The Role of Treg Cells and FoxP3 Expression in the Immunity of B Thalassemia Major and B Thalassemia Trait Patients

Thesis

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Heba Osman Mohamed

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List of Abbreviations

Abb.	Full term
APCs	*Antigen presenting cells*
APTT	*Activated partial thromboplastn time*
ATRA	*All Trans retinoic acid*
BIM	*BCL-2-interacting mediator of cell death*
CD	*Cluster differentiation*
CMR	*Cardiac magnetic resonance*
CTL	*cytotoxic T lymphocyte*
CTLA-4	*Cytotoxic T lymphocyte-associated antigen 4*
DCs	*Dendretic cells*
DFO	*Deferoxamine*
EAE	*Experimental autoimmune encephalomyelitis*
EDTA	*Ethylene diamine tetra-acetic acid*
FITC	*Fluorescein isothiocyanate*
FOXP3	*Forkheadboxes P3*
FVIII	*Factor VIII*
GALT	*Gut-associated lymphoid tissue*
GFP	*Green fluorescent protein*
GITR	*Glucocorticoid-induced tumor necrosis factor*
GRAIL	*Gene related to anergy in lymphocytes*
HLA	*Human leukocyte antigen*
HLA	*Human leukocytic antigen*
IDDM	*Insulin dependent diabetes*
IFN	*Interferon*
Ig	*Immunoglobulin*
IL	*Interleukin*
IL1RAcP	*IL-1 receptor accessory protein*

List of Abbreviations (Cont...)

Abb.	Full term
IQR	*Inter-quartile range*
iTreg	*Inducible regulatory T cell*
Kip1	*Kinase inhibitor p27*
LAG-3	*Lymphocyte activation gene-3*
MAP	*Mitogen-activated protein*
MAP	*Monoclonal antibody*
MCH	*Mean corpuscular Hb*
MCL1	*Myeloid leukemia cell differentiation 1*
MCV	*Mean corpuscular volume*
MHC	*Major histocompatibility complex*
mTOR	*Mechanistic target of rapamycin,*
NAG	*N-acetyl-D-glucosaminidase*
NKT	*Natural killer T cell*
NTBI	*Non-transferrin-bound iron*
nTreg	*Naturally occurring regulatory T cell*
PC5	*Phycoerythrin-Cyanine 5*
PE	*Phycoerythrin*
PI3K	*Phosphatidylinositol 3-kinase*
PQC	*Protein quality control*
PSA	*Polysaccharide A*
pTreg	*Peripheral induced regulatory T cell*
RORγt	*retinoid-related orphan receptor γt*
SD	*Standard deviation*
TAA	*tumour-associated antigen*
TCR	*T cell receptor*
Teff	*Effector T cell*

List of Abbreviations (Cont...)

Abb.	Full term
TF	*Tissue factor*
TGF	*Transforming growth factor*
TH	*T helper*
TR	*T regulatory*
TRAIL	*TNF-related apoptosis-inducing ligand*
Treg	*Regulatory T cell*
tTreg	*Thymus induced regulatory T cell*
UPS	*Ubiquitin – proteasome system*
B-TM	*Beta Thalassemia Major*
γc	*Common γ chain*

Abstract

Background: Immunological abnormalities in thalassemia are caused by either the illness, or therapy methods. The basis of the pathogenesis of the immunodeficiency in thalassemia is iron overload and allogenic stimulation caused by multiple blood transfusions. T regulatory cells (Tregs) are a component of the immune system that constitutes a key mechanism in maintaining peripheral self-tolerance through the control of auto-reactive lymphocyte activation. **Aim:** To determine the frequency of Tregs ($CD4^+CD25^+CD127^-$) in children and adolescents with β-thalassemia major and assessed their relation to the clinical and laboratory characteristics of patients including transfusion therapy, iron overload and alloimmunization. **Methods:** Sixty β-TM patients studied focusing on transfusion history and chelation therapy. Hematological and biochemical data including markers of hemolysis and serum ferritin were assessed. Patients' records were analyzed for the presence of alloantibodies. Assessment of T regulatory cells ($CD4^+CD25^+CD127^-$) using flow cytometry. Patients were compared with 30 healthy controls. **Results:** The percentages of $CD4^+$ T lymphocytes and Tregs were significantly reduced in β-TM patients compared with the control group ($p<0.05$). Patients with patients with splenectomy, heart disease and PH risk had lower levels of these cells than those without. The percentage of Tregs was decreased among patients with serum ferritin ≥2500 μg/L compared with patients below this cutoff and in patients with history of alloimmune antibodies. There were significant negative correlations between Tregs and each of total and indirect bilirubin and ferritin levels while they were positively correlated with WBCs counts and hemoglobin. Multivariable linear regression analysis showed that WBCs count, hemoglobin and serum ferritin are the significant independent variables related to Tregs in patients with β-TM. **Conclusion:** Children and adolescents with β-TM may encounter a state of immune dysfunction. Low frequency of Tregs in β-TM patients may contribute to the formation of alloantibodies. Alterations in Tregs are more evident in relation to iron overload and in patients with cardiovascular complications which may reflect involvement of Tregs in disease severity. It is important to assess the suppressive activity of these cells in standard proliferation assays to examine whether their function is disrupted in β-TM together with the alteration in their frequency or not.

