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REVIEW ARTICLE

Immunopathology of Autoimmune Hemolytic Anemia: A Systematic Review of Molecular Mechanisms and Emerging Therapeutic Strategies

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Abstract

Autoimmune hemolytic anemia (AIHA) has become recognised as a complex and heterogeneous disease characterised by a wide range of immunopathological and biochemical mechanisms. Several treatment options exist in the field of AIHA however; there is as yet little or no evidence of therapeutic strategies targeted on molecular aspects of the disease. An up-to-date comprehensive systematic review covering immunopathology for emerging areas of experimental therapy of AIHA will help direct future research and optimize clinical decisions, by focusing on issues needing improvement. This structured systematic review will focus on the immunopathological molecular mechanisms and the most recent emerging therapeutic strategies for AIHA or emerging therapeutic strategies targeted at the molecular mechanisms of the disease. The aim of the review is to collect, critically evaluate, and systematically summarise all available evidence on the immunopathology and experimental therapy of AIHA. For the immunopathology of AIHA, the main issues of interest are: the initiation and development of the disease, the immunological and biological mechanisms of red blood cell destruction, diagnostic markers predicting the response to therapy, the relationship with other autoimmune disorders and aspects of the genetics of AIHA. Experimental therapy will include newly applied or planned treatment protocols and compounds, or medications presenting disease-modifying and non-conventional mechanisms of action. This review will be oriented toward researchers and clinicians engaged in the management and research of AIHA, and results will be analysed and presented in light of their potential to translate findings into practice. Eligibility for inclusion criteria, salience assessment, and data extraction analysis aims to critically synthesize available data, identify research gaps and assess the quality of evidence. Substantial advances in the comprehension of the immunopathology of the disorder or the emergence of new advanced science verified drugs and other compounds in therapy will be explored. Summarised results will point toward research fields most urgently needing further investigation and provide a comprehensive synthesis of evidence helpful for the optimization of clinical decisions and the initiation of innovative therapies.

Keywords: Immunopathology; Autoimmune Hemolytic Anemia; Therapeutic Strategies

1 Introduction

Autoimmune hemolytic anemia (AIHA) develops due to reactive immune mechanisms directed against the red blood cells' (RBCs) antigen or involving autoantibodies on RBCs either by itself or associated with pathogens or malignant cells. AIHA includes a variety of syndromes with the presence of autoantibodies with or without complement, or the involvement of complex pathway activation in specific clinical settings. AIHAs can be divided into warm and cold, while cold AIHA can manifest as cold agglutinin disease or paroxysmal cold hemoglobinuria [1]. Due to the complexity of interactions among the immune system components, and between the immune system and the varied antigens on and in the RBC membrane, AIHAs still feature many unanswered questions. Early diagnosis and accurate subclassification of AIHA are of paramount importance to know the best management approach. Most cases of AIHA remain idiopathic, with no associated disorder found after a careful search, while others are found in association with diseases as varied as systemic lupus erythematosus or lymphoproliferative disorders, mainly chronic lymphocytic leukemia and non-Hodgkin lymphoma. The prevalence of AIHA in the United States is 17.5 cases per million per year with the prevalence in women being four per million per year, while the prevalence in patients older than 60 years is 36.0 cases per million per year. AIHAs can become a therapeutic challenge especially when they are refractory or relapsed cases with poor prognosis. Ongoing research efforts are focused on understanding immunopathology for the development of effective and safe personalized therapeutic strategies with ruxolitinib being identified as a promising therapeutic agent based on its simultaneous inhibition of several immune mechanisms that induce anemia or AIHA and on preclinical results. In the concept of AIHA, the RBCs, autoantibodies, and macrophages are involved in a cytophagic three-way model in the pathogenesis of AIHA, while in CAD, the pathogenesis involves a disease model far more complicated than originally proposed, and currently, it is believed that the antigen density on RBCs, the Igs affinity, and the cooperation of both immune and molecular pathways are more important than direct temperature activation [2]. See Figure 1.

1.1 Background and significance

Historical context: Autoimmune hemolytic anemia (AIHA) is an antibody-mediated disease, as evidenced by the presence of IgG, IgM, or, rarely, IgA antibodies, alone or in combination, on the surface of erythrocytes, leading to extramedullary erythrocyte destruction. AIHA is classified as idiopathic if there is no discernible underlying cause such as neoplasia, infection, or autoimmune disease, or secondary if associated with an underlying condition.

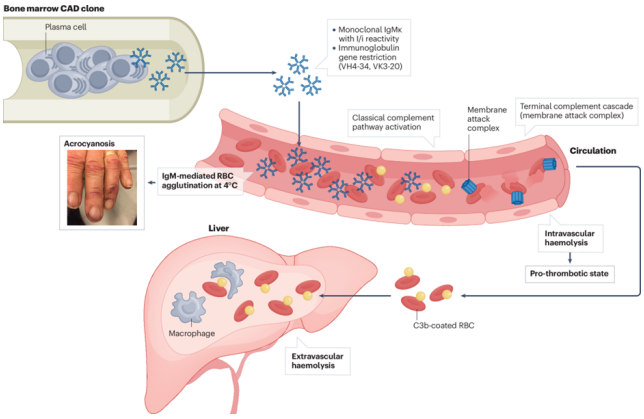

Figure 1: Autoimmune hemolytic anemia [2].

AIHA has historically been associated with certain characteristics, so much so that in the past the acquired hematological defect was understood as specific to a single cell lineage [3].

Prevalence: AIHA affects approximately 1 in 80,000 people and is recognized as one of the least frequent autoimmune diseases. Among various populations, the prevalence of AIHA defers the risk, with a lower rate in African Americans and a higher rate in Caucasians. AIHA is more common in adults than in children but this distribution pattern is not observed in warm AIHA (WA). Neither gender is affected more frequently in cold autoimmune hemolytic anemia (CAIHA). In idiopathic AIHA, the survival rate is 60% in the first 5 years and 50% thereafter [4].

Clinical implications: The relatively low frequency of AIHA tends to make it not as easily recognized as diseases with similar signs but higher prevalence. As the course of the disease can be very rapid with circulatory collapse and even immediate death due to the destruction of red blood cells caused by the antigen-antibody reaction the disease should be quickly recognized for timely treatment. A delay in recognizing the disease can have serious clinical implications and result in intravascular hemolysis, leading to hemoglobinuria, severe anemia, aplastic crisis, and even shock [5].

