Review article

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CD4$^+$ T CELL PROFILES IN AUTOIMMUNE HEMOLYTIC ANEMIA

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Autoimmune hemolytic anemia (AIHA) is an immune-mediated disorder characterized by the reduced lifespan of red blood cells (RBCs) due to enhanced intravascular and extravascular destruction. Traditionally, the immunopathogenesis of AIHA has been considered in the context of the immunological tolerance breakdown of B cells, since the autoantibodies are the main disease mediators. However, more recent data suggest that the production of anti-RBC antibodies by B cells is only an epiphenomenon and that the tolerance breakdown in the CD4$^+$ T cell compartment is a key point in early AIHA development. In AIHA, there are numerical and functional alterations of the essential CD4$^+$ T cell subpopulations, including Th1, Th2, Th17, regulatory T cells and follicular helper T cells. In this review, the main characteristics of the cellular immune response during the development of AIHA, as well as the potential mechanisms by which CD4$^+$ T cells promote the initiation and maintenance of the autoimmune process, are summarized. Identification of these characteristics and mechanisms would be of practical importance in the therapeutic sense because it opens up the possibility of designing more specific immunotherapy that is still not available for AIHA patients.

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AIHA pathophysiology

Autoimmune hemolytic anemia (AIHA) is an immune-mediated disorder characterized by the reduced lifespan of red blood cells (RBCs) due to enhanced intravascular and extravascular destruction. The main pathological feature of AIHA is the production of autoantibodies directed against different RBC antigens, which are only the reflection of much profound dysregulation of immune mechanisms involving multiple cell types.

Based on the clinical features and laboratory findings, there are three basic types of AIHA: worm AIHA, cold AIHA, and mixed-type AIHA (1). These different types of AIHA are usually distinguished by the use of direct antiglobulin test (DAT), also known as direct Coombs test, which detects autoantibodies and/or components of the complement system (C3d most commonly) on the surface of the RBCs.

Worm AIHA (wAIHA) is the most common type, comprising 70%–80% of all adult cases and 50% of childhood cases (2). It is named after the autoantibodies that optimally react with RBCs at 37 ºC, worm agglutinins. These antibodies are mainly of IgG class and polyclonal by nature—directed against different RBC membrane proteins, such as the Rh antigen system but also the antigens of Kell, Kidd, Duffy, and Diego blood group systems (3). When attached, these antibodies facilitate and accelerate RBC phagocytosis by spleen macrophages that express multiple receptors for the Fc fragment of IgG antibodies (FcγR), thus leading to extravascular hemolysis. However, more often, only the parts of the RBC membrane are phagocytized but not their cytoplasm, which disrupts their typical, discoid biconcave appearance, i.e., RBCs become smaller in size and assume a more spherical shape, which is designated as spherocytosis. Spherocytes are subjected to further degradation during their re-passage through the spleen. Antibody-dependent cellular cytotoxicity (ADCC) involving NK cells and CD8$^+$ T cells is also proposed as a possible mechanism of extravascular RBC destruction in the spleen and other secondary lymphoid organs. IgG antibodies are able to activate the complement cascade by classical pathway; however, to a much lesser extent compared to IgM antibodies. During this process, the C3b

component of the complement is formed and deposed at the RBC surface, rendering them as a target for phagocytosis by complement receptor (CR) expressing Kupffer cells in the liver. Besides extravascular hemolysis, which is the dominant form of hemolysis in wAIHA, intravascular hemolysis can also occur due to the formation of membrane attack complex, the end product of the complement system activation, which perforates cell membrane of circulating RBCs (4). Based on etiology, wAIHA can be classified as primary, idiopathic, when it is presented as an isolated clinical syndrome (no underlying disorder can be identified) or as secondary when it is associated with various other disorders including lymphoid malignancies (chronic lymphocytic leukemia, non-Hodgkin's lymphoma), autoimmune disorders (systemic lupus erythematosus and other systemic autoimmune disorders of connective tissue), immunodeficiencies, viral infections, drugs etc. Considering these pathological features of wAIHA, DAT is positive for IgG only or IgG and C3d complement component (1, 4, 5).

