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The Role of Immune System in Thalassemia Major: A Narrative Review

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Abstract

Context: Thalassemia is a genetic disorder of hemoglobin production. Patients with thalassemia major (TM) require regular blood transfusions to keep a compatible hemoglobin level for oxygenating organs. These patients suffer from different complications such as infections, autoimmunity and alloimmunization due to transfusion. Such complications link the immune system to TM pathogenesis. In the present study, we have reviewed the latest data available on interactions of TM pathophysiologic determinants and immune system components.

Evidence Acquisition: A comprehensive search was performed on PubMed, Scopous, and Web of Knowledge databases using keywords thalassemia, immune system, autoimmune, alloimmune, adaptive immunity, innate immunity, complications, and immunesenescnce.

Results: It seems that persistent antigenic stimulation and oxidative stress from excessive iron are the two main pathophysiologic factors of TM impacting the immune system. Regarding innate immunity, functional activity of neutrophils, and natural killer cells (NKCs) is decreased in TM. On the other hand, higher levels of TNF-α and IL-1β, IL-6, IL-8, and C-reactive protein proinflammatory cytokines have been observed in the serum of patients. TM patients have demonstrated higher ratios of regulatory B lymphocytes (CD19+, CD38+, CD24+), helper T cells, suppressor T cells, and T regulatory (CD4+/CD25+/Foxp3+) lymphocytes. TM patients have shown significant higher levels of IgA immunoglobin respective to normal counterparts that may predispose them to diabetes and coeliac disease. Immune cells, however, rendered lower than optimal activity in TM patients, which may be due to nutritional insufficiencies. Potential relationships have been suggested between immune system and various thalassemia compilations including heart infraction, hypertension, atherosclerosis, diabetes, thyroid dysfunction, and osteoporosis.

Conclusions: Immune genetic determinants may be involved in modulating the clinical picture of TM. TM patients generally represents with higher immune cell counts, likely as a result of persistent antigenic challenge from blood transfusions. However, these patients face compromised immune cell functions. The role of immunologic interactions in pathogenesis of TM needs to be further divulged in future studies.

Keywords: Beta-Thalassemia, Immunity, Immunosenescence, Erythroblastosis, Narrative Review

1. Context

Thalassemia is the most common monogenic disorder worldwide. In the most extreme conditions, thalassemia patients suffer from severe anemia necessitating regular blood transfusions (thalassemia major-TM) (1). TM is particularly common in Southeast Asia, Middle East, and Mediterranean countries. TM represents a major health problem in Iran, with estimated general frequency of 3% - 4% rising up to 8% - 10% in some penetrant regions (2, 3). TM patients usually present during the first month of their lives with failure to thrive, poor nutrition, and pallor, if left untreated, may be complicated with severe hepatosplenomegaly, intense bone deformities, and death. Iron overload has been suggested as the main culprit responsible for various organ disabilities such as cardiac dysfunction, endocrine insufficiencies, bone fractions, overwhelming infections, and other related complications (4).

Iron overload related complications are still encountered in a significant ratio of TM patients. Due to this, it is logical to think of other possible contributors influencing development of TM complications. Immune system, as a sophisticated and complex collection of cellular and humoral components, may be involved in the organ dysfunctions in patients with TM. Possible role of immune system

components on clinical course of TM has been focused only in recent years.

In the present review, the last updates on the roles and alternations of cells and humoral components of immune system in TM have been discussed. The understanding of immune system interactions with TM pathogenesis can help to provide new modalities for management of the disease and its complications.

2. Evidence Acquisition

A comprehensive search was performed on PubMed, Scopous, and Web of Knowledge Databases using keywords of thalassemia, immune system, autoimmune, alloimmune, adaptive immunity, innate immunity, complications, and immunesenescnce. The time span included published articles related to immune system and thalassemia during 2000 - 2015. Only manuscripts written in English were included. Those studies that had correct methodological and scientific structure (either case-control, cross-sectional, clinical trials, and review studies) based on recommendations of ICMJE (available at: http://www.icmje.org/) were studied. The initial screening of studies was based on reading titles and abstracts excluding unrelated works. Discussions of immunological interactions were drawn based on results and conclusions of these studies.

