Autoimmune Hemolysis: A Journey through Time

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Summary
The existence of autoimmune diseases in humans has been known for almost 100 years. Currently, autoimmune pathogenesis has been attributed to more than 40 human diseases; yet it is still not clear what immune abnormalities conclusively prove underlying autoimmune pathogenesis. Hence, although much has been learned, research is still needed for complete elucidation of the mechanisms of the immune dysregulation in AIHA. Better understanding of the underlying mechanism(s) may allow for development of more specific therapies of these not uncommon and often difficult-to-treat disorders.

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'The history of medicine is in fact the history of humanity itself, with its ups and downs, its brave aspirations after truth and finality, its pathetic failures … the subject may be treated as … the very bone and marrow of cultural history.'
(Fielding Garrison, 1870–1935).

The existence of autoimmune diseases in humans has been known for almost 100 years. Currently, autoimmune pathogenesis has been attributed to more than 40 human diseases, yet it is still not clear what immune abnormalities conclusively prove underlying autoimmune pathogenesis. Autoreactivity, by definition, designates a specific adaptive immune response against self-antigens. Normally there is tolerance to self-antigens, and this may be achieved by physical deletion or functional silencing of specific T and B cells. How tolerance breaks down and autoimmune disorders arise remains incompletely understood. Organ-specific autoimmune diseases are directed primarily at the autoantigens of particular tissues, for example, platelets in autoimmune thrombocytopenic purpura (AITP) and erythrocytes in autoimmune hemolytic anemia (AIHA). These particular diseases are the major autoimmune disorders in hematology. As we have previously outlined [1], this paper will review the historical odyssey of how we have learned about the clinical manifestations, etiology, and management of AIHA and how we have come to the current understanding that, although mediated by autoantibodies, T-cell defects are the major driving force behind autoantibody production.

The AIHA Disorders

In AIHA the patient's RBCs are selectively attacked and destroyed (hemolyzed) by autoantibodies produced by the patient's own immune system, resulting in the common symptoms of anemia: weakness, pallor, fatigue, jaundice, and often mild splenomegaly. There are 2 main types of AIHA: cold agglutinin disease (CAD) and warm autoimmune hemolytic anemia (WAIHA), differentiated by the optimal temperature of reactivity of the autoantibody. A third type is the relatively uncommon, but quite distinct, paroxysmal cold hemoglobinuria (PCH). The different types of AIHA have different pathophysiologies, depending on antibody immunoglobulin class and subclass and the ability of the antibody to activate complement. In CAD, the autoantibodies are generally IgM; in WAIHA, they are IgG. When opsonized with IgG autoantibody, the RBCs undergo extravascular destruction, primarily in the spleen; when coated with complement (mediated by IgM and some IgG autoantibodies), they can undergo either intravascular or extravascular hemolysis. Although it may occur in WAIHA, intravascular hemolysis is classically more associated with CAD (and with PCH) and leads to increased plasma hemoglobin and hemoglobinuria, resulting in red, brown, or tea-colored plasma and urine. Extravascular destruction of the RBCs (classic in WAIHA, but also common in CAD) results in increased bilirubin in plasma and urine urobilin, causing jaundice and dark yellow urine. The changes in urine and skin color were the earliest historical recognitions of AIHA.