Cold AIHA (cAIHA) encompasses three basic clinical entities: cold agglutinin disease (CAD), secondary cold agglutinin syndrome (CAS), and paroxysmal cold hemoglobinuria (PCH). Generally, these entities are characterized by the presence of autoantibodies that react with RBCs at lower temperatures, which is why they are designated as cold agglutinins (4).

CAD is the second most common form of AIHA (20%–25%), characterized by the production of autoantibodies that optimally react with RBCs at 4 ºC; however, these cold agglutinins can be also functional at higher temperatures, exceeding 28 ºC to 30 ºC, what actually makes them pathogenic. They are mainly of IgM class, in the form of pentameric or hexameric macromolecules, and monospecific, directed against the I/i antigen system of the RBCs (4). The monoclonality of these antibodies suggests underlying clonal B-cell lymphoproliferative disorder, which was recognized by the World Health Organization (WHO) as a distinct lymphoid neoplasm and a special type of monoclonal gammopathy in 2022 (6). In the distal, acral areas of the body, such as fingers, toes, nose, and ears, the temperature of the blood is lower than in the central parts, thus allowing IgM molecules to attach to the RBC surface and cause their agglutination. Being the most potent complement activator, IgM leads to the excessive formation of the C3b component which also deposes on the RBC surface. When the blood temperature rises again, in the central parts of the body, IgM detaches from the RBCs allowing them to separate from each other, but C3b molecules remain bound to their surface. A portion of C3b-coated RBCs is recognized by the cells of the mononuclear phagocytic system, predominantly by the Kupffer cells of the liver, and removed from the circulation. In this case, the entire RBCs are phagocytized rather than the parts of their membrane, which is why spherocytosis is rarely seen in CAD. On the other hand, C3b can be enzymatically cleaved to C3d, which allows RBCs to survive. In the circulation, complement activation at the RBC surface can proceed to the final phase, resulting in the formation of membrane attack complex and intravascular hemolysis. In CAD, DAT is IgG negative but C3d positive and typically, a spontaneous RBC agglutination occurs at room temperature (4, 7).

In contrast to CAD, CAS develops in the context of various other diseases. It is also characterized by the presence of cold reacting autoantibodies mainly of IgM class, however, these antibodies can be both monoclonal if CAS accompanies lymphoid malignancy (non-Hodgkin lymphoma, Waldenström macroglobulinemia) and polyclonal if it is associated with infections (M. pneumoniae, Epstein-Barr virus) or autoimmune disorders (systemic lupus erythematosus, rheumatoid arthritis) (4, 7).

PCH is a rare form of cAIHA mainly affecting children, which almost always arises as a postinfectious complication (8). It is mediated by Donath-Landsteiner hemolysins which are cold reacting, polyclonal IgG antibodies directed against the P antigen of RBCs. These antibodies are biphasic by nature, meaning that they attach to the RBC surface at lower temperatures and fix the components of the complement system; however, at high temperatures (37 ºC) IgG detaches, but complement components then become active, causing massive intravascular hemolysis. In contrast to CAD and CAS, in PCH there is no RBC agglutination and the hemolysis is completely complement-dependent (4).

Mixed type AIHA (mAIHA) is defined by the presence of both warm and cold agglutinins. In this case, DAT is positive for IgG and C3d; however, cold IgM agglutinins with high thermal amplitude are also present in high titers. They cause spontaneous agglutination at 20 °C which diagnostically differentiates this condition from IgG^+C3d^+ wAIHA (9). Due to the difficulties in the diagnosis, particularly, clinically irrelevant cold agglutinins with a low thermal amplitude that can also be present in patients with wAIHA as well as in healthy persons; mAIHA is probably much less common than it was previously believed (10).

In some patients with wAIHA, autoantibodies of the IgA class can be found in addition to IgG and IgM molecules; however, there are also very rare cases of IgA exclusively mediated wAIHA. They are characterized by severe clinical presentation, refractoriness to glucocorticosteroid therapy and interestingly, very little free autoantibodies detectable in the serum, probably due to their high RBC binding affinity (11, 12). The precise mechanism of hemolysis in this type of wAIHA is still not clear; however, massive hemagglutination and subsequent RBC sequestration in spleen is often seen. It appears that complement activation, FcαRI mediated phagocytosis, ADCC, and RBC apoptosis due to

membrane alterations do not play substantial role in RBC destruction (12).

