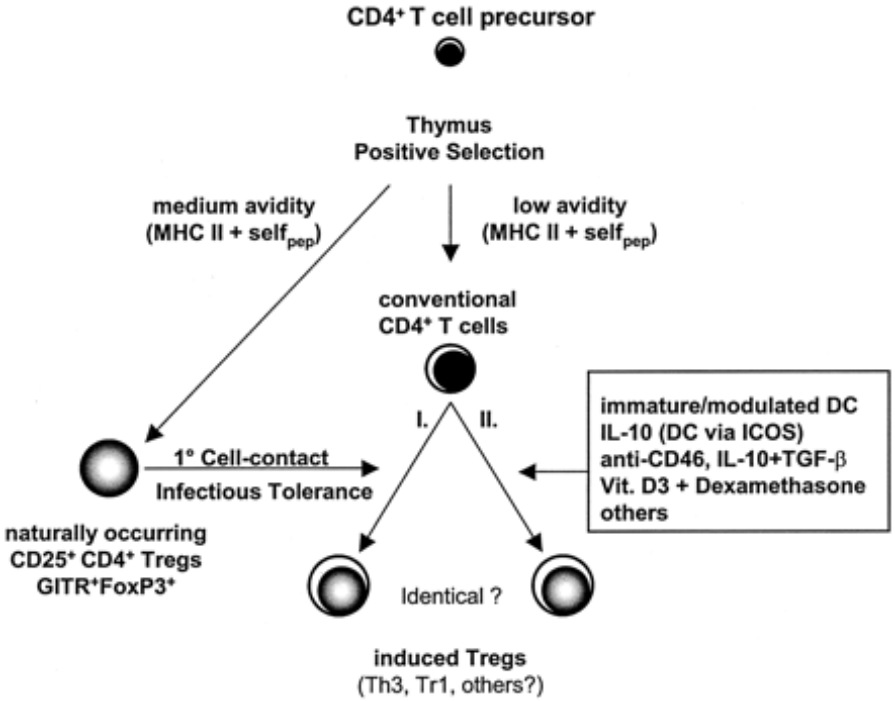

Figure (1): Model for the development and interrelations of different Treg subsets. Naturally occurring $CD4^+CD25^+$ Tregs develop directly from $CD4^+$ T cell precursors during positive selection under the influence of medium avidity interactions with thymic epithelial cells. Induced Tregs develop from naive conventional $CD4^+$ T cells either as a result of cell contact-dependent interaction with naturally occurring $CD4^+CD25^+$ Tregs or under the influence of suppressive agents like IL-10, TGF-β, dexamethasone, vitamin D_3, modulated DC, and potentially other inhibitory mechanisms.

Classification of CD4+CD25+ regulatory T cells:

1. Naturally occurring $CD4^+$ $CD25^{high}$ regulatory T-cells:

Naturally occurring T cell (nTregs) are considered to be the most pivotal players in the maintenance of immune tolerance. Numerous studies have indicated their role in the prevention of detrimental autoimmunity in animal models ***(Sakaguchi et al., 2005)***.

Consistent with this observation, a number of alterations of Tregs, whether quantitatively or qualitatively, have been observed in patients with different autoimmune diseases ***(Baecher-Allan et al., 2006)***. The isolation and investigation of Tregs have been hampered due to the lack of Treg-specific markers. For instance, CD25 (the IL-2R α-chain), glucocorticoid-induced TNF receptor family-related gene (GITR), and CTLA-4, molecules constitutively expressed by Tregs, are also induced by recently activated conventional T cells ***(Sakaguchi et al., 2008).***

The thymic development of nTregs

All T cells originate from haematopoietic stem cells in the bone marrow, Haematopoietic progenitors (Lymphoid progenitor cells) from haematopoietic stem cells populate the thymus and expand by cell division to generate a large population of immature thymocytes ***(Schwarz, 2006).*** The earliest thymocytes express neither CD4 nor CD8, and are therefore classed as double-negative ($CD4^-CD8^-$) cells. As they

progress through their development they become double-positive thymocytes ($CD4^+CD8^+$), and finally mature to single-positive ($CD4^+CD8^-$ or $CD4^-CD8^+$) thymocytes that are then released from the thymus to peripheral tissues ***(Schwarz et al., 2006).***

The latest research suggests that regulatory T cells are defined by expression of the forkhead family transcription factor FOXP3 (forkhead box p3), Expression of FOXP3 is required for regulatory T cell development and appears to control a genetic program specifying this cell's fate ***(Nadakumara, 2009).***

