HEMATOLOGY

Commentary

Warm autoimmune hemolytic anemia: transfusion lessons and therapeutic perspectives learn from a particular patient

Jun Yu Woon[1], Li-Te Chin[2,3*], Yu-Ting Lee[4,5*]

[1]*School of Medicine and Dentistry, Menzies Health Institute Queensland, Griffith University, Gold Coast, Queensland, Australia;*
[2]*Department of Microbiology, Immunology and Biopharmaceuticals, National Chiayi University, Chiayi City, Taiwan;*
[3]*Graduate Institute of Medical Sciences, National Defense Medical Center, Taipei City, Taiwan;*
[4]*Department of Hematology and Oncology, Institute of Medicine, Ditmanson Medical Foundation Chiayi Christian Hospital, Chiayi City, Taiwan;*
[5]*Department of Biomedical Sciences, National Chung Cheng University, Chiayi County, Taiwan*

***Li-Te Chin and Yu-Ting Lee are both Corresponding Authors**

Arrived: 10 July 2024
Revision accepted: 17 October 2024
Correspondence: Li-Te Chin
e-mail: dr.litechin@gmail.com

INTRODUCTION

Previously, we have described a 23-years old female patient with warm autoimmune hemolytic anemia (wAIHA) successfully treated with fresh, ABO, Rh, Kidd, MNS and Miltenberger (Mia) blood groups-matched erythrocyte transfusion alone without using steroids; the above blood type was instantly identified in the blood registry because the patient herself had been a regular blood donor with multiple donations, thus two units (derived from 500 mL whole blood of a single donation) of match, leukocyte-depleted packed RBCs was immediately issued and transfused within 24 h from harvesting[1]. About a year after the recovery, she returned to her place of residence abroad, and then a little more than one year later, her wAIHA relapsed. During this 24-month interval, she did not take any corticosteroid regimen either. Soon after the relapse, she urgently received red cell transfusions, rituximab, intravascular immunoglobulin (IVIG) and standard steroid treatments as indicated in **Figure 1**; however, the symptoms have persisted for more than 280 days. At hemoglobulin (Hb) level of 11.7 g/dL, the patient had an autonomous withdrawal of the prednisolone tapering because of ecchymosis, severe hypokalemia, parchment-like skin, leg edema, sleep disturbance, and recurrent infections. The patient subsequently passed the medical record to us for reviewing and future surveillances.

Depending on the geographical location, healthcare may be delivered very differently and thus provide a unique opportunity for a longitudinal study. In this particular case-based longitudinal study, we critically appraise and summarize the key evidence for possible mechanisms which may contribute to a specific manifestation of wAIHA, as indicated in **Figure 1**, and propose interventions to ameliorate the risks. Aiming at the present knowledge, the results are useful, albeit debatable, but not to be ignored, on the implications of the disease management. We did not suggest that our finding could provide a substitute for the current first-line corticosteroid consensus; rather it was proposed that a mix of precautions might satisfy the needs of most scenarios and may provide some tangible advantages to steroid refractory patients.

BAND 3 AS ERYTHROCYTE SENESCENCE AND AUTOIMMUNE ANTIGENS

Comprising 50% of the RBC membrane protein, band 3 is the hub of cellular organization. While being at the center of multiprotein complexes that contains CD47, the glucose transporter, glycophorins, the Rhesus (Rh) proteins, Rh-associated glycoprotein (RHAG), and other blood group glycoproteins, the cytoplasmic domain of band 3 also provides the main anchorage site for the cytoskeleton through adducin/ankyrin-mediated attachment