CD4+ T cells in AIHA pathogenesis

Traditionally, the immunopathogenesis of AIHA has been considered in the context of the immunological tolerance breakdown of B cells, since the autoantibodies are the main disease mediators. However, the production of RBC-specific antibodies by B cells is only an epiphenomenon, caused by more profound immune dysregulation involving multiple other cell types. From the immunological point of view, AIHA can be classified as either IgG or IgM mediated, taking into account the different immune mechanisms that mediate the production of these classes of antibodies as well as the mechanisms of RBC destruction. Namely, B cells need to cooperate with antigen-specific $CD4^+$ T cells to undergo the process of immunoglobulin class switching and produce antibodies of the IgG class. IgG antibodies mainly induce hemolysis by FcγR-mediated phagocytosis or cytotoxicity. On the other hand, although IgM antibody production is a T cell-independent process, $CD4^+$ T cells also have the ability to facilitate B cells production of this antibody class (13). In this case, hemolysis is mainly complement dependent. Therefore, RBC-specific $CD4^+$ T cells are of particular importance during AIHA pathogenesis, especially wAIHA which is the most common disease type. Accordingly, it has been demonstrated on a murine model, that RBC-specific B cells are constantly present in healthy animals and are able to respond to $CD4^+$ T cell stimulation by autoantibody production. On the contrary, RBC-specific $CD4^+$ T cells, although also present in healthy animals, following antigen stimulation become functionally non-responsive, and anergic. Taken together, these findings suggest that the breakdown of immunological tolerance in the T, but not the B cell compartment is actually a key point in early AIHA development (14). Also, splenic T cells isolated from New Zealand Black (NZB) mice which spontaneously develop AIHA, underwent an extensive clonal expansion *in vitro* after RBC antigen challenging, predominantly in the $CD4^+$ T cell compartment (15). In the same model, anti-CD4 monoclonal antibody treatment was able to prevent and abrogate the production of RBC-specific antibodies, but not the development of anemia, indicating that $CD4^+$ T cells are crucial for the autoantibody production but also normal erythropoiesis (16–18). Similar findings were reported in humans. In contrast to healthy donors, T cells of AIHA patients were able to mount recall response to Rh protein epitopes *in vitro*. This effect was abrogated by anti-HLA-DR antibodies, specifying that $CD4^+$ T cells were the main responding cell population, since their process of antigen recognition is HLA class II-restricted (19). Recently, the expansion of a special subset of $CD4^+$ T cells ($CD3^+CD4^+CD28^-$) was documented in patients with idiopathic wAIHA (20). This T cell subset is highly pro-inflammatory profiled and interestingly has cytotoxic capacities due to the secretion of cytolytic enzymes, perforin, and granzyme A and B; therefore, besides the supportive role, $CD4^+$ T cells could be also directly involved in RBC destruction (20, 21).

Phenotypically, $CD4^+$ T cells are a very heterogeneous cell population and depending on the microenvironment conditions, they can differentiate into diverse polarization profiles, classified as Th1, Th2, Th17, Th9, Th22, regulatory T cells and follicular helper T cells. Each of these $CD4^+$ T cell subsets has unique characteristics including the master transcription factor, cytokine profile and specific functions.

While the importance of $CD4^+$ T cells in the pathogenesis of AIHA is currently being intensively studied, there are still many unknowns related to the precise phenotypic characteristics that determine the pathogenicity of these cells during the disease development. Identification of these characteristics as well as the mechanisms by which $CD4^+$ T cells promote the disease onset and maintenance would be of practical importance in the therapeutic sense because it opens up the possibility of designing more specific immunotherapy that is still not available for AIHA patients.

