Warm autoimmune hemolytic anemia: Advances in pathophysiology and treatment

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Autoimmune hemolytic anemia (AIHA) is a rare acquired autoimmune disease in which auto-antibodies directed toward autologous red blood cells (RBCs) membrane antigens lead to their accelerated destruction [1–3]. The diagnosis of AIHA is thus based on the presence of a hemolytic anemia with a positive direct antiglobulin (or Coombs) test (DAT) and on the absence of any other hereditary or acquired cause of hemolysis [1]. AIHA can affect both children (mainly before the age of 5) and adults (mean age at diagnosis is around 50 years) and its estimated overall (not age-adjusted) annual incidence is approximately 1 to 3 per 100,000 individuals [3–5]. Among AIHAs, AIHA due to warm antibody (wAIHA) accounts for 70 to 80% of all cases in adults and for almost 90% of the cases in children [2,4,6]. wAIHA can be either primary (or idiopathic) or associated, in at least half of the cases, with or reveal an underlying disease and be therefore classified as “secondary” wAIHA [1]. The aim of this review is to provide an update on the recent advances on pathophysiology mostly extrapolated from animal models and also on the treatment of adult’s wAIHA taking into account some recent data from the very first prospective studies that have been recently reported.
**Pathophysiology**

The pathogenesis of wAIHA is a complex multistep process involving not only the auto-antibodies directed towards RBC’s membrane antigens but also various effectors of the immune system including the complement system, macrophages as well as B and T-cells [7]. Whereas the mechanisms leading to hemolysis are partially elucidated (antibody-dependent cell-mediated cytotoxicity and complement dependent cytotoxicity are primarily involved), the mechanisms leading to the breakdown of self-tolerance are still far from being understood.

In wAIHA, the autoantibody targeting RBCs is mainly of IgG1 isotype and less frequently IgG3 [2]. It is able to bind macrophages via the Fc gamma receptor and therefore hemolysis is extravascular and takes place mainly in the spleen. Autoantigens on RBC’s membrane targeted by autoantibody are by decreasing frequency:
- peptides from the Rhesus system;
- band 3 protein;
- glycophorin A [8].

In approximately 10% of the cases of wAIHA, no specificity is found. The abnormal production of autoantibody directed towards RBC’s antigens could be the consequence of different and non-mutually exclusive mechanisms: an immune response towards some cryptic antigens and/or a molecular mimicry with a cross-reactivity between external antigens and auto-antigens. The presence of cryptic antigens has been suggested by Barker et al. who have shown that synthetic peptides with a sequence homologous to the one of the rhesus antigen were able to stimulate in vitro peripheral blood monocyte cells (PBMCs) from AIHA patients but not PBMCs from healthy donors [9]. In a mouse model described by Playfair et al., the mice transfused by RBCs from rats develop some autoantibody towards autologous RBCs suggesting cross-reactivity promoted by the presence of allo-antigens and the resultant hemolysis [10]. The membrane glycoprotein CD47 plays a protective role towards the splenic RBCs’ phagocytosis in mice [11]. In combination with proteins of the Rhesus system, CD47 binds the signal recognition protein (SIRP)-alpha protein signal on macrophages blocking their activation and avoiding phagocytosis. Conversely in the absence of CD47, RBCs are rapidly eliminated by the reticuloendothelial system as it has been shown in CD47 -/- NOD mice [12]. There is no evidence so far in humans suggesting that a defect of CD47 is involved into the pathogenesis of wAIHA. In another model, Miwa et al. have shown that mice lacking complement receptor 1 (CR1 or CD35) and DAF or CD55 have a complement-mediated hemolysis [13]. In humans, an acquired CR1 deficiency has been reported in some autoimmune diseases such as systemic lupus erythematosus (SLE) but an abnormal expression of CD35 and CD55 in AIHA has not been reported.

A polyclonal activation of both B and T-cells is likely to play a role in AIHA. As an example, in mice infected with lactate dehydrogenase-elevating virus (LDV), or mouse hepatitis virus, and treated with anti-erythrocyte or anti-platelet monoclonal auto-antibodies at a dose insufficient to induce clinical disease by themselves, the infection sharply enhances the pathogenicity of auto-antibodies, leading to severe anemia or thrombocytopenia [14]. This effect is observed only with antibodies that induce disease through phagocytosis. In humans, a positive direct antiglobulin test is more frequently observed in patients with chronic infections leading to hypergammaglobulinemia such a HIV infection or leishmaniosis [15,16]. In non-infectious settings combining hypergammaglobulinemia and immune dysregulation such as in the autoimmune lymphoproliferative syndrome (ALPS) [17] or yet the angioimmunoblastic T-cell lymphoma [18], a significant proportion of patients have a positive DAT with or without active hemolysis.

