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Red blood cells, still vital after all these years: Commentary on Canadian Blood Services' International Symposium 2017

Syed M. Qadri[a,c], David A. Donkor[a,c,1], Matthew Yan[d,1], Shuoyan Ning[e,1], Donald R. Branch[b,d,f], Jerard Seghatchian[g], William P. Sheffield[a,c,*]

[a] Centre for Innovation of Canadian Blood Services, Hamilton, Ontario, Canada
[b] Centre for Innovation of Canadian Blood Services, Toronto, Ontario, Canada
[c] Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada
[d] Department of Medicine, University of Toronto, Toronto, Ontario, Canada
[e] Department of Medicine, McMaster University, Hamilton, Ontario, Canada
[f] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada
[g] International Consultancy in Blood Components Quality/Safety Improvement, Audit/Inspection and DDR Strategies, London, United Kingdom

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ABSTRACT

Canadian Blood Services (CBS), Canada's national blood transfusion service, has for many years sponsored an annual conference, for the education and awareness of interested participants, showcasing the latest evidence-based understanding of both basic science and clinical issues in transfusion medicine and science. The 15th iteration of this symposium took place September 9, 2017 and focused on some of the vital aspects of red blood cells (RBC), in line with the" 3Rs" concept, namely the provision of the Right red blood cell (RBC) product to the Right patient at the Right time. Presentations touched upon: the evolution of blood banking in North America; the monocyte monolayer assay as a predictor of post-transfusion hemolysis; hemoglobin-based oxygen carriers; RBC alloimmunization; serological approaches to complex RBC antibody problems; randomized clinical trials related to the age of stored RBC; RBC genotyping; pathophysiology, prevention and treatment of hemolytic disease of the fetus and newborn (HDFN); and testing and timing in perinatal serology. This commentary provides summaries of all speakers' presentations annotated with relevant references. Special thanks are due to all contributors for their praiseworthy approaches in sharing their experiences and knowledge on this interesting scientific/clinical and management theme.

1. Introduction

1.1. Initial remarks

The Symposium began with a welcome by Dr. William P. Sheffield, Associate Director, Research, of the Centre for Innovation, Canadian Blood Services. He stated that part of the mandate of Canadian Blood Services, which provides blood and hematopoietic stem cell products to all Canadian provinces and territories save the province of Québec, is to promote research and education in the field of transfusion medicine and science. He mentioned the 14 previous international symposia sponsored by Canadian Blood Services in this context; the first 10 were organized and chaired by Dr. Donald R. Branch. An organizing committee planned and delivered more recent symposia [1–4], including the fifteenth iteration, which took place on September 9, 2017. Dr. Sheffield explained the symposium's title, noting that getting the right red blood cell (RBC) product to the right patient at the right time remained as vital to the mission of Canadian Blood Services in 2017 as at its founding in 1998, and introduced the first speaker to commence the program.

1.2. The British blood banking invasion

Dr. Marion Reid, Former Director of Immunohematology and Head of Immunochemistry at the New York Blood Center, presented a history of the impactful migration of UK-trained physicians and medical laboratory technologists to North America. Dr. Reid pictorially illustrated the evolution of blood banking and immunohematology in the UK and

* Corresponding author at: Canadian Blood Services Centre for Innovation, Department of Pathology and Molecular Medicine, McMaster University, HSC 4N66, 1280 Main Street West, Hamilton, Ontario, L8S 4K1, Canada.
E-mail addresses: jseghatchian@btopenworld.com (J. Seghatchian), sheffiel@mcmaster.ca (W.P. Sheffield).
[1] These authors contributed equally to this work.

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US in the last century. She provided a timeline of important events in transfusion medicine, starting with the seminal discovery of the ABO blood groups by Dr. Karl Landsteiner, the clinical significance of which became apparent during World War I. In the 1940s, the discovery of numerous human antibodies/antigens was propelled by optimization of protocols for animal immunization and improvements in antiglobulin testing; critical work by Dr. Robin Coombs on antiglobulin testing paved the way for reliable detection of IgG antibodies.

Dr. Reid highlighted an example of a UK-trained blood banker migrating to North America in Marie Cutbush, who was mentored by Dr. Patrick Mollison, a legendary British physician known for his pioneering work in transfusion medicine [5]. Ms. Cutbush is known to have written considerable proportions of Blood Transfusion in Clinical Medicine, a classic textbook authored by Dr. Mollison [6]. Ms. Cutbush later moved to Toronto and together with Betty Croucher worked at the Blood Transfusion Laboratory at the Toronto General Hospital [6]. Other renowned physicians who trained under the tutelage of Dr. Mollison include Dr. Eloise R. Giblett, Former Director of Puget Sound Blood Center, and Dr. Hugh Chaplin, who returned to America to head the Blood Bank at NIH.

An exodus of UK technologists to North America in the 1960s also completely transformed blood banking practices. To illustrate this phenomenon, Dr. Reid highlighted the achievements of 12 of her famous British contemporaries, including Dr. George Garratty, who moved to North America from the late 1950s up until the early 1970s.

Dr. Reid noted that by 1957, immunohematology had gained recognition as a specialized scientific discipline and 36 “factors” were classified into 11 blood group systems while 13 “factors” were not assigned to any system. By 1990, the International Society of Blood Transfusion had allocated 157 antigens into 19 systems, 35 antigens into collections, and an additional 37 low incidence antigens and 13 high incidence antigens into an “orphan” group; this scheme continues to be used to the present day [7]. In closing, she emphasized the importance of new laboratory techniques in the history of the discovery of blood group antigens, especially those related to molecular genetics, and predicted an impactful future role for next generation sequencing of donor DNA.