3. Results

3.1. Innate Immunity

Both quantitative and qualitative properties of immune cells, as well as cytokine profile of innate immunity are subjects for derangements in TM. Patients with TM are often encountered with a low-grade systemic inflammatory status with higher total leukocyte, neutrophil, and lymphocyte counts (5). Neutrophils isolated from TM patients' demonstrated significantly lower functional activity in comparison with the cells derived from healthy controls (6). Higher expression of surface molecules such as CD11b, CD18, and CD69 on monocytes, and higher expression of CD11b, CD18, CD35, CD44, and CD67 on neutrophils have been described in TM (7). Mechanisms underlying attenuated neutrophilic function in TM are not well characterized. In one hand, chronic elevation of oxidative stress may interfere with function of these phagocytic cells (8). Besides, neutrophils of TM patients have been described with high expression of apoptotic markers; caspases 3,7,8, and 9 (9). This notion that chelation therapy has been associated with attenuation of apoptotic markers in TM derived peripheral leukocytes accentuates the role of iron in induction of apoptotic pathways in these cells. In contrast, Elsayh et al. found no differences in the apoptosis rate between neutrophils from TM patients and the cells of healthy counterparts (5). Overall, apoptosis may contribute to the functional dysregulation of neutrophils in TM. Other possible mediators should be investigated in future studies.

Activity of natural killer cells (NKCs) is decreased in TM. Micronutrient deficiency may be a critical participant in affecting NKC function in these patients (10), however its underlying mechanisms are not well understood (11).

In accordance, higher levels of TNF-α and IL-1β proinflammatory cytokines have been observed in serum of TM patient (7). Likewise, Neopterin, a proinflammatory mediator produced by activated macrophage, was described in higher levels in TM patients compared to control individuals (12). Furthermore, TM patients showed higher levels of IL-6 (13-15), IL-8 (14), and C-reactive protein (15). Despite the impoverished cellular components, the humoral determinants of innate immunity seem to be augmented in TM. Like the other features, role of oxidative stress has been proposed in this phenomenon (16).

3.2. Humoral Immunity

Regarding the role of B lymphocytes in production of alloantibodies and autoantibodies against transfused red blood cells, humoral immunity function is a critical issue in TM. TM patients harbor larger B cell proportion compared to normal counterparts (17). B lymphocytes with a regulatory phenotype, expressing CD19, CD38, and CD24 have been identified with a significant higher ratio in TM patients than control subjects (18). In addition, no significant difference has been reported in rate of B cell apoptosis between TM patients and normal individuals (5). TM patients showed significant higher levels of IgA immunoglobin respective to normal counterparts, however, there have been no significant differences between IgG, IgM, IgE, as well as complement components of C3 and C4 (19). In another study, TM patients received standard iron chelation therapy showed no significant differences in concentrations of immunoglobulins in comparison with healthy subjects (20).

3.3. Cellular Immunity

3.3.1. Quantitative Alternations

TM patients generally represents with higher lymphocyte counts, likely as a result of persistent antigenic challenge from blood transfusions (12, 17). Different subsets of lymphocytes, including helper T cells, suppressor T cells, NKCs, and B cells (distinguished by respective phenotypic signatures of CD3+/CD4+, CD3+/CD8+, CD3-/CD16/56+, and

CD3-/CD19+) have been reported with increased number in TM patients (21, 22). Excess iron may exert executive effects on the ratio of T cell subsets, which may be reflected by increased number of CD8+ lymphocyte while decreased CD4+ subset (23). A decreased ratio of Th1/Th2 cells has also been noted in the mouse model of thalassemia (24). In contrast, Noulsri et al. found no significant difference in frequencies of conventional double positive (CD4+, CD8+) or single positive (CD4+ or CD8+) lymphocytes between TM and healthy controls (25). In a report by Al-Awadhi et al., ratios of T cell subpopulation were of no significant difference between TM patients and healthy individuals (17). However, lymphocytes with uncommon CD4-CD8- phenotype ($\gamma\delta$-T cell receptor) as well as natural killer T (NKT) cells revealed higher ratios in TM patients (25). The most prominent feature of altered lymphocytic subpopulations in TM patients may be elevated ratio of T regulatory (CD4+/CD25+/Foxp3+) lymphocyte subset (26). T regulatory subpopulation negatively controls immune responses against both self and foreign antigens (27). These regulatory cells also inhibit proliferation of immune cells including B cells, T cell, and antigen presenting dendritic cells (28).