Why this review is timely: A systematic review of the available articles explores the immunopathological aspects of AIHA. Despite different therapeutic strategies developed over the years, AIHA is still a complex disease that is difficult to diagnose and manage. An in-depth literature review shows that few studies have addressed the clinical implications of AIHA or the disease in general, according to its multifactorial immuno-hematological nature and its potential impact on overall health outcomes. An integrated and multidisciplinary approach regarding the underlying ge-

netics and environmental factors of AIHA provides an indication of possible directions for future research.

2 Autoimmune hemolytic anemia: Clinical overview

Autoimmune hemolytic anemia (AIHA) is a rare type of hemolytic disorder characterized by the body's autoantibody-mediated destruction of native red blood cells [6]. According to the types of autoantibodies and their effect on the erythrocyte membrane, AIHA is divided into two broad categories: immunoglobulin G (IgG)-mediated "warm" hemolytic anemia and complement C3d and C4b-mediated "cold" hemolytic anemia. Warm antibodies react against erythrocytes at a temperature of 37°C with no complement activation. Cold antibodies agglutinate erythrocytes at temperatures below 37°C, with the capacity to activate the complement system upon warming [7]. Cold hemolytic anemia can be further divided into a spectrum of conditions, ranging from clinical benign to lifethreatening diseases. Approximately, half of cold hemolytic anemia cases are idiopathic and constitute CAD, which recognizes RBC antigens resulting in extravascular hemolysis. A further quarter of cases are CAHP, which are mediated by biphasic monoclonal IgG antibodies, often in the context of malignancy or viral infection and are usually biphasic hemolysis in nature. The presence of a monoclonal IFE on the "hot" side of the biphasic wave is characteristic and confirms the diagnosis. In atypical cold AIHA and mixed cold AIHA, the disease is mediated by complex mechanisms [4]. Finally, there is also a rare form of cold hemolytic anemia that is IgA mediated. Like warm antibodies, cold antibodies too can result in a spectrum of clinically manifesting disease. AIHA is a multifactorial disease with a complex immunopathogenesis. The diagnostic criteria for various forms of AIHA also differ given their different pathogeneses. Given the relatively rare, heterogeneous, and complex nature, its diagnosis can be challenging, especially with atypical features.

AIHA is very rare, with an incidence of 1-3 cases per 100,000 per year. The median age of onset is in 60-70 years old. The sex ratio is 0.57 (male: female) concerning warm hemolytic anemia. AIHA, in general, can be divided into primary and secondary according to whether it is associated with other underlying diseases. Primary AIHA includes warm hemolytic anemia, CAD, mixed AIHA, and atypical AIHA [8]. Secondary AIHA comprises warm hemolytic anemia due to lymphoproliferative diseases and CAD due to an underlying cold-agglutinin syndrome not otherwise specified. De novo AIHA occurs mainly in the senile part of the population, and the comorbidities are rarely involved. Secondary AIHA occurs mainly in males, and the underlying diseases are various. The risk factors for AIHA include the medications rituximab and immune checkpoint inhibitors, viral infections, and autoimmune diseases. There are race-related genetic risks in the development of AIHA. The typical, acute and severe clinical presentation of AIHA is pronounced, often life-threatening anemia, and laboratory diagnostics show very strong hemolysis. Sometimes the clinical presentation of AIHA is very atypical, slowly progressive, and the burden of anemia is moderate. Older studies showed that the most common symptoms in a 132-patient cohort were fatigue (82%), pallor (81%), and jaundice (70%) [9].

2.1 Definition and Classification

Autoimmune hemolytic anemia (AIHA) is an autoimmune disorder characterized by autoantibodies directed against erythrocytes. AIHA may cause red blood cell destruction by opsonization and subsequent phagocytosis mostly by the spleen and to a lesser extent by the liver. The most common autoantibodies in AIHA are directed against antigens in the red blood cell membrane or released due to damage from infection, cancer or medication, denoted as warm, cold, idiopathic or secondary AIHA. AIHA is a rare and heterogeneous autoimmune disorder. AIHA often arises in the presence of associated diseases, they further aggravate the anemia requiring a quick recognition and timely treatment to mitigate its severity. This may cause the membrane to activate the classical and/or the alternative complement pathway, causing the formation of membrane attack complex. Several studies describe different structural classes of these molecules and their implicit thermal capability of complement activation. AIHA should be considered a non-homogeneous disease [10]. Different mechanisms may work in this disease, showing the limits of pure categories. There are autoantibodies against RBC membrane proteins, lipids, glycoproteins, non-controlled exposure to intracellular antigens, autoantibodies can be directly against RBC band 3 of glucose transporter-1 proteins, immunoglobulins directed against pathogens that cross-react with RBCs, autoantibodies bound to pathogens can target the pathogen-complexed RBCs, and the possible role of apoptosis as trigger of RBC autoantibody. Nowadays it is widely recognized that AIHA is not only mediated by IgG and C3 but also by complement-independent Fc receptor-mediated phagocytosis by macrophages via neutrophils or killer dendritic cells, and monoclonal antibody drugs used to treat advanced tumors or chronic inflammation [11].

2.2 Epidemiology

Autoimmune hemolytic anemia (AIHA) is an uncommon autoimmune disease where the immune system creates autoantibodies that bind to the body's own red blood cells, marking them for accelerated removal from circulation [12]. AIHA can be broadly classified into two categories: idiopathic and secondary AIHA. Idiopathic cases have no apparent underlying cause and are the majority of AIHA cases. Population-based studies on AIHA are scarce and show huge variations in the incidence and prevalence due to the size of the study population, the age distribution, and the healthcare system of the country. Most studies still are hospital-based. As such AIHA may be underrecognized by primary healthcare professionals, and therefore many patients diagnosed with AIHA may not be included if the hospital-based cases are not extracted from a reliable local population sample. Treatment of AIHA is complex and often multidisciplinary [12]. See Figure 2.