Blood Transfus. 2025; 23(3): 266-270 doi: 10.2450/BloodTransfus.851

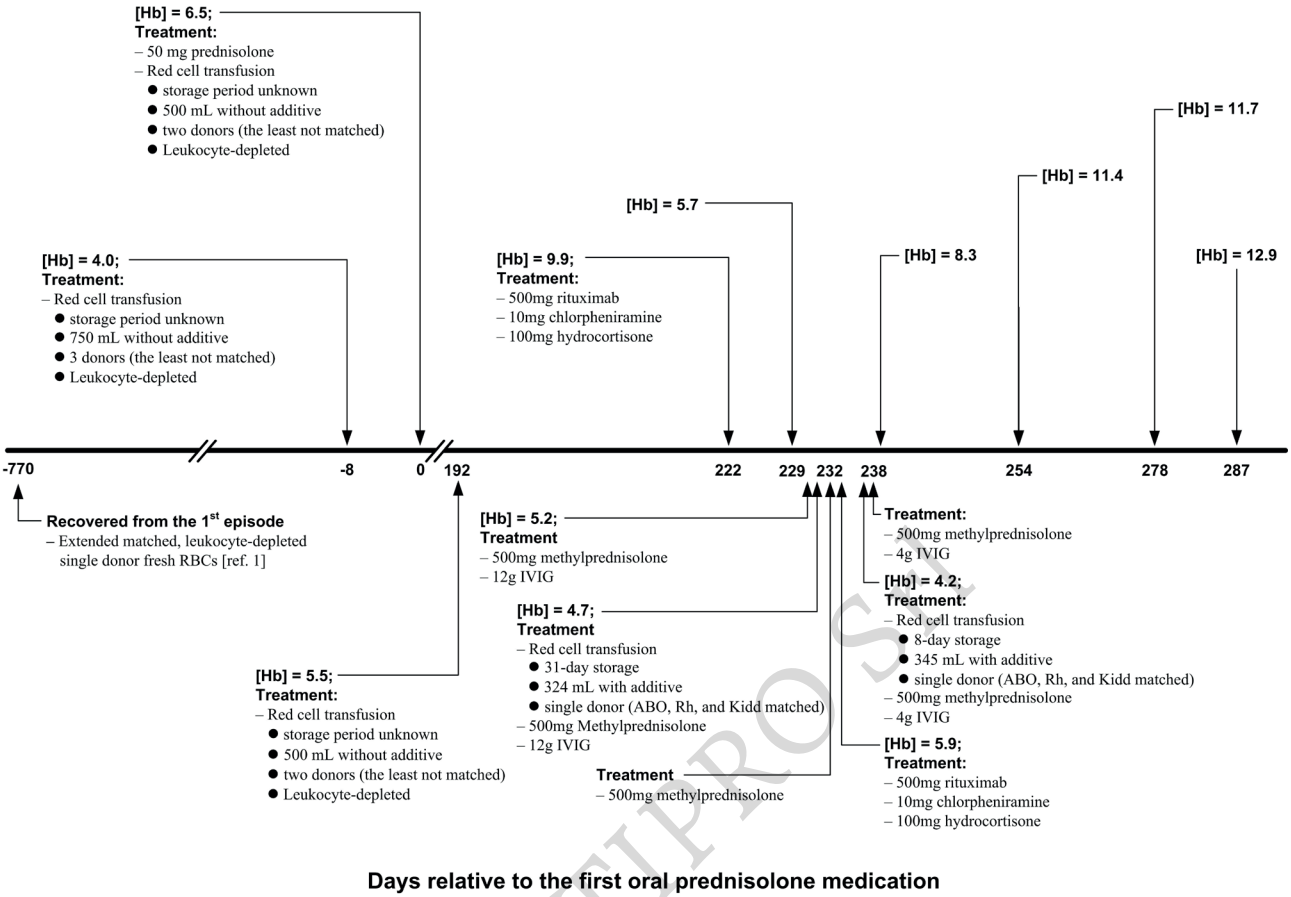

Figure 1 – Timeline for the recurrent episode of the particular patient
The serological makers of SLE, HIV, HBV, HCV, and autoimmune thyroid diseases had not been detectable during the recurrency.

of the spectrin-actin network. Thus, band 3 is at a key position in the regulation of the structure and function of erythrocytes. As recognition and elimination of senescent erythrocytes is among the first documented examples of the physiological role of the autoimmune system, senescence antigens are located on band 3 protein, in which residues 538-554 and 812-830 are two binding regions for autoantibodies[2]. Interestingly, the crystal structure revealed antigenic residues 812-830 positioned in an intracellular loop, inaccessible to extracellular autoantibodies[3]. These observations support the hypothesis that the senescent epitopes encompass residues 538-554 and 812-830 that result from a massive movement of otherwise cryptic 812-830 region in young RBCs across the lipid bilayer during ageing processes. The movement would bring the two antigenic regions in close proximity and may be prerequisites for immunological removal signals.

It seems that, as the 812-830 segment was in a transient external state, autoantibody binding would stabilize the extracellular conformational antigen until sufficient autoantibody accumulated to initiate the phagocytosis of the senescent erythrocyte through Fc receptors on macrophages[4]. Furthermore, based mainly on the degree of band 3 tyrosine phosphorylation, wAIHA patients may be interpreted in terms of severity[1] as type I, in which patients show increased band 3 tyrosine phosphorylation and autoantibodies preferentially recognize the oldest, densest RBCs; and more severe type II, characterized by an almost complete depletion of the hyperphosphorylated, oldest population.