3.3.2. Functional Alternations

Although TM patients have shown significantly higher counts for the total and activated lymphocytes, the proliferative, and cytokine production activities of these cells have been less than optimal in these patients (12). One explanation for this low activity can be Zn insufficiency, an essential element required for function of immune cells (29, 30). In fact, in used iron chelators in TM, Desferoxamine (DFO), Deferiprone (DFP), and Deferasirox (DFX), all have been shown to lead to Zn deficiency (31). Furthermore, other nutritional deficiencies may also contribute to this state of immune deficiency in TM (32). In this regard, it was shown that nutrient support of TM patients, for one month, improved proliferative responses of their lymphocytes (32). The role of iron in attenuating activity of lymphocytes may be further highlighted by the observed correlation between ferritin level and rate of cytokine production by lymphocytes (12). In accordance, a three month treatment of TM isolated lymphocytes with anti-oxidative agent, Silymarin, rendered the lymphocytes more efficient in producing of cytokines (33).

3.3.3. Immunesenescnce

A condition known as immunesenescnce, which is characterized with premature aging of lymphocyte has been described in patients with TM (16, 34). Senescent lymphocytes with reduced proliferative capacity are phenotypically distinguished by depressed expression of CD28, a major membrane co-stimulatory molecule (16). Phenotype of senescent T lymphocytes may correspond to either CD8+/CD28- or CD3+/CD95+ (34). Elevated T cell counts with higher expression of CD 95, Fas apoptotic receptor, correlates to the senescent nature of the lymphocyte population in this situation (35).

Oxidative stress has been known as a factor accompanied immunesenescnce phenomenon in TM (36). It is believed that iron induced oxidative stress to be responsible for toxic damage of DNA and premature exhausting of immune cells (37). In line with these, lymphocytes from TM patients had significantly lower levels of the intracellular antioxidant molecule, glutathione, correlating with their lower proliferative capacity (38). Exposure to silymarin resulted in retrieving both intracellular glutathione pool and proliferative capacity of lymphocyte from TM patients (38). Increased apoptotic rate is another feature of lymphocytic populations isolated from TM patients that can contribute to the lymphocyte senescence (5). It has been proposed that higher apoptosis rate is related to increased tolemerase activity in chronically stimulated T lymphocytes in TM (34).

3.4. Role of Splenectomy in Modulation of Immune System in TM

Effects of splenectomy on pathogenesis of TM are controversial. Splenectomy has been noted to boost the number of both CD4+ and CD8+ T cells in TM patients (12). Splenectomy has also been associated with higher neutrophil counts (39). In contrast, splenectomy has been reported to reduce NKCs and CD4+ lymphocytes counts in the patients (40). Cytokines produced by activated immune cells including IL-2 and TNF-α were described to be higher in splenectomized TM patients (12, 39). Splenectomy reduced levels of IgA and IgM immunoglobulins, as well as C3 level and activity of complement system in TM (41, 42). In addition to quantitative changes, reduced activity of immune cells has been reported following splenectomy (43). Splenectomized TM patients showed lower activity of macrophages in comparison with non-splenectomized patients (12). Splenectomized TM patients may also experience a transitional depressed activity of neutrophils following the operation (39, 44). The ratio of peripheral blood monocyte, as well as activated monocytes showed higher values in splenectomized TM patient respective to non -splenectomized cases (45). It has been suggested that the increased number of red blood cells exposing membrane phosphatidylserine may be responsible for the observed activated phenotype of monocytic cells in splenectomized TM patients (45). Nonetheless, immunological effects of splenectomy seem to be transitional restoring to the basal levels over time (43).

