

Immunologic Abnormalities in β -Thalassemia

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Abstract

Infectious complications are the important part of the clinical spectrum of thalassemia, being one of the main reasons of mortality rate in this disease. Data about the increased susceptibility to infections in thalassemia aroused interest in the study of the immune status in this disease. As a result of the researches considerable infringements of specific (all stages of phagocytosis), nonspecific immunity (the maintenance and functional activity of lymphocyte subsets) and humoral immunity (an increase of the immunoglobulins and a decrease of the activity of complement components) have been revealed. 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.

Keywords: Thalassemia; Immune abnormalities; Phagocytosis; Lymphocyte subsets; Immunoglobulins

Introduction

β -thalassemia is a hereditary hemolytic anemia associated with a decrease or complete lack of synthesis of β -globin chains as a result of inheriting the mutant gene. Currently there are more than 100 varieties of hemoglobin gene mutations, which can lead to β -thalassemia. In the world there are 60-70 thousand patients with major form of this genetic anomaly. The basic treatment for β -thalassemia is a lifelong hemotransfusion therapy (hypertransfusion regime) that requires to maintain normal levels of hemoglobin and to suppress enhanced but ineffective erythropoiesis. However, volumetric and multiple transfusions inevitably lead to alloimmunization, accumulation of iron in the tissues and are associated with increased risk for transmitted infections [1].

Patients with thalassemia have an increased susceptibility to infections [2]. As the results of the multicenter study conducted in Italy, infections are the second most common cause of complications in thalassemia after cardiac mortality [3]. Similar results were obtained from studies of β -thalassemia patients undertaken in Greece [4], and Taiwan [5]. In patients with E/ β -thalassemia infections were the most frequent cause of death [6].

Data on increased susceptibility to infection in thalassemia aroused interest in the study of the immune status of patients with this disease in the early 70 years of the last century.

State of immune system in β -thalassemia. The first information about the changes in non-specific immunological reactivity in β -thalassemia (decreased absorption and phagocytic ability of segmented neutrophils, reducing the levels of complement, properdin and lysozyme) were published in 1973 [7]. Further as a result of numerous studies has been described a wide range of immune abnormalities in thalassemia.

In the early 80 years of the last century, the authors of this review was first conducted a comprehensive study of morphological and functional properties of Polymorphonuclear Neutrophils (PMN) in thalassemia [determination of phagocytic activity, Nitroblue Tetrazolium Test (NBT-test) and cytochemical indicators], which revealed a reduction in both the absorptive and digestive activity of PMN, changing intracellular metabolism processes (an increase in activity of peroxidase, acid and alkaline phosphatase, chloroacetate esterase, a decrease in glycogen content), an increase in NBT-test

indicators [8,9]. Further studies have confirmed the findings about disruption of the functional activity of PMN. A study conducted in 25 patients with β -thalassemia major from Egypt has shown a decrease in phagocytic index to both salmonella and staphylococcus in patients [10]. Also found a disruption of chemotaxis and spontaneous migration of PMN in β -thalassemia major [11,12].

Several authors related detected abnormalities in phagocytic activity of PMN in thalassemia to iron overload. Thus, Bassaris et al. revealed a disruption of PMN adhesion to nylon caused by serum of thalassemia patients [13]. Van Ashbeck et al. [14] demonstrated functional defects of PMN in patients with excess iron. Skoutelis et al. [15] conducted the study PMN in 50 patients with homozygous thalassemia and identified disorders of phagocytic and bactericidal functions of these cells and related them to iron overload. Cantinieaux et al. [16,17] conducted a study to identify relationship between phagocytic function disorders in patients with thalassemia major and iron overload. The authors showed that in 13% of PMN of patients with thalassemia Perls reaction were positive, whereas in the control group were no positive result for iron cells. Incubation of PMN of healthy individuals with the serum of thalassemia patients led to disruption of phagocytic function and appearance of PMN cells with positive Perls reaction. The authors concluded that the cause of disruption of the functional activity of PMN in thalassemia is iron overload. Further, continuing their researchs in this area [17], the authors isolated from the serum of patients with thalassemia ferritin-iron compound by gel filtration chromatography and by incubating PMN with these compounds have proved that they inhibit the function of phagocytic cells in thalassemia. Another argument in favor of what has been said is that the addition chelation desferoxamine (DFO) to the incubation mixture prevented this effect. Further evidence of the role of excess iron in disruption of the PMN in thalassemia is to establish a connection between the degree of phagocytic function and the number of blood transfusions [18].