Th1 and Th2 cells in AIHA

Historically, the pathogenesis of most autoimmune disorders has been discussed in the context of a disturbed balance between Th1 and Th2 cellular immune responses, bearing in mind that these are the first two identified subtypes of $CD4^+$ T cells. Th1 differentiation program is initiated by IL-12 and/or IFN-γ, and it involves the activation of T-bet as the master transcription factor. Fully differentiated Th1 cells produce large quantities of IFN-γ, but also IL-2, TNF-α and lymphotoxin (TNF-β), thus promoting cell-mediated immunity primarily by inducing phenotype switch of phagocytes towards pro-inflammatory M1 phenotype. On the other hand, Th2 cells require IL-4 and the expression of GATA-3 as the master transcription factor for complete differentiation and produce cytokines IL-4, IL-5, IL-9, IL-10, and IL-13. Generally, this T cell lineage promotes antibody-mediated immunity by stimulating B cell antibody production but also promotes the development of M2 phenotype of macrophages that is associated with tissue regeneration and fibrosis (22, 23).

Pioneering studies investigating Th profiles in the pathogenesis of AIHA supported the notion that AIHA is a predominantly Th2-mediated disease. Namely, in basal conditions, peripheral blood mononuclear cells (PBMCs) isolated from AIHA patients produced a significantly higher amount of IL-4, a signature cytokine of Th2 cells, and less IFN-γ, a signature cytokine of Th1 cells, compared with healthy controls. When artificially

stimulated, AIHA PBMC cultures also behaved differently in the sense of higher IL-10 production and lower production of IL-12, which further favored Th2 polarization and the inhibition of IFN-γ production (24). Similar results were obtained on the whole blood cultures of AIHA patients, which following mitogen stimulation showed an increase in the production of Th2 profiled cytokines (IL-4, IL-10 and IL-13) and reduced IFN-γ production, that was more pronounced in AIHA patients with active hemolysis. Interestingly, the addition of Th2 cytokines into the cell cultures increased both autoantibody production and their binding to autologous RBCs (25). In order to minimize individual variation in cytokine production, *Kruizinga et al*. (2018) calculated the Th1/Th2 cytokine ratio in the serum of hematopoietic stem cell transplanted patients with autoimmune cytopenias, including AIHA, and also found a more pronounced Th2 cytokine profile compared with control subjects without such complications (26). Moreover, some therapeutic strategies that proved efficient, especially in wAIHA treatment, such as low doses of rituximab together with a short course of corticosteroids, were able to partially restore Th1/Th2 balance by enhancing the levels of IFN-γ, IL-12, and TNF-α and transitory reduction of IL-4 production (27).

The very origin of the Th2 immunity dominance in the pathogenesis of AIHA is still unclear, however, recently increased levels of the cytokine IL-33 have been reported in patients with wAIHA (28). Initially, this cytokine was identified as a strong inducer of Th2 cell differentiation; although more recent studies have shown that IL-33 also has stimulatory effects on a number of other cells, including Treg and Th1 cells (29). The levels of IL-33 were positively correlated with disease activity (measured by the number of reticulocytes and the levels of hemoglobin and lactate dehydrogenase (LDH)) but also with the production of anti-RBC antibodies. In PBMC cultures isolated from the patients with active AIHA, IL-33 promoted anti-RBC antibody production in a dose-dependent manner, mainly by increasing the production of the Th2 cytokines IL-4, IL-6, and IL-13 (28). RBCs contain a substantial amount of IL-33, and due to hemolysis, they can be a significant source of this cytokine during AIHA development (30).

Considering the physiological functions of Th2 cells in promoting humoral immunity, data supporting the importance of Th2 polarization during AIHA pathogenesis appear to be expected and logical. However, certain facts do not fit into the concept of AIHA as a classical Th2-mediated disease.

Firstly, the signature cytokine of Th2 cells, IL-4 as well as IL-13 preferably stimulate the production of antibodies of the IgE and IgG4 class, which do not fix the complement (31), whereas the complement is an important factor predominantly during cAIHA, but also at a lesser extent wAIHA pathogenesis (4, 32). In fact, in wAIHA, anti-RBC antibodies are most often of the IgG1 and IgG3 subclass, the latter being the most effective in RBC destruction (33, 34). In humans, such antibody isotype switching pattern is primarily associated with biological functions of IL-10 (35), which levels were indeed found to be elevated in patients with AIHA (24, 36, 37). Based on the mice studies, IL-10 was originally included in the Th2 cytokine palette; however, in humans, this cytokine can be produced by different types of immune cells including monocytes, macrophages, DCs, NK cells, B cells, T regulatory (Treg) cells, even Th1 and Th17 cells (38). Consistent with this, the higher basal production of IL-10 was observed in the monocyte cultures obtained from AIHA patients (39). Additionally, immune complexes (including IgG-coated RBCs) have the ability to polarize macrophages towards a specific M2b phenotype, which differs from the M2a phenotype typically induced by Th2 cells (40). M2b macrophages are characterized by extensive IL-10 production and the absence of IL-12 secretion, and their pathogenicity is well documented in the settings of systemic lupus erythematosus (SLE) (40, 41). Therefore, the importance of IL-10 in the pathogenesis of AIHA, as suggested by some authors (24, 25, 42), cannot be strictly attributed to Th2 cell dominance.