3.5. Immune System and Liver Damage

Liver fibrosis is a serious condition that may lead to hepatic failure in TM. Although hepatic iron overload is thought to be the main contributor to hepatic fibrosis in this condition, hepatitis C infection (HCV) seems to exert synergistic effects on this process (46). However, there is a report that contrasts with the independent role of HCV on liver fibrosis progression, and influence of immunological modulators on this phenomenon has been proposed (47). In particular, polymorphisms within IL-28 gene have been noted to influence both viral clearance and hepatic fibrosis in TM patients (48). Although studies on the role of immunologic determinants in liver lesions and fibrosis in TM patients are scarce, studies in other clinical conditions suggest pivotal roles for immunological factor in liver damage. Lower ratio of T lymphocytes producing IL-17 and higher levels of plasma CD14 have been described in HCV infected patients with high grade liver lesions (49). Higher levels of chemokine CXCL-10 within hepatic tissue have been related to fibrosis progression hepatitis (50). In addition, higher titer of autoantibodies in serum may predispose to liver fibrosis in HCV infected patients who have undergone liver transplantation (51). Deleterious or protective roles of cytotoxic reactions triggered by NKCs in hepatic disease are controversial (52). Generally, the role of immune system in either protection or predisposition to liver damage should be sought within those components that can target hepatic tissue (53). In fact, signaling pathways derived from cytokines-cytokine receptors can promote a variety of transcription factors with wide range activities. There are few studies on the role of immunologic modulators on liver biology in TM patients, which is recommended to more extensively strive in this field in future.

3.6. Immunity and Alloimmunization

Alloimmunization is one of the significant adverse effects of blood transfusions in TM (54). In addition to difficulties in finding compatible blood units for the immunized patients, alloimmunization also can lead to life-threatening hemolytic transfusion reactions (55). The rate of alloantibodies production against red blood cell antigens has been reported as 2% - 35% in different studies (18, 54, 56). Mechanisms underlying alloantibody (and autoantibody) production following blood transfusion are not well characterized. A role has been proposed for CD4+ T regulatory cells in controlling the extent of antibody response to allogenic blood transfusion in animal models (24). Increased ratio of CD4+ T regulatory cells has been descried in mouse model of TM (24). In parallel, activity of CD4+ T regulatory cells was shown to be depressed in TM patients developed alloantibody against red cell antigens (57). Interestingly, Yu et al. showed that removing of CD4+/CD25+ regulatory T cells resulted in higher alloimmunization rate in transfused mice and introduction of these regulatory cells prevented the production of alloantibodies in response to blood transfusion (58). Furthermore, regulatory T lymphocytes, Th2 subset of T lymphocytes may also contribute to the production of alloantibodies in TM (57). Understanding immune regulators involved in alloantibody response in TM, which can be useful in providing appropriate measures to prevent or reduce the rate of alloimmunization in these patients.

3.7. The role of IgA

Level of IgA is increased in TM patients (57-59). In fact, elevated IgA has been reported as the sole dysregulation of humoral immunity in TM patients (42). IgA levels may be associated with abnormal kidney function in thalassemia syndromes (60). TM patients with diabetes have had higher IgA levels (19). IgA level has been positively and negatively correlated with splenectomy and HCV infection respectively (61). The levels of IgA in TM has been higher in older TM patients respective to younger patients (19). An interaction between age and splenectomy has been noted to influence IgA level in TM. In this regard, splenectomized TM patients with age > 20 years old showed higher levels of serum IgA (42). Higher titer of IgA in TM patients has been proposed to predispose these patients to coeliac disease (62). Clinical significance of high titer of IgA in TM is to be more evident in future.

3.8. Crosstalk Between Immune System and Other Transfusion Related Complications

After cardiac failure, infections are the second most common cause of disabilities and even death among TM patients (63). This higher risk may be partly attributed to impaired immune function in TM patients (64). Various abnormalities in the immune system, including altered cell counts and cytokine concentrations, as well as compromised functions including impaired chemotaxis and attenuated phagocytic, and killing activities have been noted in TM patients rendering them susceptible to various pathogenic agents (26). The chronic stimulatory state from continuous blood transfusions may further weaken responsiveness of immune cells to the future pathogenic insults.

Hepatitis is among the most important infections jeopardizing health of TM patients (65). In particular, HCV constitutes the major hepatitis subtype affecting these patients (4). Immunological alternations, following the hepatitis infection, have been subject of numerous studies.

TM patients infected with HCV have been reported to have decreased ratio of common subsets of lymphocytic cells, NKCs, an NKT cells compared to either healthy volunteers, non-hepatitis infected TM patients, or non-thalassemic patients who were diagnosed with chronic HCV infection (40). Immunologic alternations, following hepatitis in TM, may affect treatment response to the infection, however, patterns and mechanisms involved in this process are yet to be divulged.