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Blood in Antiquity

The importance of the blood has been recognized for millennia; e.g. the Bible states that the 'soul of the flesh is in the blood' (Leviticus 17:11), 'take drink … this is my blood, which is shed for you for the remission of sins' (Mathew 26:28), and in the Koran, 'In the Name of God, the Compassionate, the Merciful recite thou, in the name of thy Lord who created: – created man from clots of blood' (Sura 96: Sūrat al-ʿAlaq (العلق) 'The Clot'). In China, 1,000 B.C., it was thought that the soul was contained in the blood. Egyptian kings bathed in blood for their health. Taurobolium, the practice of bathing in blood as it cascaded from a sacrificial bull, was practiced by the Romans. Pliny and Celsus describe Romans drinking the blood of fallen gladiators to gain strength and vitality and to cure epilepsy. Galen advised drinking of blood of a weasel or a dog for rabies. Ancient Norwegians drank blood of seals and whales as a remedy for epilepsy and scurvy [2]. Blood was recognized to be the carrier of life, the soul, heat, and the matter, which, when corrupted, leads to disease, and Hippocrates would likely have suggested a corruption of the blood (phtore haimatos) as accounting for weakness and paleness. Although red urine, the hallmark symptom of intravascular hemolysis (characteristic of CAD and PCH, but also seen in some cases of WAIHA), was recorded occasionally before 1600, in most cases it probably represented hematuria rather than hemoglobinuria. In the second century A.D., Galen recognized that not all cases of jaundice were caused by liver disease, and described an individual bitten by a viper whose 'skin turned the color of a ripe leek' [3] probably the first description of an extracellular type of acquired hemolytic anemia, albeit not autoimmune. Galen implicated the spleen in the process, an association of the spleen and hemolysis not again confirmed until the late 19th century [2]. PCH was probably the first AIHA to be clearly recognized, when, in 1529, Actuarius in Constantinople described paroxysmal hemoglobinuria, in which the urine was 'azure & livid as well as black' in patients of melancholic humor and complaining of loss of strength after exposure to cold [4]. PCH needed then to await the latter half of the 19th century to be again reported.

Early Scientific Studies of Blood

The scientific method (pioneered by Francis Bacon in the 13th century) led, in the 16th century, to the discovery of the circulation of blood by William Harvey and the seminal experiments with transfusion of blood by Richard Lower in England and Jean-Baptiste Denis in Paris in the mid-16th century. Despite this interest in blood, the discovery of the RBCs had to await the appearance of the microscope. The first 'simple' microscope (1600–1630), consisted of a pearl of glass and two foils of silver, yielding a magnification of approximately 400× [5]. Although Anaxagoras had, in the 5th century B.C., predicted the existence of RBCs ('the blood is formed by a multiple of droplets, united among them') [6], the first actual observation of an RBC was likely made in 1661, when Malpighi described their circulation in the capillaries [7], and this was followed

in 1663 by Swammerdam's description of minute globules in the blood of a frog [8]. A decade later, human RBCs were described in detail by van Leeuwenhoek, who established their size at about 1/3,000 of an inch by comparing a RBC with a grain of sand of known size [9]. However, little was written that indicates a clear diagnosis of AIHA during these centuries, except for a possible case mentioned by Morgagni who, in 1769, reported a priest with symptoms consistent with hemolytic anemia, namely red urine, fatigue, a pallid color, and an enlarged spleen [10]; this might arguably be a case representing AIHA, although a diagnosis of malaria cannot be excluded. The physical limitations of the early microscopes precluded further advances in knowledge of blood, although John Huxham, in 1770, while studying purpura by microscopy, recognized that hemoglobin came from degenerating RBCs [11].

Investigations of Anemia and Hemolysis

In 1843, Andral [12] (who introduced the study of blood as a discipline at the Paris Medical School) described a spontaneous anemia, arising without any prior blood loss. Andral proposed that the diminished quantity of blood was attributable to altered structure of the corpuscles and their 'true' destruction. Another important association between decreased RBC count and hemolytic anemia came 9 years later, when Vogel [13], in a short report, stated that the coloring matter in the urine is the same as that in the blood, and he suggested that the matter in the urine consists of a 'decomposition of blood discs'. Vogel suggested that the degree of blood decomposition can readily be ascertained by the degree of coloration in the urine, and he recorded a connection between fevers, colored urine, decomposition of the blood, and anemia, recognizing an anemia secondary to infections. Alfred Donne (1801–1878) made many contributions to hematology by microscopic study of the blood, and there was increased interest in quantitative aspects in the properties of blood in the middle of the 19th century [14], e.g. enumerating the components of the blood (Vierordt 1851), blood volume and RBC volume measurements (Weleker 1854), the hemocytometer (Malassey 1873) and the color index to evaluate anemia (Hayem 1877) [15].

