Natural Killer (NK) Cells in β-Thalassemia Major Patients

Maide Gökçe Bekaroğlu1,2, Belkis Atasever Arslan1*

1Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Üsküdar University, Turkey
2Physics Engineering Department, Faculty of Science and Letters, Istanbul Technical University, Turkey

Abstract

β-Thalassemia major is a genetic and severe blood disorder caused by absence of β-globin chain production. Regular blood transfusion treatments can lead to modifications in cytokine levels and lymphocyte subsets. Natural killer (NK) cells are lymphocyte subtypes that are a major part of innate immune responses against infectious pathogens and tumor cells. NK activity is critical for susceptibility of β-Thalassemia major patients against cancer and bacterial infections. This review discusses recent literature focused on this issue. As independent from regular blood transfusions and iron overload, effects of the other cellular and transcriptional factors causing decreased NK activity in β-Thalassemia major patients are still unknown. If NK activity mechanisms and its abnormalities in β-Thalassemia major patients were fully understood, activity rate can be regulated to reduce the risk of cancer and infectious diseases.

INTRODUCTION

Thalassemia is an inherited epidemic disease which is usually seen in Mediterranean region, Middle East countries, India, Far East and tropical Africa. As the population of world increases rapidly, thalassemia has started to be seen in the non-epidemical countries [1]. Three percent of World’s population suffers from heterozygous β-Thalassemia and in general 270 million people are carriers of mutant β-globin gene which can cause thalassemia [2]. Each year more than 300,000 children are born with a β-globin gene disorder [3,4]. It can be said that thalassemia is a very common genetic disorder which can cause mortality and morbidity. Most common causes of death in β-Thalassemia major are heart diseases, infections, liver diseases, and malignancy [5].

Increased susceptibility to infections and cancerous diseases are commonly seen in β-Thalassemia major patients [6,7]. Studies demonstrate that NK activity is decreased in β-Thalassemia major patients [8,9]. In general NK cells play an important role in the destruction of pathogen microorganisms and infected cells especially in the early phase. In addition, NK cells kill tumor cells. They play especially a vital role in defense against cytopathic viruses [10-13]. It is believed that iron overload and splenectomy causes decreased NK activity in β-Thalassemia major patients[14]. However, there are very limited amount of studies about physiological mechanisms of decreased NK activity in β-Thalassemia major patients. Many micro environmental factors causing decreased activity in NK cells are still unknown. If the physiological mechanism underlying for decreased activity of NK cells were known, it could be used to prevent infection and cancer risks in β-Thalassemia major patients.

*Corresponding author
Belkis Atasever Arslan, Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Üsküdar University, Istanbul, Turkey, Tel: +90 216 400 2222; Fax: 90 216 530 06 15; E-mail: belkisatasever.arslan@uskudar.edu.tr
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Reduced synthesis of β-globin chain causes β-Thalassemia [15]. There are 200 mutations in β-globin gene that can cause β-Thalassemia. While some of the mutations block the expression of the β-globin genes totally, some of them just reduce it. If β-globin chains are not completely expressed, it is called β-Thalassemia and when it’s just reduced it is called β-Thalassemia [16]. Absence of β-globin chain production in erythrocytes leads to over production of β-globin chain in β-Thalassemia major patients. β-globin chains are not stable, and excessive β-globin chain production increases the erythrocyte sedimentation rate. Ineffective erythropoiesis leads to apoptosis of erythrocyte precursors in bone marrows and shortens lifetime of erythrocytes [1]. Ineffective erythropoiesis can cause many complications such as anemia, free iron and oxygen radicals in the blood, altered immune responses, osteoporosis and osteopenia, damaged cytoskeletons, etc. [15,17-19]. Without treatment, life expectancy is reduced to several years [20]. Regular blood transfusions, stem-cell transplantations and iron chelation treatments are applied to elongate the life span of β-Thalassemia major patients [21]. In most cases, regular blood transfusions are used to treat thalassemia and its symptoms [20].