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Pittis et al. [19] showed a reduction in the activity of the monocyte-macrophage system against microorganisms in patients with thalassemia and related it to blood transfusions and iron overload. The author's studying the formation of phagolysosomes in monocytes of peripheral blood revealed a disruption of this process, which partially improved after adding DFO *in vitro*. In contrast, the addition of ferrous sulfate caused the decline of formation phagolysosomes in the control group. Sternbach et al. [20] studied 20 patients undergoing hemotransfusion therapy and revealed a disruption of chemotaxis and phagocytosis of monocyte-macrophage system cells, also a change in Fc-receptor dependent erythrophagocytosis and concluded that multiple blood transfusions adversely affect the functional activity of monocyte-macrophage system as a result of chronic immune stimulation by foreign proteins.

The mechanism above of the changes in the morphofunctional properties of PMN and other phagocytic cells of β -thalassemia patients is as follows. Multiple blood transfusions, iron overload, frequent infectious complications lead to the formation in the blood plasma of patients with thalassemia large number of circulating immune complexes (CIC) like antigen-antibody complex [21]. Phagocytosis and elimination of these complexes is carried by phagocytic cells. On the other hand, PMN of thalassemia patients, along with the cells of the reticulo-endothelial system are involved in the removal of haemolysis products from the body. Consequently, in the blood of patients with thalassemia have a large number of activated phagocytes in the stage of "metabolic explosion". The results above, confirm the increase in the NBT-test in thalassemia, as it is known that only activated cells have the ability to restore NBT. During phagocytosis reaction «*in vitro*» some of PMN and cells of the reticuloendothelial system of thalassemia patients were not be able to take part in this reaction, as some cells have expended their energy and resources to enzymatic digestion and absorption of hemolysis products and CIC. As for cytochemical changes identified in PMN, then in this case, definite role plays the presence of haemolysis products and CIC in blood of patients which lead to an enhanced passing into the bloodstream of young PMN with high enzymatic activity. As long as amplification of intracellular metabolism requires large amounts of energy, glycogen content of PMN decreases.

Beside disruption of the non-specific cellular immunity in patients with thalassemia was also identified significant disruption of specific immunity. In the early 80 years of the last century, the authors of this review revealed changes in lymphocyte subpopulations and their functional activity in patients with thalassemia: a significant decrease in T-lymphocytes and an increase in B-, D-, and 0-cells compared with healthy individuals [8,22,23], the reduction of transformation ability of lymphocytes to the blast in response to the insertion of phytohemagglutinin (PHA) to the culture. It has been suggested that the reason for the selective reduction of the thymus-dependent lymphocytes content in the blood of patients with thalassemia may be the hemosiderosis of thymus, which may affect the production and functions of T-lymphocytes.

Study of subpopulations of T-lymphocytes showed an increase in the number and activity of T-suppressors (CD8), reduction of proliferative volume, the amount and activity of T-helpers (CD4), decrease in the CD4/CD8 ratio, impaired activity of natural killer cells (NK cells) [8,22-29], increase of the amount, differentiation activity and inhibition of B-lymphocytes [8,22-25,28,30].

Israeli researchers found an increase in the number of T-suppressors, reducing the number of T-helpers and a decrease in the activity of natural killers (NK) in patients with thalassemia [29]. Dwyer