There were several reports on PCH in the 19th century. In 1854, Dressler [16] described PCH in a 10-year-old boy, who, after exposure to cold, passed red urine that gradually paled to a natural color; microscopic examination of the urine showed a 'dirty brown pigment' and no blood cells. The patient was treated quinine, red wine, and an infusion of China root, which led to a return to 'relatively good health.' Four more cases of PCH with similar symptoms were reported by the end of the decade [17], although in these quinine was ineffective. In 1871, an important contribution to the history of hemolytic anemia was made by Vanlair and Masius [18], who indicated that premature destruction of RBCs leads to jaundice. Their patient had anemia and splenomegaly, and reddish brown urine; they described spherical dwarf cells in the peripheral blood that they called microcytes (likely spherocytes, a hallmark of immune-mediated extravascular hemolysis), and they named the

condition microcythemia. The authors proposed two mechanisms for jaundice: 'mechanically by reabsorption, or liver-induced' and 'paradoxical icterus.' Paradoxical icterus, as in hemolytic anemia, was said to be caused by excessive release of a colored material from the blood, leading to bile formation and deposition in the tissues. More explicitly, they stated that 'there are at least a certain number of non-mechanical types of icterus which are caused by the exaggerated destruction of red cells and the transformation to bilirubin of released hematin.' In these words an idea is expressed that was essentially correct, but which was then ignored or forgotten for the next 30 years [2].

In 1879, Stephen Mackenzie, in London, did research on the pathophysiology of PCH [19]. He reported a young boy with symptoms similar to those described by Thomas Addison in 1868 [20]. Addison had described a patient with an idiopathic or primary anemia and no previous blood loss, with no organomegaly, attributing this to 'a disease of the supra-renal capsules.' Mackenzie, however, showed that the black urine contained abundant hemoglobin and no RBCs and that rewarming the child resulted in clear urine. The urine hemoglobin described by Mackenzie is likely the 'hematin' described by Vanlair and Masius [18], which was transformed into urobilinogen. Mackenzie implicated the 'proteid' hemoglobin, the coloring matter of the blood, for the range in tints from 'London porter to deep red' and recognized that 'it follows that blood solution or disintegration (hemolysis) must take place in some part of the organism' [19]. Because of the lack of jaundice in the patient, Mackenzie believed that the hemolysis occurred in the 'genito-urinary apparatus' and that 'in the highest degree of probability the seat of blood solution is the kidney'; he identified the enlarged spleen in the patient, but dismissed this organ as the seat of hemolysis because of the lack of hemoglobin in the circulation. Because chills and rigors usually preceded the liberation of hemoglobin, he regarded paroxysmal hemoglobinuria as a 'vasomotor neurosis,' suggesting that the vessels of the glomerulus constrict with such force as to push the RBCs into the glomerular lumen, where the pressure causes them to disintegrate, thus elaborating on a theory proposed by Pavy in 1866 [21]. Mackenzie's nervous disorder hypothesis and kidney-mediated hemolysis stood in contrast to that of van Rossem [19], who held that 'corpuscles are dissolved by oxalate of soda in the bladder'. Mackenzie's hypothesis was reiterated in 1899 when Mannaberg and Donath described a case of paroxysmal hemoglobinuria, emphasizing the skin reaction to be a vasomotor disturbance, suggesting an increased excitability of the vasomotor system to be an essential factor (quoted by Harris et al. [22]).

Types of Hemolytic Anemias

The late 19th century saw focus on differentiating between the congenital and acquired types of hemolytic anemia. Wilson [23, 24] in 1890 described 'a condition in which an enlarged spleen, accompanied by a sallow or subicteric complexion, appears as an hereditary condition ... showed rapidly progressing anemia, dependent upon an active hemolysis of splenic origin', leading to the name chronic splenic cachexia – this case may in fact represent an early description of hereditary spherocytosis. Hayem [25] and Minkowski [26], in 1898 and 1900, respectively, showed that the jaundice associated with hemolytic anemia was distinct from that of hepatic diseases. Hayem made the distinction between congenital and acquired hemolytic anemias, whereas Minkowski described only a hereditary condition, but both placed the blame for hemolysis on an abnormality of the spleen. Hayem has therefore been said to be the first to describe acquired hemolytic anemia, which he coined chronic infectious splenomegalic icterus [25]. Minkowski is credited with the first clear recognition of icterus due to hemolytic anemia (chronic hereditary acholuric icterus), separate from an obstructive jaundice; he associated the anemia with urobilinuria and splenomegaly and postulated that RBC destruction was attributable to lesions in the spleen [26]. In 1901 Landsteiner [27] discovered the ABO blood group system, leading to an understanding of RBC antigens, which would subsequently be found to have relevance for autoantibody specificity in AIHA. Three years later, Donath and Landsteiner [28] proposed a new mechanism for in vivo hemolysis, implicating a hemolytic substance in the serum as responsible for PCH; their study is considered the first to describe an autoantibody leading to an autoimmune disease. Their analysis showed that hemolysins in the patient's blood caused an agglutination reaction at low temperatures, and on subsequent rewarming, in the presence of complement, the hemolytic reaction occurs. Further contributing to subsequent understanding of the pathophysiology of immune-mediated RBC destruction, Metchnikoff [29] in 1905 described macrophages.