Regarding T-cell activation, based on the few data available in the literature, in patients with active wAIHA compared to healthy controls, there is a disequilibrium of the CD4+ T Helper 1(Th1)/Th2 balance with an increase of TH2 cells subsets and an increased expression of both interleukin-4 (IL-4) and IL-10 and a reduced expression of interferon-γ and IL-12 [19-21]. This ‘TH2 profile’ promotes the induction and proliferation of auto-reactive B-cell clones. More recently, it has been shown that the production of the effector cytokine IL-17 but not the one of Interferon-γ was strongly associated with wAIHA compared to healthy donors and correlated with the severity of the disease [22]. This observation may preclude some future therapeutical perspectives with the recent development of anti-IL17 monoclonal antibodies in other autoimmune diseases [23]. Lastly, the implication of a T regulatory cells’ (Tregs) defect in the loss of tolerance in AIHA has been mainly suggested through the study of animal models. NZB mice are known to develop spontaneously an AIHA at an adult age and among other mechanisms a progressive, although controversial, quantitative and/or functional defect of Tregs has been raised [24,25]. More convincing data come from IL-2 receptor and IL-2 knockout mice models developing early a severe AIHA [26,27]. In this model, the transfer in the neonatal period of Tregs from wild-type mice to IL-2 R -/- recipient mice effectively prevent the occurrence of AIHA [27]. On the other hand, mice strains with a mutation of Foxp3, the master regulator of the regulatory T-cell lineage, develop at day 24 a fatal AIHA with a multiorgan infiltration [28]. Lastly, in a mice model, the use of anti-CD25 monoclonal antibody before the immunization of mice with RBCs from rats increases the incidence of AIHA from 30 to 90% [29]. In this same model, the adoptive transfer of splenic CD4 + CD25+ lymphocytes from immunized mice to naive mice prevent from the induction of auto-antibodies and these results are not reproduced with the transfer of CD4 + CD25- lymphocytes [29]. In humans, very few data on
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the potential role of Tregs in AIHA is available. Studies from Hall et al. have shown that in the peripheral blood from AIHA patients, there are some Tregs specific of autoantigens from the Rhesus system able to inhibit in vitro the Th1 effector immune response through an IL-10 dependent mechanism [21]. More recently, Ahmad et al. have shown a significant decrease in the rate of Tregs (4.63% versus 9.76%) in patients with active AIHA compared to healthy donors [30]. Indirect evidence suggesting that a decrease in the number and/or function of Tregs is likely to play a role in AIHA in humans comes from the immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, a rare inherited disease linked to the dysfunction of the transcription factor FOXP3. Patients diagnosed with an IPEX syndrome have a high risk of developing a number of autoimmune manifestations (enteropathy, endocrinopathies including diabetes) including in a lesser extent AIHA [31].

Treatment of wAIHA

The management of wAIHA has long been and still is mainly empirical or based on retrospective uncontrolled studies [32,33]. It is only recently that the first 2 prospective studies including one randomized-controlled study focused on adult’s wAIHA have been reported.

Transfusion and other supportive measures

Folic acid supplementation (5 to 10 mg per day) is warranted in patients with active wAIHA and increased erythropoiesis in order to prevent further vitamin B9 deficiency that may be misinterpreted as a treatment failure. RBC transfusions are also indicated in patients with disabling symptoms of anemia and/or a poor underlying cardiovascular condition (i.e. coronary artery disease or heart failure). While younger patients may tolerate a stable hemoglobin (Hb) level as low as 6 g/dL, in patients with comorbidities, maintaining an Hb level of at least 8 g/dL or beyond is usually recommended. It is important for the managing physician to understand that no patient with symptomatic AIHA should be denied blood transfusions because of an “incompatible crossmatch”. The blood bank should be informed of the patient’s status. Indeed, the patient’s positive DAT almost always interferes with compatibility testing so the role of the blood bank is to provide packed red cells that are the “less incompatible” ones in regard to the specificity of patient’s autoantibody and blood group genotyping to allow antigen-matched transfusion [34]. A close communication and cooperation between the clinician and the specialist in transfusion medicine is therefore essential for reducing the risks associated with transfusion in patients with AIHA. Because transfused RBCs can be destroyed by the patient’s auto-antibodies, rapid transfusion of large volumes of RBCs must be avoided as they can have serious consequences. This risk is increased if the patient also has alloantibodies induced by previous transfusions of pregnancy. Packed RBCs units should therefore not be administered at a rate that exceeds 1 mL/kg/hour. Of note, in some patients with a severe and refractory wAIHA needing repeated transfusions and a relatively low reticulocytes count (e.g. below 250,000/µL), the transient use off-label of an erythropoietic stimulating agent at high dose (for example darbopoetin alpha at 100 to 150 µg/week) may be helpful ([35] and personal unpublished data). Conversely, there is no evidence supporting the efficacy of plasma exchanges in patients with severe wAIHA [36].