The large majority of Foxp3-expressing regulatory T cells are found within the major histocompatibility complex (MHC) class II restricted CD4-expressing (CD4+) population and express high levels of the interleukin-2 receptor alpha chain (CD25). In addition to the Foxp3-expressing CD4±CD25+, there also appears to be a minor population of MHC class I restricted CD8+ Foxp3-expressing regulatory T cells. Unlike conventional T cells, regulatory T cells do not produce IL-2 and are therefore anergic at baseline ***(Sakaguchi, 2009).***

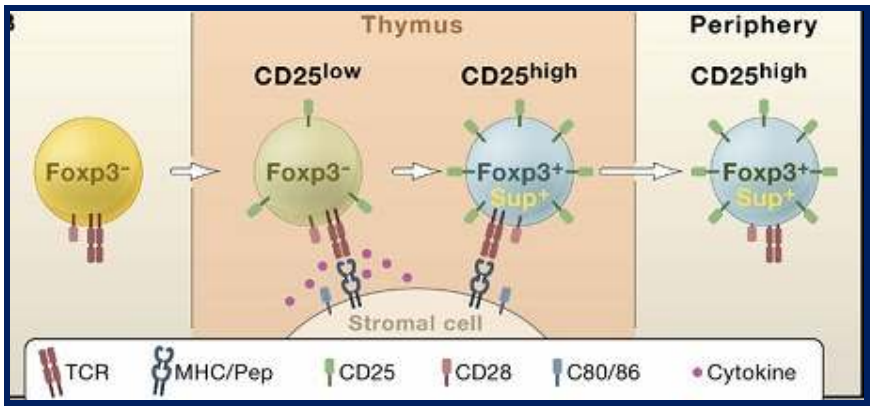

Figure (2): Development of Foxp3+ Tregs in the thymus involves interaction with thymic stromal cells via various molecules. Foxp3 thymocytes at a late CD4+CD8+ or an early CD4+CD8− stage turn on a Treg differentiation program when they receive signals produced by the interaction between their TCRs and MHC/self-peptide complexes on thymic stromal cells, between their accessory molecules (e.g., CD28) and their ligands (e.g., CD80 and CD86), and/or via stromal cell-derived humoral factors (e.g., cytokines). Foxp3 expression following cell fate determination confers suppressive activity and stabilizes Treg function and phenotype (e.g., CD25 expression). A suppressive function (Sup+). ***(Quoted from Sakaguchi, 2008).***

Marker molecules for naturally occurring $CD4^+$ $CD25^+$ Tregs (Forkhead box p3 expression)

The Foxp3 (forkhead box P3) is a member of the forkhead/winged-helix family of transcription factors Foxp3 gene is found on the X chromosome. Critical for the initiation and regulation of Treg ***(Sakaguchi et al., 2008)***.

FOXP3 protein levels in mouse and human Treg cells are decreased in inflammatory conditions, e.g. upon stimulation with LPS or IL-1β. Such a decrease is prevented by the addition of a proteasome inhibitor, suggesting that inflammatory stimuli

promote ubiquitin-mediated FOXP3 p roteasomal degradation ***(Chen et al., 2013).***

Transient treatment of mouse and human Treg cells with a deubiquitinase (DUB) inhibitor leads to decreased FOXP3 protein levels and to reduced suppressive function both in vitro (co-culture proliferation assay) and in vivo (mouse T cell transfer colitis model) ***(van Loosdregt et al., 2013)***.

The mechanisms of FOXP3 regulation described by ***Chen et al.*** and ***van Loosdregt et al***. occur at the post-translational level, it is likely that they can affect FOXP3 levels in both iTreg and nTreg cells. Indeed, both studies provide evidence of Stub1- and USP7-mediated regulation of FOXP3 protein levels in nTreg cells (although these nTreg cells were isolated based only on the expression of CD25) ***(van Loosdregt et al., 2013)***.

Further studies may provide additional information as to the post-translational regulation of FOXP3 in more finely defined populations of Treg cells and in other in vivo settings, however the findings that inflammatory stimuli can affect Treg cell plasticity via regulation of FOXP3 protein turnover, and the unraveling of the molecular processes involved may already suggest ways of manipulating Treg cells in the context of autoimmune diseases, transplantation or tumor immunology ***(van Loosdregt et al., 2013; Chen et al., 2013)***.