et al. [25] investigating the lymphocyte subpopulations at β -thalassemia major, revealed a significant increase in circulating B-lymphocytes, a decrease of T4 cells and the change in the T4/T8 ratio. Khalifa et al. [27] studied 35 patients with β -thalassemia major and revealed a decrease in the number of T-lymphocytes, reduction in the number of T-helpers and decreased helper/suppressor ratio. Similar changes in the distribution of lymphocyte subpopulations identified also researchers from Argentina [28]. Decreasing the number of T-lymphocytes and increasing the number of B-lymphocytes in patients with β -thalassemia major revealed researchers from India [24]. Researchers from Turkey investigating 38 patients with β -thalassemia major revealed abnormal correlation of CD4⁺/CD8⁺ [26]. Scientists from Greece [31] studied 50 patients with β -thalassemia major, received multiple blood transfusions, and revealed a decrease in the total number of T-lymphocytes and T4/T8 ratio decrease mainly due to the decrease of T4 cells. Decrease in helper/suppressor ratio inversely correlated with the number of blood units that transfused and the annual iron balance. The authors concluded that the immunological abnormalities associated with immunological stimulation and an excess of iron, which occur as a result of multiple blood transfusions. German researchers examined 15 thalassemia major patients and identified in some of them increased number of T-lymphocytes, T-helpers and B-cells [30]. The authors attribute these disruptions with nonspecific stimulation of antibody-producing cells as a result of multiple blood transfusions. Akbar et al. [32], identifying patients with β -thalassemia major increase part of B-cells that containing cytoplasmic immunoglobulin, suggested that these cells are terminally differentiated B cells and the cause an increase of these cells are multiple blood transfusions.

Another study by the same group of authors, allowed establishing a decrease in natural killer activity in patients with β -thalassemia major and a negative correlation between NK-activity and the number of transfusions. Pre-incubation of effector cells with iron chelators led to enhance killer activity. These results indicate that the decrease in activity of NK-cells associated with blood transfusions and iron overload [33].

In the study of humoral immunity was found inappropriate secretion of immunoglobulins, which was expressed in increased levels of IgG, IgM, and IgA [19,23,25,34,35] and depressed functioning of the complement system reducing the levels of C3 and C4 complement components [7,19,34].

Considering the increased sensitivity of thalassemia patients to infections, have been studied cytokines-the important components of the inflammation-interleukin-6 (IL-6) and interleukin-8 (IL-8) in the blood plasma in this disease [36]. Found that in patients with thalassemia concentration of IL-6 and IL-8 increased in comparison with the norm and there is a direct positive correlation between the concentration of IL-8, ferritin and total number of transfusions. The authors associated increased levels of cytokines with stimulation of macrophages due to iron overload in patients.

Pathogenesis of immunological disturbances in β -thalassemia. Analysis of the available data in the literature suggests that the immunological abnormalities in patients with thalassemia caused by both disease itself and therapies. We cannot exclude the fact that some patients have immune disorders congenital.

I. The causes leading to changes in the immune system caused by the disease itself:

- Iron overload;
 - Autoimmune stimulation.
- II. The causes due to therapies:

1. Blood transfusion:

- Iron overload;
- Allogeneic stimulation;
- Transfusion infections.

2. Chelation therapy with deferoxamine:

- Yersinia
- Zinc deficiency

3. Splenectomy.

Iron overload: Iron overload is considered the main factor of immune deficiency in β -thalassemia. It is a complication of both the disease and the treatment. It was established that iron and its protein compounds have immunoregulatory characters, and therefore, an excess of iron may adversely affect the immune balance [37]. Numerous studies have shown the negative effect of excess iron on immunological functions, which include suppression of monocyte-macrophage system, changes in subpopulations of T-lymphocytes increasing CD8 and suppression of CD4, increased secretion of immunoglobulins and inhibiting the function of the complement system [16,37-39].

It is shown as *in vitro*, also *in vivo*, that iron plays an important role in the regulation of the expression of surface markers of T-lymphocytes, affecting the expansion of different subpopulations of T-cells and possibly affecting the function of immune cells [37]. Weak ability of lymphocytes to isolate the extra iron into ferritin may also help to explain the reasons of deviation in the immune system in patients with iron overload [37].

PMN and monocyte-macrophage system takes part in the toxic iron purification. Indeed, lysosomes of these cells capable of endocytosis of free iron and ferritin and it contribute to the protection of iron [37]. Additional oxidative stress can destabilize the secondary lysosomes of phagocytic cells, leading to loss of their protective role. In addition, phagocytosis of microorganisms, dyserythropoietic precursors and aging or damaged red blood cells (inside and/or outside the vessel) produces oxidative stress [40]. The fact that the excess of iron has a negative effect on the function of PMN and inhibits phagocytosis indicated above [13,14,16-18].