Fist-line treatment for primary wAIHA

Primary wAIHA usually has a chronic course and except for the very few unusual patients with a mild compensated hemolysis with a normal or almost normal Hb level, a treatment is needed in the large majority of the cases in order to improve RBC survival and significantly and durably increase the Hb level. Corticosteroids are the cornerstone of therapy and they must be given as first-line treatment [32,33]. Intravenous immunoglobulin (ivIG) has only little efficacy in wAIHAs [37] and due to its cost should be considered (at 2 g/kg over 2 days) only in severe and transfusion-dependent wAIHA and in the absence of response to corticosteroids (see figure 1). The corticosteroids regimen is usually based on oral prednisone (or prednisolone) given at an initial daily dose of 1 to 2 mg/kg keeping in mind that there is no robust data showing that both the time to achieve a response and/or the magnitude of the response are modified by the use of a daily dose of more than 1 mg/kg [32]. By analogy with other autoimmune diseases, the use of intravenous methylprednisolone at a dose range of 250 to 1000 mg per day for 1 to 3 days or repeated pulses of dexamethasone [38] may be considered in patients with profound anemia although, again, no clinical trial has proven their greater efficacy in this setting [32]. The starting dose of oral prednisone is usually maintained for 3 to 4 weeks and then slowly tapered in case of at least partial initial response defined by a Hb level > 10 g/dL with at least a 2 g increase from baseline in the absence of recent transfusion. The total duration of treatment is not consensual, but since the likelihood of early relapse is really high if the treatment is prematurely stopped, corticosteroids should be maintained at least 3 months after a complete response (i.e. an increase of the Hb level back to normal and no active hemolysis) is achieved [33]. In adults, there is no evidence for recommending the use of alternate day prednisone before stopping the treatment. The efficacy of corticosteroids can take few days to 2 weeks and one or several blood transfusions are often necessary at wAIHA onset, especially in case of severe anemia in young children or elderly. After 2 to 3 weeks of treatment with corticosteroids, a clinically significant response is observed in 80 to 85% of the cases [6]. Except for the few patients who are truly refractory to corticosteroids, the major issue that clinicians have to deal with when treating patients with wAIHA is that approximately 50 to
60% of them turn to be dependent to corticosteroids [1,3,6]. Thus, exacerbation or relapse or wAIHA may occur either within weeks after corticosteroids withdrawal or even on treatment when the daily dose prednisone is decreased below a threshold which is usually between 10 to 15 mg [6], a dose which is associated with several adverse events on a long-term. Overall in our experience, only one third of the patients are considered as in complete remission off-treatment one year after the disease onset [6].

Second-line treatments

Danazol
In patients dependent to corticosteroids, the use of danazol, an attenuated androgen analog, at 400 to 800 mg per day, can be helpful as a corticosteroid-sparing strategy in adults requiring a dose of daily prednisone greater than 15 mg to maintain a remission [39,40]. However, due to its common androgenic side effects its use is usually rather limited in females and the potential liver toxicity makes its long-term use difficult also in men.