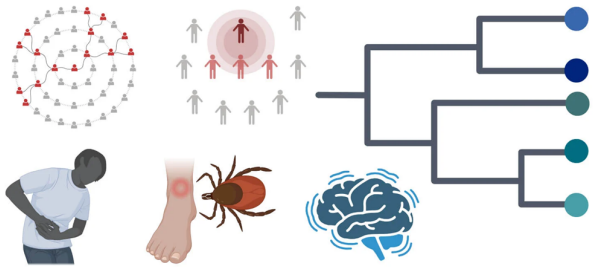

Figure 2: Epidemiology [12].

AIHA has a reported incidence of 0.8 per 100,000 person-years in northern France and 3.9 per 100,000 in Spain—however it is 17.8 per million by the US Immune Hematology database. Two peaks have been identified in AIHA incidence, the first, primarily in children, at less than 10 years of age, and the second, in 70 and above. AIHA is more common in females than males, and a female to male ratio higher than 1.5 has been reported. The pathophysiology of AIHA is still uncertain, but many risk factors have been associated with the development of AIHA. Family members have a higher chance of developing similar illnesses (genetical predisposition) [13]. AIHA may occur in patients with other hematological diseases in which exact etiology is still unknown, including severe sickle cell anemia, lymphoma, and myeloma. After the introduction of immune system-related drugs, there have been increased numbers of case reports of AIHA secondary to drug use, with symptoms of AIHA presenting 1 week to 1 year after. Similarly, a reduction in immune suppression raises the risk of AIHA reoccurrence. The immune system may develop IgG-type autoantibodies against red blood cells in association with a viral infection or due to B-cell proliferation in Sjögren's syndrome or chronic lymphocytic leukemia. Another form of AIHA is observed in patients with ethyl alcohol abuse, due to the reactive anti-F blood group antigen [14].

As AIHA occurs less frequently in teenagers and especially in children aged 5-10, preventing AIHA children from gaining RCA could be a factor responsible for the different exposures to infections in the early stages of life. According to some researchers, another important factor for this purpose could be the different immune response to the pathogen in children compared to the adult form. While children are more prone to stimulate immune response against the new RCAs, which is highly expressed in viral bacteria and they first infect humans. Another influential factor potentially causing differences between children and adults is the Th-cell response to simile and/or vaccination. The early variations of Th-cell response could be directly or indirectly activated by some dominant cytokine secretory cells, increasing the likelihood of autoantibodies [15]. Developed in the context of common antigens. A third prevention mechanism for the development of AIHA in children is immune dysregulation, especially in the immune checkpoint molecules CTLA4 and PD-1. Furthermore, immunological diseases in children could be the culmination of an AIHA disease progression that began in adult or even within utero. The rules according to many family members, it is not possible to establish exactly the period of exposure and the link with infections. Many respondents say it is over 50 years old. NCAs are relatively newly created viruses and bacteria, although many questions are still unanswered. Finally, they confirm the hypothesis that AIHA in humans is a common immune disease that children with RCA may, for different reasons, still do not have. In 2002, a population-based study was conducted to determine the prevalence of AIHA in an extensive area in the province of Pavia in northern Italy, with 470,265 inhabitants totaled. However, there are two significant methodological limitations. The first is that cases are collected from hematologists with previous experience in diagnosing and treating AIHA, and the second is the highly selective nature of the extracted sample because only patients prescribed in most clinical cases are included, while those followed with a more benign form were not included [16]. The results showed that the prevalence of AIHA in the overall area was 5.5 per 100,000 people, based on a positive extraction sample, and those higher than those extracted from a hospitalization file. Most patients diagnosed with AIHA are therefore not included, resulting in an underestimation of the disease. Prospective population observation studies on AIHA are scarcely available and show great variations in sex and geographical spread from 0.6 to 16.4 cases per million people per year due to study size, age dis-

tribution study population, and country health system. We performed a population-based retrospective study to determine the incidence of AIHA in the area of Rome and its province starting from the positive extraction list. A total of 638 cases were diagnosed from 2011 to 2017. Overall, the age-adjusted incidence rate was 2.8 million people per year. Two incidence peaks were identified in children under 10 years and in individuals over 70. The disease appears to be more common in females than males, within marked deviations, ranging from 1.7: 1 to more than 2: 1 in red vessel complications. More than 10 years in age, priority for treating autoimmune disorders, active fine production based on early caution, and comprehensive training prior form [17].

2.3 Clinical presentation and diagnosis

Autoimmune hemolytic anemia (AIHA) refers to a group of hemolytic disorders characterized by the production of autoantibodies against red blood cells (RBCs). The antibodies function in extravascular hemolysis. AIHA is a rare hematologic disorder with an estimated prevalence of 17 per million individuals. This disorder impacts patients at all ages, with a median age of 56 years at diagnosis [18]. The disease is well-recognized in children and young adults, while the incidence increases in the elderly. There is significant racial and gender predilection, with a female-to-male ratio of 2:1 to 4:1 or higher. There are many factors to consider when examining the patient population of AIHA. The etiology of AIHA remains unclear. Autoimmune diseases are closely associated with AIHA. There are also environmental and genetic factors increasing the risk of developing AIHA. For example, patients infected with certain viruses and those with malaria are found to have developed AIHA [19]. The complexity of the pathophysiology and etiology underlying AIHA necessitates the establishment of new models. Thus, certain strains have also been applied as alternative models. Given the increased patient population and the complexity of the etiology and pathophysiology of AIHA, it is timely and important to review these aspects in order to implement better prevention, timely diagnosis, and optimal treatment for AIHA patients. Understanding the development of AIHA can help in formulating treatment plans and guiding follow-up care [20].