Observed changes in phagocytic activity due to iron overload is the result of the harmful effect of ferritin bounded iron [17]. However, high serum ferritin levels in thalassemia may cause the formation of antiferritin antibodies, which in turn leads to the formation of CIC [37].

Direct evidence of the influence of iron overload on the deviation of the immune system is the fact that some of the symptoms improve during intensive chelation therapy [17]. Desferoxamine (DFO), being the first iron chelator drug is still used in most cases of thalassemia. However, DFO predisposes to bacterial infections as family Yersinia. Under normal conditions, Yersinias have low pathogenicity, but at the same time, an important characteristic of these bacteria is unusually high requirement for iron. Having receptors for feroxsamine, complex free iron with DFO, in conditions of high iron levels in the body, they

are becoming more and more pathogenic in patients with iron overload treated with DFO [1].

Allogeneic stimulation: Multiple blood transfusions are major pathogenetic mechanisms of immune abnormalities. Currently, transfusion therapy of patients with thalassemia consists of hyperhemotransfusion regime, which aims to maintain pretransfusion hemoglobin level of 100 g/L or higher [1]. Repeated transfusions lead to a continuous allo-antigen stimulation, which leads to disruption of the immune balance in patients [12,15,17,18,20,38-44]. Finally, β -thalassemia itself leads directly to a constant immune stimulation. Decrease of synthesis β -globin chains leads to an excess of α -chains which precipitate in the precursors of erythrocytes, resulting structural changes of the cell membrane. The presence of these abnormal erythrocytes leads to a continuous activation of monocytes that responsible for immune clearance [45]. For this reason, multiple blood transfusions lead to autoimmune hemolysis [44] change of T-and B-lymphocytes [42] and the changing of the functions of monocytes and macrophages.

Recently, Vamvakas and Blajchman [46] in his review of the literature summed positive and negative effects of blood transfusion. Associated with blood transfusion immunomodulation can lead to all the changes of immune system discussed above. Transfusion also leads to a decrease in delayed-type hypersensitivity, by that causing the production of anti-idiotypic anti-clonotype antibodies. Allogenic mononuclear cells and soluble substances which are produced during storage of blood components play the leading role in the pathogenesis of immunomodulation associated with the transfusion. Moreover, soluble peptides of HLA class I, which freely circulate in allogeneic plasma, also contribute to the formation of transfusion related immunomodulation.

Zinc deficiency: Zinc deficiency is a usual feature of thalassemia, may also play a role in the pathophysiology of immunodeficiency [47,48]. Like iron, zinc is an immunoregulator [48]. A low level of zinc in patients with thalassemia is associated with changes in lymphocyte subpopulations and thymulin deficit, which is adjusted after zinc administration [48].

Splenectomy: Splenectomy in thalassemia is carried out at excessive increasing need for blood transfusions [1]. Spleen-reservoir of immunocompetent lymphocytes is primary organ of immunological surveillance. After splenectomy immune system is modified, expressed in quantitative changes of lymphocytes, without any functional disorders [49-51].

Immunotherapy in thalassemia: Numerous data about cellular and humoral immunity disturbance in thalassemia led to ideas about the feasibility of a complex therapy for this disease with immunocorrective drugs. One of the first attempts in the immune complex therapy of patients with thalassemia was the proposal to apply Levamisolum that suggested by azerbaijani scientists [52-54]. Proved the effectiveness of therapy as immunocorrective therapy in thalassemia major [55], as well as the β -thalassemia intermedia [53,54]. Similar results were obtained by researchers from Georgia [52].

Conclusion

Infectious complications are an important part of the clinical spectrum of thalassemia, as one of the leading causes of death in this disease. Increased sensitivity to infections in thalassemia based on immunodeficiency associated with both the development of the disease and the treatment (secondary immunodeficiency). Requires a separate

study for the possibility of primary immunodeficiency in individual carriers of thalassemia genes. Few attempts for immunocorrective therapy in this disease that carried out up to date do not lead to the conclusion that they are sufficiently effective. In recent years developed and introduced into clinical practice number of immunocorrective drugs that could be applied to the treatment of patients with thalassemia. The urgent need for further research in this direction is obvious, which allows determining the effectiveness of the correction of immunity to prevent infectious complications in patients with thalassemia, thereby improving survival and quality of life of the patients.

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