French scientists at the time explored the mechanisms of hemolytic anemia, dividing acquired hemolytic jaundice into 2 groups: cryptogenetic and secondary. Chauffard et al. [30–32] were the first to describe autohemolysins in patients with acute acquired hemolytic jaundice. They described patients with hemolytic icterus whose serum had the capability of hemolysing RBCs, and they termed the condition 'hemolysinic icterus'; it was acute in course and associated with hemoglobinuria. Thus, a definitive distinction was made between acute paroxysmal hemoglobinuria and congenital hemolytic icterus. Chauffard et al. implied that the hemolysin was endogenous and the primary cause of the hemolysis. Chauffard et al. also standardized the osmotic fragility test (a diagnostic tool of the age), described reticulocytes and their increased numbers in congenital hemolytic icterus (later to be known as hereditary spherocytosis), and drew attention to the 'microcytic' nature of the RBC in some hemolytic anemias. Between 1908 and 1912, Widal et al. [33, 34] introduced the term acquired hemolytic anemia, noting several cases in which the disease was not congenital but rather associated with various infections and intoxications and in which the course was either gradual or sudden; they remarked on a slight difference in the fragility test as compared with the congenital type, marked reticulocytosis and, importantly, the autoagglutination of RBCs (consistent with IgM-mediated AIHA). Hence, at this time the 2 types of hemolytic anemia were recognized: the congenital form of Minkowski and Chauffard et al. and the acquired form of Hayem and Widal et al.

Mechanisms of RBC Destruction

While Landois [35] had, in 1875, reported that agglutination of RBCs can be induced by the serum of an animal of another species and while Landsteiner [27] had made the first observation of agglutination of human RBCs by serum of the same species, these were due to alloantibodies, and it was Widal et al. [33, 34] who observed autoagglutination of erythrocytes, and their work, as well as others, was summarized at the 12th Congres Francais de Medicina, at Lyon in 1911, where the role of hemolysins in pathology was a major topic [2]. By now the role of the spleen was widely accepted as being the major site of hemolysis and that of the liver had been acknowledged although it was not generally regarded as a significant site of erythrocyte destruction. Therefore, it is not surprising that in 1911 Micheli [36], in Turin, performed the first splenectomy for acquired hemolytic anemia. Banti, in 1912, introduced the term hemolytic splenomegaly, when he observed that the spleens of animals undergoing hemolysis were enlarged and congested [37], and noted that the rate of hemolysis in animals with immune hemolytic serum was slower in splenectomized animals. Banti implicated the splenic endothelial cells as erythrophagocytes and described agglutinated RBCs within the splenic pulp. He also described the same activity in the Kupffer cells of the liver, but only under conditions of extreme hemolysis. Thus, Banti effectively described the role of the reticuloendothelial system (RES) in RBC hemolysis; he recognized the importance of the spleen to the disease, but that it was not the only, nor even the prime, site of RBC destruction. The observations of Micheli and Banti entrenched splenectomy as a treatment for hemolytic anemia, representing the first specific therapy for AIHA. Despite the widespread acceptance of the benefits of splenectomy, however, some, such as Antonelli [38] in 1913, refuted Banti's hemolytic splenomegaly as a separate disease, pointing out that it did not differ from acquired hemolytic anemia. By this time, much had been learned about RBC fragility, reticulocytosis, autoagglutination, and hemolysis, but then a period of relative quiet occurred in elucidation of AIHA. Dameshek and Schwartz [39] described a patient in 1914, who, after splenectomy, was found to have 'dense, hemoglobin-laden microcytes,' subsequently called spherocytes by Naegeli (1919) [40], although Dacie [41] showed that the first description and the coining of the term spherocyte was rather by Christophers and Bentley (1909), who had observed spherocytes while studying blackwater fever in India [42] and suggested that a change in the elasticity of the RBCs accounted for its altered morphology, preventing it from appearing as the common flat disc. Treatments other than splenectomy were applied for cold-induced paroxysmal hemoglobinuria; e.g., Tileston [43] used cholesterol to inhibit hemolysis with an effect in the test tube but of only temporary benefit in patients.