Secondly, FcγR-mediated phagocytosis is the most important mechanism of RBC destruction in wAIHA (43). In monocytes, IL-4 down-regulated the expression of stimulatory FcγRs (FcγRI, FcγRIIa, and FcγRIIIa), while stimulating the expression of inhibitory FcγRIIb and thus actually compromised their ability to internalize IgG coated particles (44, 45). In contrast, peripheral blood monocytes of AIHA patients showed higher expression levels of FcγRI compared with healthy controls (46). On the other hand, in cAIHA, the dominant mechanism of hemolysis was CR-mediated phagocytosis of C3b-coated RBCs (4). Th2 cytokines, IL-4 and IL-13 were also found to down-regulate the expression of immunoglobulin superfamily complement receptor (CRIg) and decrease the ability of macrophages to phagocytize complement opsonized particles (47). Considering these data, one could speculate that Th2 cells actually have a protective function during the development of AIHA as they reduce the degree of RBC hemolysis.

Third, AIHA does not show the typical features of classical Th2-mediated inflammation, such as atopic reactions and parasite infestation, in which, in addition to an increased titer of IgE antibodies, there is also an increased infiltration and activation of eosinophils. By producing IL-5, Th2 cells support eosinophil hematopoiesis in the bone marrow, while IL-4 and IL-13 induce the expression of eotaxin I (CCL11) in stromal cells and thus enhance eosinophilic infiltration. However, the cooperation between Th2 cells and eosinophils appears to be much more complex in nature and of particular importance in initiating and maintaining the Th2 immune response (48,

49). To our current knowledge, there are only a few reported cases of the coexistence of AIHA and eosinophilia in the literature and their interconnection was not investigated (50–52).

Although most studies point to Th2 cell polarization, it is clear that the pathogenesis of AIHA cannot be explained by a simplistic model of Th1/Th2 imbalance. In concordance to that, new subtypes of $CD4^+$ T cells were described as well as many different intermediate phenotypes, which have also been shown to play an important role during the AIHA onset and development.

Th17 and Treg cells in AIHA

The established Th1/Th2 paradigm was questioned by the discovery of Th17 cells, which were shown to mediate protection against fungi and extracellular bacteria, but at the same time have an important role in the development of various autoimmune diseases. This subpopulation of $CD4^+$ T cells is characterized by the production of cytokines IL-17, IL-22, IL-21, and IL-26, expression of RORγt as a master transcription factor, while their differentiation program, although still enigmatic, most likely includes IL-6 and TGF-β for initial Th17 development, and IL-21, IL-1β, and IL-23 for phenotype stabilization (53).

The first evidence to suggest the pathogenicity of Th17 cells in AIHA was based on higher levels of their signature cytokine, IL-17 in the patient's serum, which closely correlated with disease activity (54, 55). Moreover, PBMCs of AIHA patients when co-cultured with autologous RBCs as well as Rh peptides produced significantly higher amount of IL-17 compared with healthy controls (55). These results were reconfirmed by the work of Xu et al. (2012), who also detected elevated frequency of Th17 cells in the patient's blood. In this study, both Th17 cell frequency and serum IL-17 levels were closely correlated with the levels of anti-RBC antibodies, hemoglobin, the C3 component, and the activity of LDH (56). The important role of Th17 cells during disease development was additionally emphasized in the murine models of AIHA. Adoptive transfer of Th17 cells purified from AIHA mice was able to induce a higher incidence and more severe clinical presentation of AIHA in healthy animals compared with the adoptive transfer of Th0 cells. IL-17 was proposed to be the main mediator of Th17 cell pathogenicity, considering a significantly lower incidence of AIHA induction in the animals pretreated with IL-17 neutralizing antibodies or in *IL-17*$^{-/-}$ animals (56).