The role of inflammatory mediators has been described in cardiovascular conditions including heart failure (66), hypertension (67), and atherosclerosis (68). There has been a proposition that elevated level of IL-1α along with increased level of TNF-α, can be involved in cardiovascular events in TM (69). In addition, abnormal pulmonary function has been associated with higher level of IL-8 and TGF-β in TM patients (70). Cardiovascular complications are leading cause of mortalities in TM patients; however, potential effects of immunological mediators in pathogenesis of these abnormalities are less studied.

TM patients diagnosed with diabetes have shown significantly higher levels of IgA and IgG, while lower levels of IgM and C3 component of complement (19). Furthermore, inappropriate immune reactions against pancreatic beta cells may also share a part in pathogenesis of diabetes in TM (71). On the other hand, role of T regulatory and Th17 lymphocytes has recently been described in development of thyroid dysfunction in TM (72). An interaction between immune components with bone generating osteoblasts has been proposed to be involved in regulating bone metabolism in TM (73). These findings highlight indispensable role of immune components in clinical progression of TM that need to be addressed in future studies. Table 1 summarizes immune dysregulations in TM and their suggested clinical implications.

Table 1. Immune Alternations and Their Respective Clinical Impacts on Clinical Course of Thalassemia Major

Immune Component		Immune Alternations	Potential Contributors and Clinical Implications
Innate immunity	Cytokine changes	Higher levels of IL-1α, TNF-α and IL-1β, IL-6, IL-8, C-reactive protein, Neopterin, Reduced C3 level and complement activity	Role of oxidative stress, splenectomy, increased risk of cardiovascular events, compromised pulmonary function, predisposing to diabetes
	Cellular changes	Low neutrophil functional activity (chemotaxis, phagocytosis), Increased activated monocytes, decreased natural-killer cells activity	Chronic oxidative stress condition, higher rate of apoptosis, positive correlation with splenectomy, and increased PS on the RBC membrane, Micronutrient deficiency such as vitamin C and selenium
Humoral immunity	B lymphocytes	Increased B lymphocyte counts, Increased regulatory B cell count (CD19+/CD38+/ CD24+)	Higher alloimmunization rate against transfused red blood cells
	Immunoglobulins	Increased IgA level	Potential role of splenectomy in this process, contributing to abnormal renal function, predisposing to coeliac disease, diabetes
Cellular immunity	Quantitative changes	Increased helper T cell, suppressor T cells, NKT cells, regulatory T cells (CD4+/CD25+/Foxp3+), Increased CD8+/CD4+ lymphocytes ratio	Modulating alloimmunization against allogenic red blood cells, contributing to thyroid dysfunction, and osteoporosis
	Functional changes	Decreased proliferative and cytokine production activity	Increased susceptibility to infections

3.9. Immune System and Perspectives in TM

The main goal in managing TM patients is to ameliorate the clinical phenotype of the disease. Phenotype modifiers of TM (known of quantitative trait loci) include Xmn-1 polymorphism (74), BCL11A genetic variations, and polymorphism in HBS1L-MYB locus (75). Interestingly, genetic variations within immune specific transcription factor, CEBPε, which is expressed in myeloid lineage of immune cells, has been described in correlation with an intermediate clinical picture in homozygous thalassemia patients (76). The role of immunity in pathogenesis of TM is further highlighted considering that cells of immune system can modulate iron turn over through regulating levels of hepcidin, a master regulator of iron metabolism (13). Furthermore, components of the immune system may contribute to the rate of ineffective erythropoiesis (IE), which is the main factor determining iron absorption and bone deformities in TM (77). In particular, expression of CD 177, human neutrophil antigen-2a (HNA-2a), has been associated with the rate of IE in TM patients (78), Erythropoietic activity of bone marrow has been noted to be under regulation of macrophage activity through controlling iron availability, and regulating hepcidin expression, iron cycle, and EPO signaling pathway (79). Regarding emerging evidences of immune participation in TM pathogenesis, it deems necessary for immune system to share a more pronounced role in management of TM in future.

4. Conclusions

TM represents a major health problem in the world with a wide range of complications affecting different organs. Application of immune system modalities in clinical practice in TM is hindered by a lack of knowledge regarding role of immune system in course of the disease. By emerging the significance of various components of immune system in pathogenesis of TM, it is a necessity to conduct more elaborating studies to reveal potential clinical implications of immune factors in the management of TM.

Immunesenescnce is a respectively new concept in TM and its contribution to the clinical course of the disease needs to be more studied.

Footnotes

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