The general understanding of the mechanism of RBC destruction to the 1920s was that it resulted from autoagglutinin-induced agglutination, the first step in hemolysis. Lederer (1925, 1930) [44, 45] and Brill (1926) [46] described transfusion-responsive acute hemolytic anemia associated with infectious diseases; because much of the prior French work had been forgotten these were thought to be a new disease, known as Lederer's anemia, but it is likely that they were examples of AIHA [2]. Neglect of the prior literature resulted in a loss of the distinction between the congenital and acquired forms of hemolytic anemia – as well as a neglect of the serological tests for autohemolysins, isohemolysins, and heterohemolysins. This led to claims in the 1930s of autoagglutination in hemolytic anemia being observed for the first time. In England at that time it was believed that hemolytic anemia in adults was a form of hereditary spherocytosis [47]. Dawson (1931) [48] claimed that 'in the sense that latent defects may be accentuated so as to become clinically manifest, hemolytic anemia can be acquired, but the defects themselves are inborn'. Doan et al. [49] disagreed, stating that 'we believe that a sharper differentiation should be made between the various mechanisms responsible for the nonhereditary forms of hemolytic anemia … designating them as "acquired", "atypical", or "pseudo" with full realization that these latter symptoms are fundamentally different …'

With no laboratory tests to identify RBC-bound IgG autoantibodies and complement, the obvious clinical signs and symptoms of PCH (and CAD) led to focus on these patients. In 1936, Witts [50] recognized that hemoglobinuria is most always the result of intravascular hemolysis and follows hemoglobinemia, and that, although the symptoms of shock, anemia, reduced urine production and thrombosis are common to all types paroxysmal hemoglobinurias, 'haemolytic anaemia with paroxysmal haemoglobinuria' is the most common type of paroxysmal hemoglobinuria. During the same period, Dameshek and Schwartz [51] described cases of acute hemolytic anemia responding to splenectomy, reporting that the patients' sera were able to lyse allogeneic as well as autologous RBCs and that the amount of spherocytosis was proportional to the hemolysin titer. Although before 1940 it was hypothesized that WAIHA was the result of 'undue stasis and destruction of blood in the splenic pulp' [52]; by 1940, Dameshek and Schwartz [39] had confirmed the existence of acquired hemolytic anemia, with spherocytosis, increased fragility, and abnormal serum hemolysins, and suggested an immunologic cause resulting from the action of hemolysins on RBCs rather than abnormal RBC production in the marrow. In short, they described what would become the 'outline for our modem concepts of the clinical and serological implications of autoimmune hemolytic anemia' [2].

Advances in Diagnostic Tools and Therapies

1940–1960 saw important advances in diagnostic tools, hypotheses for cause and pathogenesis of AIHA, and new therapeutic approaches. This was in large part due to growth in serological studies. In 1944, Race [53] and Weiner [54], separately concluded that there were 2 types of Rh antibody, one that bound to the RBC surface and caused agglutination (the 'complete' antibody; IgM), and one that also adsorbed to the RBC surface but did not cause agglutination (the 'incomplete' antibody; IgG). A major diagnostic advance, critical to elucidation of the pathophysiology of AIHA, occurred in 1945 with the description of the antiglobulin test by

Coombs et al. [55] using rabbit anti-human sera to detect incomplete antibodies bound to human RBCs. A similar procedure had in fact been developed by Moreschi in 1908 [56], but because the concept of incomplete antibody was unknown at that time, the clinical value of this test was not then appreciated. In 1946, Boorman et al. [57], using the direct antiglobulin test (DAT), recognized the association of idiopathic hemolytic anemia and incomplete antibody on the RBC surface, and this became the definitive test for the diagnosis of AIHA, allowing clear distinction from congenital hemolytic icterus. It was found that some patients had AIHA despite a negative DAT, and in 1971 Gilliland et al. [58] showed that DAT-negative patients with AIHA do have increased RBC-bound IgG autoantibody, but at levels below the sensitivity of the serological DAT.