3 Immunopathological basis of autoimmune hemolytic anemia

Autoimmune hemolytic anemia is a disorder characterized by the production of autoantibodies against autologous red blood cells. These autoantibodies cause premature destruction of erythrocytes, so that these patients may develop hemolytic anemia. Autoimmune hemolytic anemia may sometimes be induced by specific drugs and/or be seen in the course of malignancies. Nevertheless, most of the cases of autoimmune hemolytic anemia arise de novo and are classified as primary [21]. Lose of tolerance for self-erythrocyte antigens as well as a stimulation of auto reactive immune defense cells are considered to be the main causative mechanisms. AIHA patients can suffer from severe anemia and its consequences, such as general weakness, shortness of breath, rapid heart rate, and/or other symptoms. An extensive deletion of erythrocytes causes bilirubinemia and may result in further complications, such as cholelithiasis, jaundice, and pruritus. The early diagnosis and the prompt introduction of therapy are fundamental to avoid complications linked to the severe decrease in blood cells. Notwithstanding the improvement in the diagnostic procedures, which are now quite refined, the treatment of AIHA remains challenging, as there are still several unmet needs in the therapeutic management of both naïve and multirefractory cases. Mechanisms responsible for the expression of AIHA have been indeed elucidated in depth only partially. Several experimental models have contributed to better understand the pathophysiology of AIHA, which encompasses a complex synergy between different branches of the immune system, in particular autoantibodies, phagocytes, and T and B lymphocytes [19].

3.1 Overview of immune system in hemolytic anemia

Two key caspase cascades and intrinsic mitochondrial-apoptosis cascades are modulated by Bcl-2-mitochondrial interaction and release of cytochrome C in the caspase-dependent and -independent pathways, indicating that the Bcl-2 family is one of the key regulators in the life and death of erythroid cells [22]. Mismanaged apoptosis of erythroid progenitors and immature erythroblasts is associated with ineffective erythropoiesis in anemia of chronic disease and β-thalassemia, accompanied by enhanced release of adhesive erythroblasts that may contribute to anemia by increasing phagocytosis in the reticuloendothelial system, and extravasation into bloodstream. Excessive erythrocyte apoptosis due to destruction triggered by complement, antibodies or oxidants underlies anemia of congenital red cell membrane and metabolic disorders and autoimmune hemolytic anemia, characterized by accelerated RBC clearance [23]. The clearance of cell corpses/released mitochondria is primarily dependent on the phagocytic function of macrophages, and dying cells may also affect the mitochondria of the

recipient cell, such as caspase-mediated mitochondrial fragmentation followed by cytochrome C release in caspase-mediated but Bax/Bak-independent pathway.

3.2 Mechanisms of autoantibody production

Autoimmune hemolytic anemia (AIHA) is a rare autoimmune disease with pathology manifestations of accelerated destruction of red blood cells by autoantibodies. Despite numerous studies have been carried out in recent decade, the specific mechanism of AIHA remains unclear, although it is considered involving both humoral immunity and cellular immunity. In AIHA, production of autoantibody (ies) against red blood cell (RBC) self-antigen(s) is the key pathway leading to RBC destruction. Moreover, adaptive immune responses through T-cell class switching are necessary for sustained inhibition of protective immunity [24]. Unfortunately for patients suffering from harmful autoimmunity, the methods by which de novo formation of an isotype would have clinical utility are counterindicated by existing strategies. Therefore therapeutic approaches are needed to interrupt cellular mechanisms that sustain established autoimmune responses while leaving the remaining immune defenses intact. In AIHA, production of autoantibody (ies) against red blood cell (RBC) self-antigen(s) is the key pathway leading to RBC destruction [25]. Regulatory T (Treg) cells play a crucial role in the maintenance of immune tolerance and homeostasis by controlling autoreactive T cells. However, results from animal models and patients indicate that Treg cells do not efficiently inhibit the production of autoantibodies. Traditionally, B cells, the precursors of antibody-secreting plasma cells, are thought to mature in the bone marrow or the spleen. Successful VDJ rearrangement allows the emerging immature B cell to escape the developmental impasse formed by RAG gene shutdown. After emigration from primary lymphoid organs, immature B cells complete their maturation in the periphery to become follicular (FO) B cells [26].

3.3 Role of complement system in hemolysis

The complement system is a cascade of more than 30 plasma proteins that recognize and eliminate pathogens and pretransformed cells. This ancient system of innate immunity is activated on bacteria or virus-infected cells via three main initiation pathways: the classical pathway, the lectin pathway, and the so-called alternative pathway. Complement activation triggers the formation of reactive complexes called the membrane attack complex, leading to target cell lysis. Red blood cells that lack the cell surface expression of targeted antigens are usually resistant to complement. However, numerous reports illustrate how perfectly formed red blood cells can be destroyed by complement activation, either specifically or as collateral damage to immune complexes [27]. Complement-based hemolysis is rapid and virtually 100% efficient. Furthermore, fragments generated during target cell lesions negatively affect nearby red blood cells that are not within reach of the initial attack. This concept of bystander hemolysis may be important in the manifestation of autoimmune hemolytic anemia, emphasizing that such cytopenias do not need the complete surface reactivity of the red blood cells, but are more likely to manifest when the lipid rafts content of bystander red blood cells is altered. Increased resistance of activatory C3 convertases to inhibition with microparticles may alleviate the sensitivity of autoantibody-exposed bystander cells to complement attack; at variance, the weakened reduction of C3dc by factor I could still strongly sensitize them to hemolysis [28].

This suppositional model matches the earlier observation that not all exposed red blood cells lyse upon re-exposure to anti-p specter despite the deposition of the membrane attack complex on them. The chronic lysis of bystander red blood cells, combined with the supply of new epitopes by them, could generate consecutive rounds of hemolysis contributing to the persistence of the autoimmune process, despite the preferential initial attack of a particular red blood cell population. Although individual complement components have been shown to induce mechanistically distinct modes of target recognition, anti-red blood cell IgM generally activates the classical pathway, similarly to how it activates complement on bacteria. Would targeting the alternative pathway be beneficial in reducing this bystander hemolysis? As documented in model fluid-phase classical pathway activation and elsewhere, the classical pathway is also capable of activating the alternative pathway directly, bypassing both the C1 complex and MBLs [29]. This process relies on the exchanging assembly on the ligand patterns of classical pathway components with C3 and properdin as the initial activator.

Model red blood cells are poor activators of either pathway for the same reason – they lack the ligands capable of binding to and properly orienting pathway factors in situ. It was shown whether autoantibody opsonized red blood cells through the spread of immune complexes would be better in plating C3b on bystander cells and thus could promote their more efficient complement killing. However, red blood cells are poor ligand surfaces for activatory C3 convertases. Collectively, these data imply that in red blood cell models, [30] the classical pathway–alternative pathway amplification loop is inefficient. Amplify the classical pathway output with properdin – to promote more ef-

ficient alternative pathway amplification? Implement an intracellular signal upon membrane attack complex deposition? More than 90% of membrane attack complex-mediated lysis occurred in the first 30 seconds after the injection.