Rituximab
The efficacy of rituximab, the well-known chimeric monoclonal antibody that targets CD20 antigen on B lymphocytes has first been shown in refractory wAIHA in children with a response rate reaching up to 100% in some studies [41]. In adults, few retrospective series have shown that, both in primary and secondary wAIHAs, rituximab could have a great efficacy [42,43]. Of note and as previously shown in ITP, rituximab could be also effective in patients who have failed to respond to splenectomy [44]. Conversely, patients who do not respond to rituximab are able to achieve a response after splenectomy [42]. Toxicity is usually mild, although late onset neutropenia and opportunistic infections such as pneumocystis jirovecii pneumonia have rarely been reported [42,44]. In the absence of any official recommendation, the systematic use of a primary prophylaxis with cotrimoxazole seems wise in patients with wAIHA exposed to a great cumulative dose of corticosteroids and treated with rituximab. The great and promising efficacy of rituximab has recently been confirmed throughout two prospective studies, one of which
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was a randomized-controlled study [45,46]. In the first study, low doses of rituximab (i.e. 100 mg fixed-dose weekly for 4 weeks) were used in patients with either a newly-diagnosed (30%) or relapsing AIHA (70%) [44]. In the second one, efficacy and safety of rituximab given at "standard" dose (i.e. 4 weekly doses at 375 mg/m²) in combination with prednisolone was compared to prednisolone alone in patients (n = 32 in each group) with newly-diagnosed primary or secondary wAIHA [46]. Taken together and waiting for the results of another ongoing prospective controlled study, the data from the literature and especially this last study clearly support, whenever possible, the use of rituximab off-label in patients with chronic active and/or relapsing wAIHA who need to pursue a daily dose of prednisone (or prednisolone) equal or greater of 15 mg to maintain at least a partial remission (figure 1). If rituximab is administered prior to splenectomy, vaccination against Streptococcus pneumoniae ± Haemophilus influenzae type B and Neisseria meningitidis must be systematically performed whenever possible 2 weeks before rituximab as a subsequent splenectomy can be required thereafter. Whether rituximab will be officially approved and licensed in the future as a second (or even first)-line treatment in combination with corticosteroids for wAIHA is not yet established.

Splenectomy

Splenectomy has long been the main and preferred second-line option for the treatment of primary wAIHAs. The rate of sustained response after splenectomy is approximately 60–70% according to the most recent data from the literature [47,48] but predicting factors of response option are still lacking. The peri-operative risk of laparoscopic splenectomy is low and acceptable with a mortality rate of less than 1% [47]. The most feared complication remains the rare but unpredictable risk of overwhelming sepsis and laparoscopy does no more reduce the risk of postoperative thromboembolic complications especially in the portal vein system [49,50]. While this risk is well-known in some hereditary hemolytic disease, it is likely to be underestimated in patients with wAIHAIs [51]. A systematic peri-operative course of low molecular weight heparin is therefore strongly recommended in wAIHA patients who undergo splenectomy and especially in those who have positive antiphospholipid antibodies [52]. The best time for splenectomy is currently controversial now that alternatives such as rituximab are available at least in some countries. In children aged of less than 5 to 7 years, this procedure should be avoided and delayed as long as possible. In adults, it must be considered early in the course of the disease in patients who fail to respond to corticosteroids (or need high and unacceptable dose to maintain at least a partial remission) and rituximab (figure 1).

Other treatment lines

In patients with refractory wAIHA who have failed splenectomy and rituximab, the management is mainly based of everyone experience and on the few retrospective data available in the literature. The efficacy of azathioprine, cyclophosphamide, and to a lesser extent cyclosporine and mycophenolate mofetil has been reported in small cases series [32,53–55]. The good benefit/risk ratio of azathioprine makes it the better option to be considered after splenectomy or when splenectomy is contra-indicated keeping in mind however that its efficacy can take up to 3 months. More recently, the efficacy of high dose-intravenous cyclophosphamide (50 mg/kg/day for 4 days) without autologous stem cell transplant rescue has been reported in 8 patients with refractory wAIHA [55]. Cyclosporine has been used successfully in a few patients with refractory AHA [53], the usual daily dose is 3 to 5 mg per kg. Mycophenolate mofetil also lead to complete or partial responses in patients with active or refractory wAIHA in small retrospective studies [54]. In severe forms of wAIHA refractory to several treatment lines including classical immunosuppressors, the use of Campath-1H or yet autologous hematopoietic stem cell transplantation can be exceptionally considered [56,57].