In the 1940s studies focused on mechanisms of AIHA and the involvement of the spleen. In 1948, Wagley et al. [59] observed that, after splenectomy, the amount of 'sensitizing agent' on RBCs was decreased, suggesting the spleen as primary site of production for the agent. However, cases of failure of splenectomy to result in remission led Evans and Duane [60] to propose the lymphatic and RES as alternate sites, although it was appreciated that the reason for failure of splenectomy might be the presence of an accessory spleen. By 1949, it was recognized that the sensitizing plasma protein was a globulin, but there were still two distinct views held: i) that agglutination was a result of the disease and ii) that the number of antibodies bound per RBC determined the state of the disease; and the literature still stated that 'evidence is needed to show that the hemolytic agent is a specific immune response to an antigen common to erythrocytes' [60]. Evans and Duane delineated the symptoms most often associated with AIHA, i.e., chronic anemia, reticulocytosis, increased serum bilirubin, increased fecal urobilinogen, and erythroid hyperplasia of the bone marrow. While recognizing the importance of antibodies in AIHA, Evans and Duane believed that there was no evidence that hemolysis occurs as a result of the fixation of complement. Thus, the first half of the 20th century closed with a more complete clinical understanding of the disease and beginnings of understanding its mechanisms.

The Latter Half of the 20th Century

Young et al. [61] in 1951 were the first to coin the term autoimmune hemolytic anemia; it was theorized that production of autoantibody resulted from a breakdown in the 'regulatory contrivances,' leading to autoimmunization. Yet some refused to believe that the RBC-coating globulin was in fact an autoantibody and the name 'antiglobulin-positive hemolytic anemia' was employed [2], but by 1960 this term had disappeared and the autoantibody theory was entrenched.

Studies in the early 1960s examined the variable age ranges for AIHA. Although WAIHA was believed to be rare in the young, Iafusco and Biffa [62] in 1962 documented WAIHA in a newborn. There was further recognition of the variety of illnesses associated with AIHA, and Pirofsky found 18% of AIHA to be idiopathic [2], in contrast to the studies of Dacie (1962) [47] and Dausset and Colombani (1959) [63] reporting 70% of cases to be idiopathic. In this period, there were investigations on the properties of the antibodies involved in AIHA. For example, Dacie [47] reported on the variations in temperature range and pH amplitude of the agglutinating and hemolysing properties of patient sera in CAD, and Schubothe [64] suggested that the pathological cold agglutinins were modified paraproteins with many individual differences. Immunosuppressive drug therapy was widely employed during this period, in response to the emerging concept that an immunologic inadequacy (failure of immunosurveillance) resulted in a buildup of the cells that make autoantibodies. In 1971, Dacie [65] summarized the existing hypotheses of autoantibody formation: response to modified RBC antigens, not true autoantibodies but a cross-reactivity to viruses, and spontaneous development of 'forbidden' clones of antibody-forming cells. Dacie, like most others, favored the third hypothesis, a failure in immunologic surveillance.

Studies in the 1970s focused on the antibodies involved in AIHA, and it was recognized that in WAIHA IgG1 and IgG3 predominate but that IgG2 was not uncommon, nor were IgA antibodies [66]. The mechanisms of complement-mediated hemolysis were elegantly elaborated by Schreiber and Frank [67]. Before these studies, it was thought that CAD manifested itself at low temperatures because of a temperature-related change in the surface membrane of RBCs or a change in the IgM antibody, but Frank et al. [67, 68] elucidated the significance of RBC-bound complement and its involvement in both intravascular and extravascular hemolysis. They observed that in AIHA, RBCs coated with complement are sequestered in the liver, whereas those coated with IgG only are taken up in the spleen. They postulated three mechanisms for destruction of RBCs in AIHA: i) C5b-9 terminal attack complex complement-mediated intravascular lysis, seen in IgM-mediated CAD and less commonly in WAIHA (with IgG autoantibodies lgG1 and IgG3 and less commonly IgG2 capable of activating complement); ii) adherence of C3b-coated RBCs to complement receptors on hepatic macrophages, seen in both CAD and WAIHA; and iii) adherence of lgG-coated RBCs to fragment cristalline receptor (FcR) on splenic macrophages, seen in WAIHA. Quantitative studies were performed on the relation of the number of IgG and of C3 molecules bound to RBCs and the severity of the hemolysis [69–72]. Variant and mixed forms of IgG and IgM autoantibodies were recognized [73–75].