4 Molecular mechanisms of autoimmune hemolytic anemia

A coordinated effort spanning human and murine studies that combined cellular, protein, and genetic analyses of RBC autoreactivity is explored. It is reported that multiple genes encode autoantibody specificities against a limited set of RBC proteins in mice and humans, and develop a model of disease pathogenesis involving interferon-γ expression by CD4 T cells and RBC destruction by CD8 T cells and RBC phagocytes. [31] An expanded set of RBC self-antigens recognized by patients and corresponding to many different anion exchange, calcium, and potassium channel family members is identified; some of these proteins have not been previously implicated in AIHA or any other autoimmune disease. Focusing on a protein family critical for RBC biology reveals that clinical diagnostics and experimental studies generally underestimate the extent of antibody recognition and complexity of protein targets. This work demonstrates that a molecular understanding of RBC proteins alterations can provoke autoimmunity and how autoantibodies target RBC proteins and mediate disease. Relatively few RBC proteins can be immune targets, but a diversity of genes in patients may shape the spread of antibody specificities and course of AIHA. Prompt treatment of the underlying infections would be expected to resolve the immune stimulation and associated autoantibody responses, potentially ameliorating AIHA [32].

Autoimmune hemolytic anemia results from molecular and cellular mechanisms, some of them are defective structure or function of RBC antigens or regulators, while others are defective structure or function of immune cells. Enacted or facilitated by dysregulatory T cells, it is also suggested that B cells can participate in some molecular stages of attacks. RBCs can be altered to be recognized by immune cells, by missing/not expressing antigens or by molecular modification of glycophorins due to disease or aging. Complement activation is a key molecule of attack that generates membrane-coating complexes which attract immune cells. This can produce a strong intracellular stress and promotion of early apoptosis, irreversible alterations of globins lose membrane lipid asymmetry, lose mitochondrial potential and phosphatidylserine exposure. Erythrophagocytosis is crucially controlled by RBC rigidity and it can cause systemic inflammation. During the oldest age, the ability to control membrane damage after membrane attack complex action decreased, promoting the onset of immune attack [33].

4.1 Autoantigens in autoimmune hemolytic anemia

Autoimmune hemolytic anemia (AIHA) is an autoimmune disease (AD) in which immune cells target red blood cells (RBCs) or erythrocytes. AIHA is very similar to other autoimmune hematological diseases and it has a wide range of autoantigens to trigger the immune response. This very large spectrum of autoantigens allows for the classification of AIHA according to the autoantigen; according to the best-known autoantigens, it can be classified as primary, secondary, or tertiary AIHA [34]. The different AIHA forms are associated with different autoantigens, several of which can be considered as environmental factors because the exposure of a certain autoantigen can trigger AIHA. The spectrum of AIHA should be better considered during the developing of diagnostic and classification criteria in order to find each molecule in each specific AD. On the other hand, the molecular mechanisms clarified in AIHA can be extended to other ADs, providing new therapeutic strategies. In this context, a review of the autoantigens acting in AIHA is presented, and new emerging therapies based on different factors of the etiopathology of AIHA are shown.

Autoantibodies are the pathological autoantigens playing a pivotal role in the immunopathology of AIHA [4]. The wide spectrum of autoantigens in AIHA can be divided into two classes: external and internal autoantigens. According to the location, external autoantigens can be divided into membrane and outmembrane autoantigens. Internal autoantigens are substances inside of the erythrocyte membrane and include simultaneously two subclasses namely, normal and pathologic autoantigens. Considering the structures and functions of the various autoantigens, the mechanisms that trigger the production of vicious autoantibodies are analyzed. Taking in account the spectrum of autoantigens acting in AIHA, it can be classified by autoantigen type and environmental factor, strokes provide a guide on the therapy and the diagnosis of the disease. Additionally, research on the genetic predisposition to produce pathologic autoantigens in AIHA is reviewed, which may increase knowledge on the etiopathology of AIHA and AMHAs [35]. The common underlying features are worsening the response to viral infections in patients and the formation of polyspecific responses in both autoimmunity and polyclonal lymphoproliferations. Dissecting the relationships of external autoantigens, normal internal autoantigens, and AD in AIHA may allow a better understanding of the etiopathology of the disease

while revealing new targets in diagnostic and therapeutic fields.

4.2 B cell dysregulation in autoimmunity

Dysregulated B cells are a fundamental component of autoimmune hemolytic anemia (AIHA) pathogenesis, both in primary and in the context of other immune diseases. Aberrant B cell function may contribute to autoimmune hemolysis through the generation of antibodies, against either well-characterized or cryptic self-antigens of erythrocytes. This review sheds light on B cell alterations during AIHA and subsequent development, underlying mechanisms, emerging therapeutic biologics, and critical areas for future investigation [36]. Normal B lymphocytes are part of complex immune systems and are at the crossroads in the pathogenesis of AIHA for their capability in contributing and coordinating immune responses. Dysregulation of B cell activation results in the production of pathogenic autoantibodies against red blood cells [37]. Although erythrocyte-specific autoantibodies usually belong to IgG, and AIHA can be mediated independently from B cells through other effector mechanisms. Crucial aspects of communication between B and T cells in the field of autoimmunity are mechanistically discussed, encompassing cytokines, signaling pathways, and functional responses. Furthermore, features of B cell development and maturation in the autoimmune guardian are investigated. Intrinsic B cell genetic susceptibility in immunodeficiencies, including defects of central and peripheral tolerance, leading to B cell dysregulation is reviewed, overall impacting on the immune balance [38].