ADDITIONAL EXACERBATING FACTORS

With respect to the recent wAIHA sub-grouping principle, we may classify the present patient as having the more severe type II wAIHA. Nevertheless, how the

conformational changes induce wAIHA is obscure, especially for the patient whose profound and rapid exacerbating anemia has not been associated with known infection or autoimmune diseases[1]. One possible explanation may be the disturbance of potential synergism between the CD47 "don't-eat-me" signals and the senescent band 3. CD47 is a ubiquitously expressed glycoprotein and its interaction with signal regulatory protein a (SIRP a or CD172a) expressed on macrophages, dendritic cells (DCs), and cytolytic T lymphocytes, results in an anti-phagocytic ("don't-eat-me") signal. Since CD47 and band 3 are near-at-hand, it is possible to generate a decreased and altered exposure of CD47 while the intracellular 812-830 region travels a long distance to the extracellular site. Such a transformation would demoralize the original anti-phagocytic signal to an accelerated level of removing aged RBCs as found in type I patients. However, ultimately additional signals are needed to exacerbate the processes of erythrocyte depletion; only in this way can a particular effector function be responsible for promoting hemolysis, as in type II wAIHA. Based on our case report[1], lipopolysaccharides (LPSs) may fit the bill. Through direct interaction with red cell membranes, LPS can compromise their integrity and contribute to hemolysis independently without the involvement of Toll-like receptor (TLR4) signaling or complement activation[5]. If the notion of a transitional, extraintestinal LPS leakage exacerbates hemolysis holds true, we may observe a higher prevalence of AIHA in patients with inflammatory bowel disease (IBD), wherein LPSs are released from damaged intestinal barriers. Indeed, associations between AIHA and gastrointestinal disorders, particularly in the context of IBD[6], have been highlighted. For example, a significantly elevated prevalence of AIHA in IBD and ulcerative colitis (UC) patients has been reported, with a prevalence of 125 per 100,000 in IBD compared with 18 per 100,000 in non-IBD patients[7] and of 150 per 100,000 in UC patients[8], respectively. This implies that the gut-LPS axis may represent another possible risk factor in exacerbating wAIHA.

Transfusion considerations

During an episode, most type II wAIHA patients have a low Hb level below 6.0 g/dL, thus requiring transfusion support with red cell products[9]. However, in this particular condition, an initial alloresponse is likely to cause release of immunogenic self-antigens and senescence antigens, leading to the formation of autoantibodies. Transfusion of donor units compatible for extended blood group antigens has been proposed to replace the use of "least incompatible" units[10]. To avoid the patient being immunized to antigens absent from their own erythrocytes, phenotype matching of ABO, Rh, Kell, Kidd, Duffy, and MNS are thus suggested. Additionally, the Miltenberger blood group has been routinely screened in Taiwan not only it is the second most important blood group antigen following ABO[11], but also the antigens are located on glycophorins A and B, which are physically associated with band 3 and CD47. Although it is inherently very difficult to characterize, lack of extended compatibility may help to explain why the first three red cell transfusions might have failed to stabilize the Hb to >10 g/dL (**Figure 1**).

Transfusing a stored RBC product may fulfil most medical needs; however, the storage conditions are far from being normal and physiological situations thus placing a burden on the circulation when they are reinfused into wAIHA patients. In fact, typical storage lesions like hemolysis and hemoglobulin oxidation, which represents a risk factor in band 3 aggregation, can be detected as early as day 7 during blood banking conditions[12]. This is corroborated by the fact that a 31-day old (~324 mL) RBC concentrate provided little effect and the Hb dropped from 5.7 to 4.2 g/dL even under an extensive regimen including methylprednisolone, hydrocortisone, and antihistamine (**Figure 1**).

Monoclonal antibodies as new drugs and research tools on the horizon

Chronic corticosteroid regimens for AIHA often fail to achieve long-term remission in patients with IBD (**Table I**)[6,8,13,14]; moreover, long-term treatments often associate with toxicity and substantial quality-of-life burden, thus alternative approaches are needed. Since B cells are responsible for both generating autoantibodies as well as presenting self-antigens, producing cytokines, and promoting further hemolysis, thus it's the scientific rationale behind targeting B cells. To this end, novel agents are becoming an approach which would represent an advancement of the treatment paradigm from one of global immunosuppression to one of targeted modulation of the immune response in wAIHA. CD20 is a general B-cell marker expressed by the majority of B cells starting