Investigations in the 1980s on the immunologic mechanisms of AIHA and the relationship of the autoantibodies to the antigens showed that type II MHC glycoproteins mediate the presentation of antigens by macrophages to T cells, and cytokines were shown to be important in the macrophage-dependent activation of antigen-specific T cells. Studies on the substrate-receptor interactions showed that monocytes and macrophages have receptor sites for the Fc portion of IgG and the C3b component of complement [76], and the role of these receptors in phagocytosis was confirmed [77–79]. As reviewed by Garratty [80], knowledge of the autoantibody specificities for blood group antigens was refined.

Pathogenesis of Autoimmunity

The existence of autoimmune diseases in humans had, in fact, been known for almost 100 years, and over time we have learned that autoimmune diseases result from the failure of normal self-tolerance mechanisms. We have learned that this might be the result of several non-mutually exclusive mechanisms, such as a failure of central tolerance leading to the abnormal accumulation of self-reactive T cells, environmental stimuli that can mimic self-antigens (antigenic mimicry), aberrant self-antigen expression, cytokine secretion, and/or defects in co-stimulation. We have learned that AIHA is mediated by autoantibodies that opsonize erythrocytes leading to their enhanced destruction by Fc receptor-mediated phagocytosis by cells of the RES primarily within the spleen. Although the disease is mediated by autoantibodies, there is increasing evidence to support that their production is crucially dependent on abnormal T-cell reactivities and understanding these T-cell-mediated mechanisms is important for designing immunotherapies to specifically downregulate the autoantibody production.

In the early 1900s, Ehrlich and Morgenroth [81] showed that animals did not produce antibodies to their own RBCs and introduced the concept of 'horror autotoxicus' to describe the mechanisms that prevent the adverse effects of autoimmunization through production of an 'autotoxin.' At the same time, presence of autoantibodies under normal conditions or after immunization was observed [82]. Nonetheless, the opinion that autoimmunization could not occur persisted. However, the experiments of Owen [83] in 1945 and Medawar [84] in 1953 led to the hypothesis that in the embryo the immune system learns to tolerate self-antigens, and autoreactive lymphocytes are deleted. These observations and Jeme's natural selection hypothesis [85] led Bumet [86] to propose the clonal selection theory of antibody formation. It was believed that, with this self-tolerance, autoantibodies could only arise because of somatic mutation and expression of forbidden clones. It was shown that induction of autoantibodies against non-sequestered antigens can result in disease [87] and that autoantibodies could occur under normal conditions (natural autoantibodies) [88], and there was recognition of autoreactive B lymphocytes [89]. So it appeared that the precursor B cells that produce these autoantibodies must escape deletion. Autoantibody production could result from uncontrolled polyclonal stimulation of normal autoreactive B cells, resulting in increased levels of natural autoantibodies [90], and/or pathological autoantibodies from an antigen-driven selection of autoreactive B cells, which, under the selective pressure of autologous antigen, undergo somatic mutations, producing pathological autoantibodies [91]. Thus, we have gone from 'horror autotoxicus' to the forbidden clone theory to autoimmunity being a natural physiological process.