5 Emerging therapeutic strategies

Autoimmune hemolytic anemia (AIHA) is a heterogenous group of diseases characterized by the production of autoantibodies against red blood cells with subsequent destruction, extravascular or intravascular and shortened survival of affected cells. Living the broad spectrum of pathogenesis of the autoimmune diseases and the apparently unpredictable and spontaneous onset and course of AIHA, traditional and not evidence-based therapeutic strategies have been applied in the treatment of AIHA. Currently used treatments of AIHA are mainly those routinely used to induce immune suppression or inhibition of the autoimmune response, i.e., corticosteroids and immunosuppressive agents, with a few exceptions, as well as those suggested by the knowledge of the onset of the disease or of its coexistence with other diseases. The reviewed therapies summary reports on studies of the same therapeutic strategy performed with different AHIA patients in different decades, as well as on studies of different therapeutic strategies in the same AHIA patients or with different treatment courses, none of which results in a uniform trend in the results. Given the wide spectrum of potential immunopathological roots, each AHIA case cannot always be treated with traditional and empirically determined therapy [39]. The reviewed study results identify some common, as well as individual, trends in unsuccessful therapy in AHIA cases and suggest a focus on them in attempts to characterize AHIA stratification and prognosis and also therapeutic and diagnostic strategies. The results of the reviewed studies suggest possible targets for monitoring treatment effectiveness and identifying the more common events associated with treatment failure/refractoriness. Finally, the results of the reviewed studies suggest the inefficacy of standard, similar or replicated therapeutic approaches over time, highlighting a potential underestimation in the planning of the response to the acute and long-established form of AIHA transversely in patients [40]. These considerations suggest the desirability of further logical and more careful experimental clinical trial design through, for example, the control of therapeutic drugs, the detection of relevant molecular and cellular markers at the same time in the course of monitoring, the control of responses over time the same therapeutic procedure or comparison and coordination between attempts of the different therapeutic protocols or therapeutic strategies.

5.1 Current treatment modalities

The goal of this study was to analyze the latest possible scientific articles that deal with the molecular pathogenesis of AIHA as well as with the future therapies [41]. Ion, molecular basis of the signaling pathways in question, gene-expression changes, and molecular treatments of AIHA are clarified in detail. Autoimmune hemolytic anemia (AIHA) is characterized by the deposition of antibodies (usually IgG but also IgM, IgA or complement) on erythrocyte membranes and subsequent phagocytosis of antibody-coated erythrocytes by reticuloendothelial macrophages. Two types of AIHA are defined according to the temperature, allo- and auto-immune hemolytic anemia. The latter may be idiopathic (approximately 50% of AIHA) or secondary to an underlying disease. Warm autoimmune hemolytic anemia (wAIHA) is mediated by IgG antibodies reacting at 37 °C. And, wAIHA has a female predominance (the female/male ratio ranges as 2:1 or higher) and may develop at every age with a higher incidence between 40 and 70 years in Caucasians. The finding of AIHA involves various antibod-

ies against membrane erythrocyte antigens including Band 3, SPECTRINS, ribosomal proteins, α-enzymes, and other antigens. Nearly 40% of idiopathic AIHA cases are so called "primary" cases, in which no underlying condition can explain the hemolysis. AIHA cases associated with autoimmune diseases, B-cell malignancies, viral infections, and solid tumors are named "secondary" AIHA.

AIHA causes hemolysis, characterized by anemia, jaundice, splenomegaly, and often results in severe morbidity and sometimes in death. Recent clinical studies have started the use of the expression of signaling molecules such as HeatShockProteins and proinflammatory cytokines to monitor kidney disorders induced by cisplatin treatment. Devil's claw extract is known to possess anti-inflammatory, and analgesic properties. [42] Previous data indicate changes in the biochemistry and structure of the lung tissue during the allergic reaction. The aim of the present study was to investigate the effects of intraperitoneally injections of hexanic or alcoholic devils claw solutions on this alteration of the lung tissue. Honeybee venom, a natural toxin secreted by the Hymenoptera species, has been used in traditional medicine and any form has been praised for its healing properties in folklore. However, bee stings may cause various allergic and toxic reactions. The aims of the present study were: to create a rat model of bee venom inhalation, to investigate the toxic effect by measuring the serum levels of proinflammatory cytokines, and to describe local morphological changes.

5.2 Novel therapeutic approaches

Autoimmune hemolytic anemia (AIHA) has been considered a simple example of antibody-mediated autoimmune disease. The pathogenesis of AIHA is based on the production of autoantibodies directed against erythrocytes, which are considered to be the main pathogenic mechanism. However, AIHA is a heterogeneous disease in which several immunological mechanisms are present beyond antibodies. Many review articles have been published on the topic and a rather comprehensive perspective has been reached [43]. Until the last decades, increasing attention was directed mostly to the demonstration of complement or cell-mediated mechanisms in antibody-positive cytotoxic AIHA. Nonetheless, a wider number of immunological mechanisms have been progressively appreciated. In some cases, results have not been perfectly reproducible and have been reported differently in experimental studies relative to patients. However, as a whole, accumulating evidence suggests that most patients have reduced percentages of CD4+ T-regs cells and at the same time an imbalance of T-helper (Th) 1/2 cytokines with a high ratio of IFNγ/an IL-4 cytokine 1. In addition, expanded in a higher number of cytotoxic CD8+ T lymphocytes have been documented in AIHAs, which broadly show hyperactivation signs and the production of higher amounts of pro-inflammatory and pro-apoptotic molecules. Importantly, the same cytotoxic CD8+ T lymphocytes display a restricted T-cell receptor repertoire, thus fully activating these important cells. This knowledge has driven the design of innovative therapies. Most attention has been directed to non-damped dendritic cells, since they can increase the generation of new cytotoxic/dysfunctional CD8+ T cells. AIHA can be primary or secondary to other illnesses, which may reveal various mechanisms. The prognosis is highly dependent on the underlying disease and may influence the choice of treatment. Frequently "secondary" forms show a more serious presentation and have a negative impact on the prognosis relative to "primary" ones. Prima facie, the distinction between "primary" and "secondary" AIHAs relied on starting/occurring within or in absence of hemopathy [44]. However, in some cases, laboratory analysis did not offer robust evidence on the interpretation of such condition. The data obtained from several studies analyze the largest series of AIHAs ever reported to her knowledge. Definition of "primary" and "secondary" diagnosis is more stringent in patients thoroughly analyzed by bone marrow and immunobiological studies.