Treg cells represent a special subset of $CD4^+$ T cells that mediates the suppression of the inflammatory response and the establishment of immunological tolerance. In addition to CD4, these cells express CD25 and FoxP3 as the master transcription factor, and these molecules are the most commonly used identification markers of so-called conventional Treg cells. However, there is a whole spectrum of phenotypically different cells, some of which do not express FoxP3 but perform immunosuppressive functions, and they are designated as non-conventional Treg cells. IL-2 is required for TGF-β to induce FoxP3 expression and the differentiation program of conventional Treg cells (57). When activated, these cells achieve immunosuppression by different mechanisms including the secretion of immunomodulatory cytokines IL-10, IL-35 and TGF-β, the expression of inhibitory molecules PD-1L and CTLA-4, IL-2 deprivation and the generation of cAMP and adenosine (58).

A significant role of Treg cells during AIHA pathogenesis was suggested in the Marshall-Clarke and Playfair model of murine AIHA. Specifically, mice pretreated with anti CD25 antibody, that depletes Treg cells, had a much higher incidence of AIHA following rat RBC immunization. Additionally, the adopted transfer of splenic $CD4^+CD25^+$ cells from AIHA mice was able to prevent the production of anti-RBC antibodies in healthy mice following immunization with rat RBCs. This effect was not observed when the population of splenic $CD4^+CD25^-$ cells was adoptively transferred (59). In IL-2αR deficient mice which develop systemic autoimmune disease and the lethal form of AIHA, decreased number and impaired function of $CD8^+$ Treg cells was found to be a decisive factor for the early appearance of a more severe form of the disease (60). In humans, reduced percentage of conventional Treg cells in the blood of AIHA patient was detected, and their number was closely associated with the parameters of hemolysis: reticulocytes and haptoglobin (36). Another indirect support of Treg cell role in AIHA control, comes from the frequent occurrence of autoimmune hemolysis after treatment with purine analogs, especially fludarabine (61). Fludarabine has excessive toxicity to T cells, among which are Treg cells as well. According to some authors, this could be beneficial in immune reconstitution during treatment (62), but the depletion of Treg cells might consequently lead to immune tolerance disruption and autoimmune hemolysis.

Although perform completely different functions, Th17 and Treg cells share a common differentiation factor, and that is TGF-β, meaning that additional microenvironment factors are crucial in deciding the final fate of the naïve $CD4^+$ T cell differentiation process. More specifically, TGF-β in combination with IL-2 promotes Treg differentiation; whereas in combination with IL-6 drives the Th17 differentiation program (53, 57). Higher serum levels of TGF-β were found in AIHA patients (63,64), as well as higher frequency of TGF-β single nucleotide polymorphisms (SNPs) associated with enhanced production of this cytokine, especially in patients with more severe clinical presentation of the disease (65). Additionally, in the mitogen-stimulated whole blood cultures, TGF-β production was significantly

increased whereas IL-2 secretion was reduced in patients with the active form of AIHA compared with non-haemolytic AIHA patients (25). This cytokine milieu generally favors the generation of Th17 cells and, thus, may be the cause of the disturbed balance between Th17 and Treg cells observed in AIHA patients.

T follicular helper cells in AIHA

T follicular helper (Tfh) cells are particularly interesting from the aspect of AIHA pathogenesis, bearing in mind that these cells provide necessary signals for B cell maturation, but also stimulate antibody production, class switching and affinity maturation. Due to the specific pattern of chemokine receptor expression ($CXCR5^+CCR7^-$), Tfh cells migrate towards B cell-rich zones; where they regulate B cell maturation and differentiation by the expression of both co-stimulatory (ICOS, CD40L) and inhibitory (PD-1) molecules and the production of IL-21. The differentiation of this unique $CD4^+$ T cell subset is dependent on IL-6 and ICOSL signaling, while B cell lymphoma 6 (Bcl-6) is identified as the master transcription factor (66). Recently, T follicular regulatory (Tfr) cells have been described which, in addition to ICOS, CD40L, PD1, and Bcl-6, express markers of Treg cells, CD25 and FoxP3, and thus are specialized in suppressing Tfh mediated B cell activation and antibody production (67, 68).