1.3. The monocyte monolayer assay

Dr. Donald Branch, Professor of Medicine and Laboratory Medicine and Pathobiology at the University of Toronto, and Canadian Blood Services Scientist, presented the past, present, and future of an assay used to detect the removal and destruction of red blood cells – the Monocyte Monolayer Assay (MMA). He first classified red cell destruction as arising from either intravascular or extravascular hemolysis. The former results from sensitization of red blood cells by IgM and/or IgG, activation of the complement cascade, the subsequent formation of the membrane attack complex on the RBC surface, and the resultant rupture of the cells within blood vessels. The latter arises from extravascular hemolysis, in which opsonized RBCs with bound IgG molecules are recognized via an Fc γ Receptor (FcγR) present on phagocytic cells of the mononuclear phagocyte system (MPS) located in the spleen and liver. Dr. Branch pointed out the MMA can provide a more precise estimate of the likelihood of post-transfusion hemolysis than serological crossmatching, because not all serologically incompatible RBD units will be hemolysed by transfusion recipients. Finding compatible RBC units for patients requiring transfusion support who have been chronically transfused or pregnant multiple times is a challenging task.

Dr. Branch positioned the conception of the MMA and its 1984 publication [8] as building on decades of scientific discoveries by a host of talented scientists. By 1965, Archer had proposed that the removal of RBCs from the circulation was mediated through the Fc portion of RBC-binding IgG and Fc receptors, possibly via monocyte mediated phagocytosis [9]. Abramson et al supported this concept with a 1970 article in which they demonstrated that initial interactions between IgG subclasses 1 and 3 and the Fc receptors on monocytes were sufficient to lead to the onset of hemolysis [10]. By 1977, Borne et al. had elegantly confirmed the importance of Fc in immune-mediated RBC destruction by showing that when RBC were sensitized by the F(ab') fragments of IgG anti D and transfused, the cells survived in vivo [11].

Hunt et al. then confirmed the importance of IgG subclass compositions in the process, and furthermore showed a clear correlation between increased titer score and increased RBC-monocyte interactions [12]. These investigators showed that significant macrophage activity did not occur until the level of sensitizing antibodies reached a critical threshold. Once maximum antibody-RBC interactions were achieved, additional antibody molecules inhibited rather than stimulated monocyte activity. Hunt et al also confirmed clinically significant and non-significant antibodies by comparing both subclass and titer scores [12].

The establishment of these links among RBC binding to IgG subclasses, titer score, and RBC-monocyte interactions leading to phagocytosis and hemolysis permitted Dr. Branch to develop the monocyte monolayer assay. He worked with Michael T. Gallagher, Angie P. Mison and Anita Sy Siok Hian in the laboratory of Dr. Lawrence Petz, at the City of Hope National Medical Center in Duarte, California [8,13]. The assay is comprised of these general steps [14]. First, the mononuclear cells are isolated from anticoagulated whole blood using a density gradient, ideally from the prospective transfusion recipient. They are then washed and diluted in cell culture medium containing fetal bovine serum and applied to sectioned slides. After incubation, cells adhering to slides are exposed to diluted donor RBC (R2R2 phenotype) that have been challenged with recipient serum. A section on the slide is used as a control for phagocytosis inhibition. Anti-Rh (D) opsonized R2R2 test panel RBCs are used as positive control. The opsonized RBCs as well as samples to be analyzed are then added to designated sections on the slide and incubated. Hematological stains and light or phase contrast microscopy of the fixed slides are then used to detect adherent RBCs (which exhibit rosetting) or phagocytosed RBC (which are fully engulfed). A phagocytic index can then be calculated, which corresponds to the number of phagocytosed RBC divided by the total number of monocytes multiplied by 100. A high phagocytic index is predictive of clinical hemolysis for either autoantibodies or alloantibodies [8,13].

Dr. Branch's group has recently established optimal conditions for the MMA that permit use of whole blood samples drawn into acid citrate dextrose anticoagulant and stored for up to 36 h at ambient temperature [14]. With this and other planned optimizations, Dr. Branch envisions the MMA as the future chosen method for added-value crossmatch in the selection of RBC units for transfusion.

1.4. Hemoglobin-based oxygen carriers (HBOC)

Dr. Ronald Kluger, a Professor in the Department of Chemistry at University of Toronto, discussed the promise and limitations of using hemoglobin (Hb)-based acellular oxygen carriers (HBOC) as alternatives to RBC transfusions. Dr. Kluger first enumerated the characteristics of an ideal HBOC: effective oxygen carrying capacity; sterility; freedom from immunogenicity; circulatory stability; extended shelf-life; and minimal side effects. He stressed the instability of the Hb tetramer outside its cellular environment, where it dissociates into its constituent αβ dimers and heme, rendering it nonfunctional. Accordingly, prevention of extracellular dissociation of the Hb tetramer is essential in exploiting it as an oxygen carrier [15]. The development of a “blood substitute”, was pursued with widespread enthusiasm by scientists in the 1980s and 1990s, a period that saw the involvement of both new and established pharmaceutical companies and considerable military interest [15]. However, clinical assessment of the HBOC products developed during this time raised concerns about both efficacy and safety, especially with respect to the adverse effect of hypertension. A major limitation of early HBOC agents likely related to the scavenging of nitric oxide (NO), resulting in vasoconstriction [15]. A 2008