While AIHA is characterized by the production of antibodies directed against self-RBCs and idiopathic or primary AIHA shows no apparent association with an underlying disorder, given the frequent association between AIHA and other autoimmune disorders, generalized immune system dysfunction probably also plays a role, supporting the concept that a generalized dysfunction of immune surveillance is present in patients with AIHA. Studies in animal and human AIHA suggested that loss of immunological tolerance to RBC self-antigens might originate by different, non-mutually exclusive mechanisms that include antigenic mimicry, apoptosis, and immunoregulatory disorders including cytokine network alteration. It was found that IgM autoantibodies or cold agglutinins generally react with polysaccharide antigens on the RBC surface and are usually associated with neoplastic B-cell populations [92, 93]. By contrast, IgG warm autoantibodies generally react with protein antigens on the erythrocyte surface and are typically panagglutinins, reacting with all cells [94]; immunoblotting studies implicated Rh antigens, membrane protein band 4.1, protein band 3, and glycophorin A as universal RBC targets. The association of WAIHA with systemic autoimmune disorders suggested that these autoantibodies, in contrast to the clonal cold-reactive autoantibodies, might arise from polyclonal activation rather than from activation by specific (self-)antigen [95]. Infectious agents were shown to be associated with the development of anti-erythrocyte antibodies, and it was suggested that virus-induced AIHA is a T-helper-dependent autoimmune event [96]. Studies showed that activation-induced programmed cell death may also play an important role in regulating autoimmunity, and this is also true for AIHA [97–99], e.g., defective apoptosis of auto-aggressive T cells expressing IL-2 receptors can play a role in initiating AIHA pathogenesis.

In vitro and in vivo experiments have suggested that quiescent T and/or B cells specific for self-antigens might be activated in AIHA if adequate antigen presentation and co-stimulation occur [100]. Synthetic peptides corresponding to the Rh polypeptide sequence, the most frequent target for autoantibodies in human WAIHA, were shown to provoke T-cell activation and their proliferation could be blocked by anti HLA-DR antibodies. It was suggested that autoreactive T cells in AIHA might not be deleted, but are anergic to autologous Rh polypeptides and thus immunologically ignorant against RBC self-antigen [95, 101]. There was also experimental evidence that autoantibody production in some AIHA cases is caused by the activation of class-II-restricted helper T cells specific for cryptic Rh epitopes [102]; these autoreactive T cells seem to escape the clonal deletion and anergy during the induction of self-tolerance and remain quiescent even if the autoantigens they recognize are present. Several mouse strains, e.g. NZB and NZB/NZW, which spontaneously develop a complex autoimmune syndrome including AIHA, have been extensively studied to identify the immunological factors contributing to the autoimmune onset of AIHA [102–109] and have provided experimental evidence to support an antigen induction model of AIHA. In mice, RBC membrane band 3, a RBC anion exchange protein, appeared to be the major antigen for RBC autoantibodies.

Thus, an ongoing autoimmune process can be viewed as a rather fine-tuned and fragile equilibrium of aggressive and regulatory components, and the precise activation kinetics and survival times of all lymphocyte types implicated in the process will determine the outcome.

Therapies

Over the past half century, there have been extensive efforts to find effective therapies, particularly for patients refractory to first-line treatments. Glucocorticoids, first used in 1951 [110], have remained the mainstay of first-line treatment of WAIHA and splenectomy for patients who do not respond to steroids. Intravenous immunoglobulin, cyclosporine, and other immunosuppressive drugs may be used, but with variable success, and after 50 years of relatively little progress in management of refractory AIHA, the discovery of the anti-CD20 antibody rituximab has offered promise. There is a considerable literature showing the successful (albeit sometimes short-term) benefit of rituximab in both CAD and WAIHA [111]. Elucidation of the mechanisms of AIHA is contributing to development of novel immunotherapy strategies, e.g. alemtuzumab, ofatumumab. Identification of optimal cellular targets, forms of antibody, targeted antigens, and disease-related cytokines will allow soluble receptors, monoclonal antibodies, and molecular mimetics to be developed as specific novel immunotherapeutics. Of interest is the recent novel concept of administering erythropoietin to AIHA patients (despite their usually having compensatory increased RBC production) showing effectiveness in refractory cases of both CAD and WAIHA [112, 113].

Hence, although much has been learned, research is still needed for complete elucidation of the mechanisms of the immune dysregulation in AIHA. Better understanding of the underlying mechanism(s) may allow for development of more specific therapies of these not uncommon and often difficult-to-treat disorders.

'This story should also serve as a cautionary tale for us today. The history of science and medicine tells us that not everything that we regard as true today will hold up with the passage of time. Science and scientific medicine are always works in progress. Their conclusions are tentative and provisional, subject to correction as new data appear.'
(Irving Kushner) [114].

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