The disturbed balance between Tfh and Tfr cells was previously implicated in the pathogenesis of various autoimmune disorders including rheumatoid arthritis, SLE, Sjögren's syndrome and others (68). In the mouse model of AIHA, a higher frequency of $CD4^+CXCR5^+CD25^-$ Tfh cells was documented in autoantibody-positive mice, as well as a high ratio of Tfh/Tfr cells. Moreover, the adoptive transfer of $CD4^+CXCR5^+CD25^-$ T cells, but not $CD4^+CXCR5^-CD25^-$ T cells, was able to promote the induction of autoantibody production (69). In antibody-positive mice, serum levels of Tfh-associated cytokines IL-6 and IL-21 were also found to be elevated as well as the T cell mRNA expression of IL-21 and transcription factor Bcl-6 (69). In IL-2 deficient BALB/c mice which early develop a lethal form of AIHA, a higher number of $CD4^+$ but also specific $CD8^+$ Tfh cells was documented, considering that IL-2 is a negative regulator of the differentiation program of this T cell subset (70, 71). In humans, the expansion of the circulating Tfh cell population was reported in the peripheral blood of AIHA patients (72). Besides germinal center B cells, Tfh and Tfr cells also regulate the development and the activity of regulatory B (Breg) cells which perform immunosuppressive functions (73). Previously, it has been shown that Breg cells have a significant role during autoimmunity development (74); however, we were unable to find studies investigating the importance of Breg cells in the pathogenesis of AIHA.

Conclusion

Today, the importance of $CD4^+$ T cells in the pathogenesis of AIHA, especially wAIHA, is indisputable; however, there are many conflicting results regarding the phenotypic characteristics of these cells. The reason for this discrepancy may be the pronounced heterogeneity of the study designs and participant inclusion criteria, as well as the differences in the animal models of AIHA used to study the disease. On the other hand, AIHA per se is a very heterogeneous disease, so there may be several different mechanisms that result in the production of anti-RBC antibodies, depending on the cause of the disease itself. In general, the altered $CD4^+$ T cell compartment is in the background of pathological B cell activation and the production of autoantibodies therefore should be considered as a potential target of future therapeutic strategies. To achieve this goal, additional studies are needed that will determine the precise pathogenic profile of $CD4^+$ T cells active during the onset of AIHA.

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CD4⁺ T ĆELIJSKI PROFILI U AUTOIMUNOJ HEMOLITIČNOJ ANEMIJI

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Autoimuna hemolitična anemija (AIHA) predstavlja imunoposredovanu bolest koju karakteriše skraćenje životnog veka eritrocita usled pojačane intravaskularne i ekstravaskularne destrukcije. Tradicionalno, imunopatogeneza AIHA sagledavala se u kontekstu prekida imunske tolerancije B ćelija, budući da su autoantitela osnovni medijatori bolesti. Međutim, skorašnji podaci sugerišu da je produkcija antieritrocitnih antitela od strane B ćelija samo epifenomen i da je prekid tolerancije u odeljku CD4⁺ T ćelija zapravo centralni događaj u ranom razvoju AIHA. U AIHA postoje i numeričke i funkcionalne alteracije osnovnih subpopulacija CD4⁺ T ćelija, uključujući Th1, Th2, Th17, regulatorne T ćelije, kao i folikularne pomoćničke T ćelije. U ovom preglednom radu prikazane su osnovne karakteristike celularnog imunskog odgovora tokom razvoja AIHA, kao i potencijalni mehanizmi kojima CD4⁺ T ćelije promovišu inicijaciju i održanje autoimunog procesa. S obzirom na to da otvara mogućnost dizajniranja specifične imunoterapije, još uvek nedostupne bolesnicima sa AIHA, definisanje ovih karakteristika i mehanizama bilo bi od praktičnog značaja u terapiji.

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***Ključne reči**: autoimuna hemolitična anemija, eritrociti, Th1 ćelija, Th2 ćelija, Th17 ćelija, Treg ćelija, Tfh ćelija*