6 Conclusion and future directions

Autoimmune hemolytic anemia (AIHA) is a heterogeneous, phenotypically diverse group of disorders characterized by the premature destruction of red blood cells due to the binding of autoantibodies on the cellular surface. The exact underlying mechanisms of the development of these autoantibodies have not yet been fully elucidated. This systematic review evaluated the current knowledge on the immunopathological and molecular mechanisms that play a role in the pathogenesis of AIHA, and it presents an updated perspective for emerging therapeutic strategies. Articles discussing the pathogenesis of AIHA (e.g., molecular pathways, immune mechanisms, and genetic risk) were systematically searched up to December 2020. While the genetic and environmental basis of AIHA remains intricate and multifactorial, research advances may offer new approaches for personalized therapies in the future.

AIHA is a rare hematological autoimmune disease characterized by the loss of tolerance to self-antigens on the surface of red blood cells or facilitated by an underlying condition. AIHA can be an isolated disorder or associated with lymphoproliferative malignancies,

most commonly chronic lymphocytic leukemia (CLL), or other autoimmune diseases. Accumulating evidence suggests that several molecular pathways and immunologic mechanisms regulate the immune response promoting the generation of autoimmune antibodies. New insights into the molecular mechanisms underlying the pathogenesis of the disease have allowed previewing several new potential molecular inhibitors as possible targeted therapies. In conclusion, the advances in the molecular knowledge about the pathogenesis of AIHA might improve the understanding of the disease, allowing new multiple therapeutic approaches and the development of novel targeted agents. However, ongoing and future research, including clinical studies, is necessary to confirm these treatments for improving patient care.

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References

[1] Gehrs BC, Friedberg RC. Autoimmune hemolytic anemia. American Journal of Hematology. 2002;69(4):258-71. doi:10.1002/ajh.10062. [Backref page 41]

[2] Barcellini W. Immune hemolysis: Diagnosis and treatment recommendations. Seminars in Hematology. 2015;52(4):304-12. doi:10.1053/j.seminhematol.2015.07.001. [Backref page 41]

[3] Barcellini W, Fattizzo B. The changing landscape of autoimmune hemolytic anemia. Frontiers in Immunology. 2020;11:946. doi:10.3389/fimmu.2020.00946. [Backref page 41]

[4] Jäger U, Barcellini W, Broome CM, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: Recommendations from the First International Consensus Meeting. Blood Reviews. 2020;41:100648. doi:10.1016/j.blre.2019.100648. [Backref page 41], [Backref page 42], [Backref page 46]

[5] Barcellini W, Fattizzo B. Clinical applications of hemolytic markers in the differential diagnosis and management of hemolytic anemia. Disease Markers. 2015;2015:635670. doi:10.1155/2015/635670. [Backref page 41]

[6] Duffy TP. Autoimmune hemolytic anemia and paroxysmal nocturnal hemoglobinuria. Rossi's Principles of Transfusion Medicine. 2009;3. [Backref page 42]

[7] Gertz MA. Updates on the diagnosis and management of cold autoimmune hemolytic anemia. Hematology/Oncology Clinics of North America. 2022;36(2):341. doi:10.1016/j.hoc.2021.11.001. [Backref page 42]

[8] Mitchell KD. The acute phase response in canine autoimmune hemolytic anemia. University of Guelph; 2008. Doctoral dissertation. Available from: https://hdl.handle.net/10214/22224. [Backref page 42]

[9] Miya W. Initial Sputum Smear Grading as a Factor in Predicting Treatment Outcome in Newly Diagnosed Sputum Smear Positive Pulmonary Tuberculosis Patients. Rajiv Gandhi University of Health Sciences (India); 2018. Doctoral dissertation. [Backref page 42]

[10] Hill QA, Stokes M, Massey E, Grainger JD, Provan D, Hill A. The diagnosis and management of primary autoimmune haemolytic anaemia. British Journal of Haematology. 2017;176(3):395-411. doi:10.1111/bjh.14478. [Backref page 42]

[11] Jalink M, De Boer EC, Evers D, Havinga MQ, Vos JM, Zeerleder S, et al. Halting targeted and collateral damage to red blood cells by the complement system. In: Seminars in Immunopathology. Springer Berlin Heidelberg; 2021. p. 1-18. doi:10.1007/s00281-021-00859-8. [Backref page 42]

[12] Barcellini W, Fattizzo B. The changing landscape of autoimmune hemolytic anemia. Frontiers in Immunology. 2020;11:946. doi:10.3389/fimmu.2020.00946. [Backref page 43]

[13] Cooper GS, Bynum ML, Somers EC. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. Journal of Autoimmunity. 2009;33(3-4):197-207. doi:10.1016/j.jaut.2009.09.008. [Backref page 43]

[14] Sharma SK. B Cells. In: Basics of Hematopoietic Stem Cell Transplant. Springer Nature Singapore; 2023. p. 87-120. doi:10.1007/978-981-19-5802-1_10. [Backref page 43]

[15] Street NE, Mosmann TR. Functional diversity of T lymphocytes due to secretion of different cytokine patterns. The FASEB Journal. 1991;5(2):171-7. doi:10.1096/fasebj.5.2.1825981. [Backref page 43]

[16] Mossong J, Hens N, Jit M, Beutels P, Auranen K, Mikolajczyk R, et al. Social contacts and

mixing patterns relevant to the spread of infectious diseases. PLoS Medicine. 2008;5(3):e74. doi:10.1371/journal.pmed.0050074. [Backref page 43]

[17] Group DD. Prevalence of small vessel and large vessel disease in diabetic patients from 14 centres: the World Health Organisation multinational study of vascular disease in diabetics. Diabetologia. 1985;28(Suppl 1):615-40. doi:10.1007/BF00290267. [Backref page 44]

[18] Loriamini M, Cserti-Gazdewich C, Branch DR. Autoimmune hemolytic anemias: classifications, pathophysiology, diagnoses and management. International Journal of Molecular Sciences. 2024;25(8):4296. doi:10.3390/ijms25084296. [Backref page 44]

[19] Salama A. Complement-mediated autoimmune hemolysis and its treatment. Clinical and Developmental Immunology. 2012;2012:181258. doi:10.1155/2012/181258. [Backref page 44]

[20] Andrew M Kaunitz M, Deanna C McCullough M, Erin H Burnett M. Medical management of abnormal uterine bleeding in reproductive-age women. OBG Management. 2021;31(9):31 34. [Backref page 44]