Mutations of the human gene FOXP3 are the cause of the genetic disease IPEX (immune dysregulation, polyendocrino-pathy, enteropathy, X-linked syndrome), which is the human counterpart of Scurfy ***(Ochs et al., 2005).***

Foxp3 gene has been expressed in nTregs in thymus and peripheral blood of normal mice, and it is a key regulatory gene for the development of regulatory T cells ***(Hori, 2003).***

What emerges, is that many factors, some positive, some negative, interact to collectively drive Foxp3 gene expression and then maintain its expression in Foxp3 regulatory T cells. TCR signaling is imperative for Foxp3 gene expression and TGF-β is a key cytokine for initiating Foxp3 gene expression in naïve T cells. But other signaling pathways are also known to play a role in properly orchestrating Foxp3gene expression and regulatory T cell expansion ***(Maruyama et al., 2011)***.

Some studies indicate that CD25 (IL-2 receptor) appears to be the most specific, cell surface marker for the nTreg. $CD4^{+}CD25^{high}$ nTregs are the only $CD4^{+}$T cells to constitutively express CD25 as well as CTLA4 and the tumour-necrosis factor (TNF) superfamily member glucocorticoid-induced TNF receptor (GITR) family-related protein (TNFRSF-18) ***(Bruder et al., 2004).***

Glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR, also known as TNFRSF18) is

predominately expressed on the surface of $CD4^{+}CD25^{high}$nTregs, $CD4^{+}CD25^{+}CD8^{-}$ thymocytes and activated $CD4^{+}$ T-cells ***(Shevach et al., 2006).***

However, Identification of Treg cells remains problematic, because accumulating evidence suggests that all the presently-used $CD4^{+}CD25^{+}$nTregs markers represent general T-cell activation markers rather than being truly Treg-specific ***(Corthay, 2009).***

Immune regulation by nTregs:

Regulatory T (Treg) cells, whose identity and function are defined by the transcription factor Foxp3, are indispensable for immune homeostasis ***(Samastein et al., 2012).***

The most remarkable feature of nTregs cells is their ability to dampen immune responses; they appear capable of suppressing a wide variety of immune cells, encompassing those of both innate and the adaptive immune system ***(Fehérvari and Sakaguchi, 2004)***.

Of particular interest, Foxp3 nTregs cells suppress the proliferation of naive T cells their differentiation to effector T cells in vivo. They can also suppress effector activities of differentiated CD4+ and CD8+ T cells and the function of natural killer cells, natural killer T cells, B cells, macrophages, osteoclasts, and dendritic cells ***(Tang and Bluestone, 2008).***

In accordance with this nTregs cells achieve their immuno-suppressive effects, when non-professional APC present antigens to T cells and there is an absence or reduced expression of costimulatory molecules like CD80 or CD86, it results in deficient immune activation and anergy. The latter could also result in the presence of CTLA-4 on T cells. These interact with CD80 and CD86 on dendritic cells and result in the production of indoleamine 2, 3-dioxygenase (IDO), which metabolizes tryptophan to kynurenines that are toxic to T cells and leads to a decrease in the activation of T cells both appear to be dependent on the expression of CTLA-4 by Tregs ***(Maggi et al., 2005; Romagnani, 2006; Frew, 2008).***

Alternatively, absorption of cytokines by Tregs may induce apoptosis in responder T cells**.** Tregs might also kill responder T cells or APC through cell-to-cel contact by a granzyme or perforin dependent mechanism or through delivery of a negative signal to responder T cells ***(Pandiyan et al., 2007).***

Possible negative signals include upregulation of intracellular cyclic AMP, which leads to inhibition of T cell proliferation and IL-2 production, or the generation of pericellular adenosine catalyzed by CD39 (ectonucleoside triphosphate diphosphohydrolase 1 and CD73 (ecto-5′-nucleotidase) expressed by Tregs ***(Tang and Bluestone, 2008).***

The presence of a greater amount of antigen or a high degree of T cells activation could also lead to immune suppression by activation induced cell death. This process is predominantly mediated by FAS-FAS-ligand. This interaction leads to the activation of caspase enzymes which results in the degradation of chromosomal DNA and apoptotic cell death. Activation induced T cell death also occurs as a result of activation of $CD4^{+}CD25^{+}Foxp3^{+}$ cells by CD3 and CD46. Activation of the Tregs upregulates the expression of granzyme A and eventually the Tregs can eradicate activated $CD4^{+}$ and $CD8^{+}$T cells in the presence of perforin ***(Romagnani, 2006)***.