Table I – *Results of IBD patient with AIHA treated by different modalities*

Characteristics	Number (No. 66)
Sex	
Male	34
Female	32
Activity of IBD at diagnosis	
Active	53
Inactive	8
Simultaneous	5
Resolution modalities	
No treatment	1
Steroids	11
Steroids + immunosuppressant	22
Immunosuppressant	6
Splenectomy	5
Splenectomy + colectomy	6
Colectomy	11
(Deceased during treatment)	4

Data were obtained from case reports and retrospective cohort studies[6,8,13,14]. Notably, only 11 out of 66 patients received steroids alone, while 22 required a combination of steroids and immunosuppressants, highlighting the complexity to manage this condition.

from late pre-B lymphocytes. CD20 has been the target of clinically relevant monoclonal antibody (mAb), rituximab, for steroid-refractory wAIHA[15]. However, a few cases still remain incurable because of emerging resistance. Conversely, B-cell activating factor receptor (BAFF-R) is a mature B-cell survival receptor, which is minimally expressed in immature B cells[16]. This limited expression has made the BAFF/BAFF-R axis successfully targeted for the disease treatment, particularly with mAbs directed against the BAFF ligand, e.g., belimumab for the treatment of systemic lupus erythematosus (SLE). Furthermore, harnessing both antibody-dependent cellular cytotoxicity to deplete BAFF-R-bearing mature B cells together with blockade of BAFF-R-mediated signaling may provide a greater hope. Thus, a fully human mAb that directs BAFF-R, ianalumab (Novartis, Basel, Switzerland), which incorporates two therapeutic mechanisms into a single medication, has become a new immunotherapy. It has been developed for immune thrombocytopenia (ITP) and wAIHA[17]; however, it remains to be seen whether ianalumab can induce and maintain durable responses beyond treatment completion.

As anti-band 3 autoantibodies are omnipresent and are responsible for the removals of naturally senescent erythrocytes; however, do any functional and phenotypic differences exist between physiological and pathological anti-band 3? In the recent understanding of wAIHA development, this is an important and rarely discussed lacuna. An intriguing possibility that deserves consideration is that the anti-band 3 autoantibodies currently available are polyclonal encompassing a heterologous mixture of IgGs. On the contrary, the discovery of exact antigenic target by mAbs is imperative in disease management. As illustrated by myasthenia gravis, a prototypical autoantibody-mediated disease, mAbs have allowed not only accurate subtyping and therapy but also supporting the development of advanced therapeutics[18].

DISCUSSION AND CONCLUSIONS

Corticosteroid regimens have limitations, the international consensus meeting has recommended a rituximab-prednisolone combination in the first line for the severely anemic patients (Hb <8 g/dL)[19]. As exact diagnosis is required to select the appropriate treatment, deciphering the mechanisms underlying pathological processes promises to lead us to a greater understanding of wAIHA. To put these theories into practice, mAbs isolation that links the disorder to existing senescent functions is a vital task.

ETHICAL CONSIDERATION

This study was approved by the Institutional Ethics Committee of Ditmanson Medical Foundation Chiayi Christian Hospital (approval number/protocol number IRB2024020). The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from the participant/patient for study participation and data publication.

FUNDING AND RESOURCES

A part of the present study was funded by the Ditmanson Medical Foundation Chiayi Christian Hospital (IRB2024020). The sponsor has responsibility to oversight the ethical issues, otherwise has not been specifically involved in the research.

AUTHORS' CONTRIBUTIONS

JYW: data collection, analysis, drafting the manuscript; LTC: conception of the project, design of methodology, data analysis; YTL: drafting the manuscript, critically manuscript revising for important clinical content. All

Authors have read and agreed to the published version of the manuscript.

The Authors declare no conflicts of interest.