INTRODUCTION

β-thalassemias are heterogeneous autosomal recessive hereditary anemias characterized by reduced or absent β-globin chain synthesis. Approximately 68,000 children are born with various thalassemia syndromes each year ***(Modell and Darlison, 2008; Origa et al., 2016)***.

Thalassemia was first clinically described nearly a century ago and treatment of this widespread genetic disease has greatly advanced during this period. Recently, there has been a rejuvenated interest in studying thalassemia at the basic science level, leading to the discovery of previously unknown mechanisms leading to anemia and enabling the development of novel therapies ***(Rund, 2016)***.

Patients with β-thalassemia major (β-TM) require regular transfusions of red blood cells to survive ***(Rachmilewitz and Giardina, 2011)***. However, repeated transfusions cause iron overload, with life-threatening complications, such as endocrine dysfunction, cardiomyopathy, liver disease and, ultimately, premature death ***(de Dreuzy et al., 2016)***.

β-TM have an increased risk for serious infections, due to that a basic defect in the host defense and this may be related to the iron overload, chronic immune-stimulation by repeated blood transfusions, splenectomy and immune deficiency. Changing in lymphocyte subsets include a greater number and activity of suppressor T cells (CD-8), reduced proliferative

capacity and a number and level of activity of helper T- Cells (CD-4) leading to decreased CD4/CD8 ratio, as well as defective activity of natural killer (NK) cells. High immune globulins were reported and B-lymphocytes were found to be increased, activated with impaired differentiation, impairment of immunoglobulin secretion accompanied by increased levels of IgG, IgM and IgA. Neutrophils and macrophages are associated with defective chemo taxis and phagocytosis ***(Javad et al., 2011)***.

Immunological abnormalities in thalassemia are caused by either the illness, or therapy methods. The basis of the pathogenesis of the immunodeficiency in thalassemia is iron overload and allogenic stimulation caused by multiple blood transfusions. There are not numerous data about efficiency of the immunocorrection therapies for thalassmia patients ***(Asadov, 2014)***.

A major complication of transfusion therapy is alloimmunization, which may result in life-threatening delayed hemolytic transfusion reactions in addition to difficulties in obtaining compatible blood for transfusion ***(Bauer et al., 2007)***.

T regulatory cells (Tregs) are a component of the immune system that constitutes a key mechanism in maintaining peripheral self-tolerance through the control of autoreactive lymphocyte activation and the prevention of harmful effects of such activation. These cells are involved in shutting down immune responses after they have successfully eliminated invading organisms ***(Caramalho et al., 2015)***.

The different populations of Tregs share a common characteristic of immunosuppressive capability, but differ in their cell surface markers, types, and site of formation. Among these populations, natural Tregs, characterized by the expression of $CD4^+CD25^+$ and the transcription factor (Foxp3) ***(Sakaguchi et al., 1995)***, have been well studied and accumulating evidence suggests that this population plays a crucial role in the maintenance of immunological self-tolerance and negative control of pathological as well as physiological immune responses ***(Fehe´rvari and Sakaguchi, 2004)***.

At present, Foxp3 is the most specific molecular marker for thymic or peripheral Tregs in rodents and humans ***(Valencia and Lipsky, 2007)***. It is believed that CD127 expression is down-modulated on the Treg cells and that FoxP3 interacts with the CD127 promoter and contributes to reduced expression of CD127 in Tregs ***(Liu et al, 2006)***. Recently, the frequency of Tregs ($CD4^+CD25^+CD127^-$) has been found to be decreased in children with hemophilia A with inhibitor formation ***(El-Asrar et al., 2016)***.

Literature is limited about the role of Tregs in chronically transfused patients. Alterations in immunoregulatory T cell subsets of regularly transfused SCD patients with alloantibodies has been reported compared to those without, specifically a reduction in Treg activity, an increase toward Th2 responses and lower serum levels of IL-10 in the alloimmunized cohort ***(Bao et al., 2011)***.