$CD4^{+}CD25^{high}$ nTregs themselves require TCR stimulation, as well as, IL-2 to actually trigger their suppressive effects, this is explained by the fact that the Tregs have a higher level of IL-2 receptor expression; thereby possessing a greater ability to utilize the cytokine and deprive the other T cells of IL-2. The latter is a T cell growth factor and essential for T cell proliferation with its deficiency resulting in a hampered multiplication of the T cells. This was postulated to be the mechanism of suppression that is independent of cytokines such as IL-10 and TGF-β ***(Yamazaki et al., 2006).***

Of particular interest, TGFβ plays a major role in Treg differentiation and is important for nTreg development. Deleting TGFb from Treg cells results in diminished suppressive action. In the absence of proinflammatory cytokines, TGFb induces iTreg

differentiation from naive mouse CD4 T cells ***(Li et al., 2007; Marie et al., 2005).***

TGF activates Smad3 (Mothers against decapentaplegic homolog 3) while TCR stimulation induces NFAT (Nuclear factor of activated T-cells) activation. Smad3 (Mothers against decapentaplegic homolog 3) and NFAT collaborate in remodeling the Foxp3 enhancer region and promote Foxp3 expression, IL-2–mediated Stat5 (Signal Transducer and Activator of Transcription 5) activation is also required for the induction of Foxp3 expression ***(Burchill et al., 2007; Davidson et al., 2007);*** (A recent study has shown that Foxp3+ natural Tregs predominantly produce immunosuppressive IL-35, a new member of the IL-12 family ***(Collison et al., 2007).***

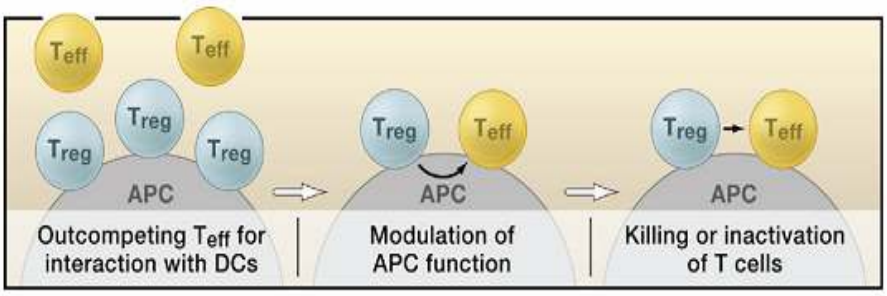

Figure (3): Possible Mechanisms of Treg-Mediated Suppression. More than one mechanism of Treg-mediated suppression may operate for the control of a particular immune response in a synergistic and sequential manner. Antigen-activated Tregs are recruited to antigen-presenting cells (APCs), especially dendritic cells (DCs), and out-compete antigen-specifc naive T cells regarding interactions with dendritic cells mainly because of the high expression of adhesion molecules (such as LFA-1) by Tregs Tregs then modulate dendritic cell function. For example, Tregs promote the downregulation of dendritic cell CD80 and CD86 by a CTLA-4-dependent mechanism. Some Tregs may further differentiate to kill or inactivate responder T cells by secreting granzyme/perforin or immunosuppressive cytokines (such as IL-10) ***(Quoted from Sakaguchi, 2008).***

Amplification of the suppressive effects of nTregs:

Given the relative physiological scarcity of nTregs, it seems likely that in vivo they would use mechanisms to amplify their suppressive action, this could occur by the "infectious" spreading of tolerance to conventional T-cells ***(Féhervari and Sakaguchi, 2004)***.

In accordance with this, some recent work has demonstrated that human nTregs can confer a suppressive phenotype to conventional $CD4^+$ T-cells in a contact-dependent manner. These newly generated regulatory-like cells then suppress by means of IL-10 or TGF-ß ***(Stassen, 2004)***.

This would constitute a mechanism of not only spreading a suppressive phenotype but also making it more efficient on a per-cell basis by engaging the action of soluble mediators ***(Féhervari and Sakaguchi, 2004)***.

Foxp3 nTregs suppress the proliferation of naive T cells and their differentiation to effector T cells in vivo. They can also suppress effector activities of differentiated CD4+ and CD8+ T cells and the function of natural killer cells, natural killer T cells, B cells, macrophages, osteoclasts, and dendritic cells ***(Shevach, 2006 and Tang and Bluestone, 2008).***

Several mechanisms of Treg-mediated suppression have been proposed, and these include secretion by the Treg of immunosuppressive cytokines, cell-contact-dependent suppression,