Treatment of secondary wAIHAs

Systemic lupus erythematosus-associated wAIHA

Autoimmune hemolytic anemia occurs in approximately 5 to 10% of patients with SLE and is more frequently observed in patient of African ancestry [58]. wAIHA may be the sole presenting sign of the disease and may precede the appearance of other disease manifestations by several years. wAIHA in the setting of SLE is often associated with the presence of antiphospholipid antibodies with or without a definite antiphospholipid syndrome and has been associated with a higher risk of thrombosis [58]. When AIHA is the leading manifestation of SLE, its initial management is similar to the one of primary wAIHAs and it is mainly based on corticosteroids. Prednison must however be maintained at the lowest effective dose and ideally not below 10 mg/day on a long-term in order to minimize the risk of relapse and/or the occurrence of other SLE-related manifestations over time. Moreover, hydroxychloroquine at an average daily dose of 400 mg must be given in combination with prednison although its efficacy on the course of AHA is not proven.

In patients refractory to corticosteroids or in whom a daily dose equal or higher than 15 mg is needed to maintain at least a partial remission, rituximab can be considered off-label in the absence of any other severe systemic manifestation. Although it could be effective in SLE-associated immune cytopenias [59], splenectomy must be avoided whenever possible in SLE as it may increase a preexisting acquired immunosuppression and also potentially the risk of thrombosis although some reassuring data has been reported [60]. In patients who fail to respond to rituximab, intravenous cyclophosphamide can be an option as well as azathioprine or mycophenolate mofetil. The efficacy of belimumab, an anti-BAFF monoclonal antibody that has been
recently licensed for the treatment of SLE in North America and Europe has not been assessed specifically in lupus-associated wAIHA but preliminary data have shown only a moderate efficacy for treating SLE-hematological manifestations [61].

Chronic lymphocytic leukemia-associated wAIHA
Patients with chronic lymphocytic leukemia (CLL) have an increased risk of AIHA and mainly of wAIHA; up to 14% of the patients have a positive DAT at time of diagnosis and the overall prevalence AIHA is 2 to 4% [62,63]. The risk of AIHA increased with age and is also increased in males, in patients with progressive disease (stage C-CLL) and in those previously treated by fludarabine or chlorambucil alone. Biological prognostic factors include a high level of beta 2 microglobulin, a high ZAP70 and/or high CD38 expression level and an unmutated IGVH genes status [62]. The management of CLL-associated wAIHA depends on the activity of CLL. Patients with stage A CLL and active hemolysis should be managed as they have a primary wAIHA keeping in mind that rituximab is less effective in this setting. On the other hand patients with a progressive CLL must be treated more aggressively with combined regimens such as rituximab + cyclophosphamide and dexamethasone (R-C Dex) [64]. The use of fludarabine alone is contra-indicated but this drug can be used safely in combination with cyclophosphamide [62]. In patients with active CLL and refractory wAIHA, the use of alemtuzumab may be considered whereas the place of bendamustine and of the recently developed Bruton-tyrosine-kinase (Btk) inhibitor ibrutinib needs further evaluation [65].

Common variable immunodeficiency-associated wAIHA
Patients with common variable immunodeficiency (CVID) are at high risk of developing immune thrombocytopenia (ITP) and/or autoimmune hemolytic anemia (AHA). Given their underlying immunodeficiency, the long-term use of corticosteroids and/or immunosuppressive treatment may increase the risk of infection and splenectomy must be avoided. Based on a retrospective series of 34 episodes of immune cytopenias in 33 CVID patients, rituximab was shown to be highly effective and relatively safe option for the management of wAIHA in patients with CVID [66]. This treatment should be the primary second-line treatment to be considered, prior to splenectomy and/or immunosuppressors, in this group of patients at high risk of infection and especially in those with wAIHA or Evans’ syndrome. To minimize the risk of infection, initiating or continuing long-term Ig replacement therapy is strongly recommended and long-term antibiotic prophylaxis must also be used in patients who have undergone splenectomy [66].

Conclusion
wAIHA has long been viewed as a rare autoimmune disease that could be treated empirically mainly by corticosteroids on a long-term. Thanks to some informative observational studies and efforts made in the classification of wAIHA (primary versus secondary) the natural history and prognosis of the disease is henceforth better known. Based on retrospective and more recently on prospective trials, rituximab has emerged in the last decade as the preferred second-line option prior to splenectomy. As in other autoimmune diseases, avoiding the long-term use of corticosteroids in wAIHA must indeed be considered as a major objective. The increasing knowledge of the pathophysiology of the disease based mainly on animal models could serve as a relevant background for testing in the next future some new therapeutic approaches targeting either autoreactive B-cells or TH17 cells.

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