**REGULAR BLOOD TRANSFUSIONS AND IRON OVERLOAD**

β-Thalassemia major patients are treated with repeated blood transfusions to prevent anemia [22]. Regular blood transfusions and ineffective erythropoiesis triggering intestinal iron absorption can cause iron overload in β-Thalassemia patients. Regular blood transfusions, done every 2-4 week, lead to approximately 15-20 mg/day iron overload in patients [23-26]. Iron overload is still the main reason for the mortality and morbidity in β-Thalassemia major patients. Because of the reducing properties of free iron in the system; iron overload, due to regular blood transfusions leads to the production of toxic oxygen radicals. These oxygen radicals mediating protein degeneration and lipid peroxidation on cell membrane and organelles causes death of cells and organ failure such as cardiac insufficiency, endocrine abnormalities, and cirrhosis [1-27]. Also, it is shown that regular transfusion treatments decrease NK activity and alter the immune responses in β-Thalassemia major patients [28]. Low activity of NK cells and immune abnormalities reduce the resistance against infections and developing malignancy [25]. Cellular iron homeostasis is important for immune functions. Both iron deficiency and iron overload can affect the immune system. Iron plays a critical role in macrophage mediated cytotoxicity by catalyzing reactive oxygen species production in phagolysosomes [29]. It’s also important for the immune response of mononuclear phagocyte system cells. The increase of unbounded iron to ferritin in cells causes decreasing of effects of IFN on mononuclear phagocytes [30]. Studies show that iron overload is not the only cause for immune abnormalities and the decreased activity of NK cells in β-Thalassemia; also there are many different functional defects in immune system components such as T and B lymphocytes, NK cells, etc. [32]. However, there are not enough studies showing these functional defects in particular.

**Immune system in β-Thalassemia major**

In β-Thalassemia major patients, infections are one of the main reasons of mortality of 12-46% of patients. Recent studies show that gram negative microorganisms are responsible for at least half of the serious bacterial infections in thalassemia patients [32]. In addition, sepsis, soft tissue infections, osteomyelitis, corneal ulcers, enteritises and abscesses on liver, lungs, kidneys, intra abdominal area, retropharynx, gingival...
and hips were reported clinically. It is found that splenectomy and iron chelation therapy are an independent risk factor in the development of infectious diseases [33]. Increased susceptibility to infectious diseases (independent of multiple transfusions) indicates the existence of immune deficiency in β-Thalassemia major patients [32]. NK cell activity plays an important role in immune system homeostasis. Factors that affect NK activity are crucial for optimizing the NK activity. Therefore, these factors are important to achieve immune system homeostasis [34,35,36].

In β-Thalassemia major patients, monocyte and neutrophil activities are declined, whereas polyclonal immunoglobulin synthesis increases and the activity of alternative complement pathway is corrupted. In addition, lymphocyte subgroups have changed functionally and numerically for these patients. It’s also reported that lymphocyte phenotypes of splenectomized patients and their C-reactive protein levels were altered [34]. Also, a different study shows that while NK cell number of children with β-Thalassemia was decreased, their T lymphocytes were increased. It’s also reported that the number of NK cells and transfusion number are inversely proportional. These abnormalities are thought to result from transusions, iron overload or splenectomy [25,34,35]. The primary mechanism causing these immunological modifications is still unknown.

**NK cells and their functions**

The presence of cytotoxic cells against the virus infected cells and tumors were found by Paul Ehrlich 100 years ago. In 1950’s MacFarlane Burnet showed that the immune system plays a role in the regulation of cellular transformation and decreasing tumor growth [37]. The identification of NK cells was made approximately 30 years ago by Rolf Kiessling [38]. In the human body, NK cells form the third subgroup of lymphocytes after T cells and B cells and they constitute the 5-15% of the total lymphocytes in peripheral blood. NK cells are classically known with CD56+ and CD3− surface markers. In addition, natural cytotoxicity receptors, leukocyte marker 7 (Leu-7/CD57), interleukin 2 receptors (IL2Rβ), CD69, several activator receptors bonded to target cell and Killer cell lectin-like receptor B1(KLRB1/CD161) are also identified as NK markers [39-42]. Also in adulthood stage the expression of NKG2A decreases and KIR expression in NK cells increases [43].

Although NK cells are part of innate immunity, also they play a role in adaptive immune response directly via dendritic cells. The interaction between NK cells and dendritic cells provides positively or negatively regulation of dendritic cell activity. Dendritic cells, antigen presenting cells, are important for T cell response. This interaction contributes to feedback regulation of T cell-mediated immune response. On T cell-mediated activation, NK cells are thought to be a regulator both as trigger and suppressor. Because NK cells can costimulate via interacting directly with T lymphocytes, CD40L (CD154) and OX40L ligands expressed by NK cells allows to transmit co-stimulator signals of NK cells to T and B lymphocytes. Thus, a bridge between innate and adaptive immunity is built [44-47]. Immune alterations can affect both the innate and adaptive immune system, these alterations can lead to functional defects in neutrophil chemotaxis, natural killer (NK) function, neutrophil and macrophage phagocytosis [48].

NK cells mediate cytotoxicity and also produce chemokines and inflammatory cytokines such as IFNγ and TNFα [41,47,49]. The killing function of NK cells against the target cell (simultaneously without antigenic stimulation) is decided by the inhibitor and activator signals located on cell surface [50-53]. Inhibitor receptor superfamily (IRS) located on the surface of NK cells prevents from harming the cells expressing self-MHC molecules [45,46]. Decreased MHC class I expression of virus infected cells or tumor cells increases their sensitivity against NK mediated lysis [40,46]. As a result, NK cells kill the cells which are absent or ineffectively expressed of MHC class I molecules [54]. Also, when bone marrow transplant procedure is used to cure thalassemia, NK cells play an important role in bone marrow transplantation and preventing graft-versus-host disease [39,14].