[21] Dhaliwal G, Cornett PA, Tierney Jr LM. Hemolytic anemia. American Family Physician. 2004;69(11):2599-607. Available from: https://www.aafp.org/pubs/afp/issues/2004/0601/p2599.html. [Backref page 44]

[22] van Loo G, Schotte P, Van Gurp M, Demol H, Hoorelbeke B, Gevaert K, et al. Endonuclease G: a mitochondrial protein released in apoptosis and involved in caspase-independent DNA degradation. Cell Death & Differentiation. 2001;8(12):1136-42. doi:10.1038/sj.cdd.4400944. [Backref page 44]

[23] Michel M. Warm autoimmune hemolytic anemia: Current and emerging treatment options. Hematology American Society of Hematology Education Program. 2017;2017(1):201-6. doi:10.1182/asheducation-2017.1.201. [Backref page 44]

[24] Michalak SS, Olewicz-Gawlik A, Rupa-Matysek J, Wolny-Rokicka E, Nowakowska E, Gil L. Autoimmune hemolytic anemia: current knowledge and perspectives. Immunity & Ageing. 2020;17:1-6. doi:10.1186/s12979-020-00208-7. [Backref page 45]

[25] Dierickx D, Kentos A, Delannoy A. The role of rituximab in adult patients with warm antibody autoimmune hemolytic anemia. Blood. 2015;125(21):3223-9. doi:10.1182/blood-2014-11-577346. [Backref page 45]

[26] Paul WE. Fundamental Immunology. Philadelphia, PA: Wolters Kluwer Health; 2013. [Backref page 45]

[27] Englinger B, Pirker C, Heffeter P, Terenzi A, Kowol CR, Keppler BK, et al. Metal drugs and the anticancer immune response. Chemical Reviews. 2018;119(2):1519-624. doi:10.1021/acs.chemrev.8b00396. [Backref page 45]

[28] Vasilev VV, Radanova M, Lazarov VJ, Dragon-Durey MA, Fremeaux-Bacchi V, Roumenina LT. Autoantibodies against C3b—functional consequences and disease relevance. Frontiers in Immunology. 2019;10:64. doi:10.3389/fimmu.2019.00064. [Backref page 45]

[29] Berentsen S, Sundic T. Red blood cell destruction in autoimmune hemolytic anemia: Role of complement and potential therapeutic targets. Frontiers in Immunology. 2019;10:1604. doi:10.3389/fimmu.2019.01604. [Backref page 45]

[30] Denomme GA. The structure and function of the molecules that carry human red blood cell and platelet antigens. Transfusion Medicine Reviews. 2004;18(3):203-31. doi:10.1016/j.tmrv.2004.03.006. [Backref page 45]

[31] Richards AL, Kapp LM, Wang X, Howie HL, Hudson KE. Regulatory T cells are dispensable for tolerance to RBC antigens. Frontiers in Immunology. 2016;7:348. doi:10.3389/fimmu.2016.00348. [Backref page 46]

[32] Barcellini W, Fattizzo B, Zaninoni A. Current and novel treatment options for autoimmune hemolytic anemia. Drugs. 2018;78(11):1093-117. doi:10.1007/s40265-018-0931-1. [Backref page 46]

[33] Turpin C, Catan A, Meilhac O, Bourdon E, Canonne-Hergaux F, Rondeau P. Erythrocytes: central actors in multiple scenes of atherosclerosis. International Journal of Molecular Sciences. 2021;22(11):5843. doi:10.3390/ijms22115843. [Backref page 46]

[34] Michalak SS, Olewicz-Gawlik A, Rupa-Matysek J, Wolny-Rokicka E, Nowakowska E, Gil L. Autoimmune hemolytic anemia: current knowledge and perspectives. Immunity & Ageing. 2020;17:1-6. doi:10.1186/s12979-020-00208-7. [Backref page 46]

[35] Gertz MA. Cold agglutinin disease: Current understanding and treatment approaches. Hematology Am Soc Hematol Educ Program. 2016;2016(1):226-31. doi:10.1182/asheducation-2016.1.226. [Backref page 46]

[36] Michalak SS, Olewicz-Gawlik A, Rupa-Matysek J, Wolny-Rokicka E, Nowakowska E, Gil L. Autoimmune hemolytic anemia: current knowledge and perspectives. Immunity & Ageing. 2020;17:1-6. doi:10.1186/s12979-020-00208-7. [Backref page 47]

[37] Hill QA, Hill A. Autoimmune hemolytic anemia. Hemolysis and Hematology. 2018;24(2):103-17. doi:10.1016/j.haem.2018.01.002. [Backref page 47]

[38] Vale AM, Schroeder HW. Clinical consequences of defects in B-cell development. Journal of Allergy and Clinical Immunology. 2010;125(4):778-87. doi:10.1016/j.jaci.2010.02.018. [Backref page 47]

[39] Ma L, Zeng X, Liu X, et al. Advances in the pathogenesis and treatment of autoimmune hemolytic anemia. J Transl Med. 2021;19(1):25. doi:10.1186/s12967-021-02718-9. [Backref page 47]

[40] Jäger U, Knöbl P, Lechner K. Mechanisms of immune-mediated hemolysis and novel therapeutic approaches. Transfus Med Hemother. 2019;46(6):349-58. doi:10.1159/000503801. [Backref page 47]

[41] Berentsen S, Randen U, Tjønnfjord GE. Cold agglutinin disease. Hematol Oncol Clin North Am. 2015;29(3):455-71. doi:10.1016/j.hoc.2015.01.005. [Backref page 47]

[42] Michalak SS, Olewicz-Gawlik A, Rupa-Matysek J, Wolny-Rokicka E, Nowakowska E, Gil L. Autoimmune hemolytic anemia: current knowledge and perspectives. Immunity & Ageing. 2020;17:1-6. doi:10.1186/s12979-020-00208-7. [Backref page 48]

[43] Barcellini W, Fattizzo B. The changing landscape of autoimmune hemolytic anemia. Frontiers in Immunology. 2020;11:946. doi:10.3389/fimmu.2020.00946. [Backref page 48]

[44] Rubin RH. Infectious disease complications of renal transplantation. Kidney International. 1993;44(1):221-36. doi:10.1038/ki.1993.234. [Backref page 48]

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