REFERENCES

1. Liu CS, Woon JY, Chiu YT, Lu SC, Chin LT. Blood donations affect disease management in a case of warm autoimmune hemolytic anemia. Blood Transfus 2023; 21: 301-304. doi: 10.2450/2022.0064-22.
2. Kay MM. Molecular mapping of human band 3 anion transport regions using synthetic peptides. FASEB J 1991; 5: 109-115. doi: 10.1096/fasebj.5.1.1991578.
3. Arakawa T, Kobayashi-Yurugi T, Alguel Y, Iwanari H, Hatae H, Iwata M, et al. Crystal structure of the anion exchanger domain of human erythrocyte band 3. Science 2015; 350: 680-684. doi: 10.1126/science.aaa433.
4. Badior KE, Casey JR. Large conformational dynamics in Band 3 protein: significance for erythrocyte senescence signalling. Biochim Biophys Acta Biomembr 2021; 1863: 183678. doi: 10.1016/j.bbamem.2021.183678.
5. Bloch EM, Branch HA, Sakac D, Leger RM, Branch DR. Differential red blood cell age fractionation and Band 3 phosphorylation distinguish two different subtypes of warm autoimmune hemolytic anemia. Transfusion 2020; 60: 1856-1866. doi: 10.1111/trf.15911.
6. Brauckmann S, Effenberger-Neidnicht K, de Groot H, Nagel M, Maye C, Peters J, et al. Lipopolysaccharide-induced hemolysis: evidence for direct membrane interactions. Sci Rep 2016; 6: 35508. doi: 10.1038/srep35508.
7. Ramakrishna R, Manoharan A. Auto-immune haemolytic anaemia in ulcerative colitis. Acta Haematol 1994; 91: 99-102. doi: 10.1159/000204264.
8. Sunkesula V, Kundrapu S. Prevalence of autoimmune hemolytic anemia in inflammatory bowel disease. Am J Gastroenterol 2020; 115: S358. doi: 10.14309/01.ajg.0000704904.45165.0a.
9. Uzzan M, Galicier L, Gornet JM, Oksenhendler E, Fieschi C, Allez M, et al. Autoimmune cytopenias associated with inflammatory bowel diseases: insights from a multicenter retrospective cohort. Dig Liver Dis 2017; 49: 397-404. doi: 10.1016/j.dld.2016.12.006.
10. Lechner K, Jäger U. How I treat autoimmune hemolytic anemias in adults. Blood 2010; 116: 1831-1838. doi: 10.1182/blood-2010-03-259325.
11. Petz LD. "Least incompatible" units for transfusion in autoimmune hemolytic anemia: should we eliminate this meaningless term? A commentary for clinicians and transfusion medicine professionals. Transfusion 2003; 43: 1503-1507. doi: 10.1046/j.1537-2995.2003.00583.x.
12. Lin M, Broadberry RE. Immunohematology in Taiwan. Transfus Med Rev 1998; 12: 56-72. doi: 10.1016/s0887-7963(98)80090-4.
13. Jana S, Kassa T, Wood F, Hicks W, Alayash AI. Changes in hemoglobin oxidation and Band 3 during blood storage impact oxygen sensing and mitochondrial bioenergetic pathways in the human pulmonary arterial endothelial cell model. Front Physiol 2023; 14: 1278763. doi: 10.3389/fphys.2023.1278763.
14. Dybowska A, Krogulska A. Autoimmune haemolytic anaemia as a rare and potentially serious complication of Crohn's disease in a 11-year-old child-case report and minireview. Children (Basel) 2023; 10: 1698. doi: 10.3390/children10101698.
15. Ahmed W, Monroe K, Essell J, Broun ER. A rare cause of anemia in inflammatory bowel diseases: a case report and review of literature. Blood 2008; 112: 5385-5385. doi: 10.1182/blood.V112.11.5385.5385.
16. Barcellini W, Fattizzo B. How I treat warm autoimmune hemolytic anemia. Blood 2021; 137: 1283-1294. doi: 10.1182/blood.2019003808.
17. Novak AJ, Grote DM, Stenson M, Ziesmer SC, Witzig TE, Habermann TM, et al. Expression of BLyS and its receptors in B-cell non-Hodgkin lymphoma: correlation with disease activity and patient outcome. Blood 2004; 104: 2247-2253. doi: 10.1182/blood-2004-02-0762.
18. Cuker A, Al-Samkari H, Barcellini W, Cooper N, Ghanima W, Michel M, et al. Ianalumab, a novel anti-B-cell activating factor (BAFF) receptor (BAFF-R) monoclonal antibody (mAb) in development for immune thrombocytopenia (itp) and warm autoimmune hemolytic anemia (wAIHA), has demonstrated a favorable safety profile in Sjögren's Syndrome (SjS), Systemic Lupus Erythematosus (SLE) and Chronic Lymphocytic Leukemia (CLL). Blood 2023; 142: 5427. doi: 10.1182/blood-2023-180055.
19. Lazaridis K, Tzartos SJ. Autoantibody specificities in myasthenia gravis; implications for improved diagnostics and therapeutics. Front Immunol 2020; 11: 212. doi: 10.3389/fimmu.2020.00212.
20. Jäger U, Barcellini W, Broome CM, Gertz MA, Hill A, Hill QA, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: recommendations from the first international consensus meeting. Blood Rev 2020; 41: 100648. doi: 10.1016/j.blre.2019.100648.