The other important effector function of NK cells is that they can induce inflammatory responses by secreting proinflammatory cytokines and chemokines [55]. These cytokines are: IFNγ, TNFα, lymphotoxins (LTαG-CSF, IL5, IL13, IL10 macrophage inflammatory protein (MIP-1α and β) CCL3[41,13,46,53,31]. NK cells are important for immune regulation, when these cells are stimulated by IL12/L1, IL12/IL15 or IL12/IL18, they secrete IFNγ, TNFα, IL10, IL13 and GM-CSF[28=13]. IFNγ is an essential cytokine, which has a central role in immune defense. IFNγ plays also an important role for regulation of hundreds of genes, which are related with immune system functions and resistance against infections. IFNγ, activates macrophage and neutrophils, stimulates the alienation of Th1 CD4+ lymphocytes, enhances antigen presenting by increasing the expression of MHC, and this leads to the interactions between IgG and B cells. Thereby IFNγ has a binding function on adaptive immunity [38, 41]. While IL2, IL12, IL15, IL18, TNFα and IFNα/β contributes to the activation of NK cells; IL4, IL10 and TGFβ suppresses NK cell activity. IL12 and IL18 are important for triggering immune response. The secretion of IL12 and IL18 increases IFNγ level [13,38,41]. NK cells trigger dendritic cells to secrete IL18. This cytokine does not increase cytoplastic activation of NK cells as distinct from other helper factors increasing NK activation but causes differentiation of NK cells to help the activation [57]. IL2, IL18 or TLR2 ligands cause activation dependent on NF-κB in NK cells. Natural cytotoxicity receptor (NKP30) causes a fast degradation of I-kβ and NF-κB nuclear translocation for NK activation [58]. Receptors like NKP30, NKP44 and NKP46 are important for NK cells to mediate cytotoxicity [59], but ligands of these receptors are not known completely.

**NK cell activity in β-Thalassemia Major**

Most of the studies show that NK activity is decreased in β-Thalassemia major patients [25,55,60]. However cellular mechanism and transcriptional regulation of NK cells of those patients are still unknown. There are limited numbers of articles on NK activity in β-Thalassemia major patients [61-64]. Some studies show, that, In vitro conditions, desferrioxamine or 2,3-dihydroxybenzoic acid (DHB) chelators can increase the activity of NK cells [55,65]. Also, it is demonstrated that Vitamin C increases the activity of NK cells [61-64]. Some other studies show that NKp30 and aberrant secretion of IL10, TGFβ1 and IL15 cytokines are possible major reasons of underlying mechanism of decreased NK activity in β-Thalassemia Major patients [66]. So, it can be
said that NK cell activity is not only dependent to environmental factors; there can be a defect at the physiological mechanisms of NK cells in β-Thalassemia major patients. Also, these studies show that lower NK activity of β-Thalassemia patients caused by iron overload and oxidative stress can be modulated.

**SUMMARY AND FUTURE OUTLOOK**

It is mentioned that β-Thalassemia major is a severe disease which can lead to infection and cancer related mortality and morbidity. This condition indicates that there are some problems in the immune system. Previously it is shown that NK cell activity is an important part of the adaptive and innate immune system and factors that affect NK activity can also affect immune responses against cancer and infectious diseases. Many studies show that NK activity is decreased in β-Thalassemia major patients. Decreased NK cell activity is a serious problem which must be fixed in order to prevent infectious diseases and cancer for these patients. Until this day iron overload and splenectomy was blamed for the decreased activity of NK cells, but cellular mechanisms and transcriptional regulations of NK cells are still unknown. There are not many studies searching these unknown mechanisms. It is stated before that some of these few studies showed that DHB chelators and vitamin C can increase NK activity for β-Thalassemia major patients. Also, NKP30 receptors and IL10, TGFβ1 and IL15 cytokine secretions were stated unusual for β-Thalassemia major patients. Many other micro environmental factors are still unknown. Considering the network of mononuclear cells, not only regular blood transfusion and iron overload causes decreased NK activity; also other mononuclear cells may affect NK activity or iron overload can affect indirectly NK activity through other mononuclear cells. If the physiological mechanisms underlying for decreased activity of NK cells were known, it could be used to modulate NK activity and prevent infection and cancer risks and improve life standards of β-Thalassemia major patients. The answer of these questions is important to develop new strategies against the disease and its complications.

**REFERENCES**

26. Kontoghiorghes GJ. Iron mobilization from transferrin and non-

