ERYTHEMPHAGOCTYSIS

ITS RELATION TO AGGLUTINATION AND HEMOLYSIS, PARTICULARLY IN

ACQUIRED HEMOLYTIC ANEMIA

A THESIS

SUBMITTED FOR THE DEGREE OF M.D.

OF

THE UNIVERSITY OF ADELAIDE.

By

J. A. BONNIN, M.B., B.S., ADELAIDE, 1946.
CONTENTS

The regulations of the University of Adelaide for the degree of Doctor of Medicine require:

(1) A declaration that the thesis is the writer's own composition. This declaration may be found on page 6.

(2) An indication of where the writer considers the thesis to advance medical knowledge or practice. This subject is contained in the Conclusion on page 102.

Page

PART I - INTRODUCTION 1 - 6
An Outline of the Study Undertaken 1
The Reasons for the Study 3
Acknowledgements 4
Publications 5
Declaration (of originality of the study and personal composition of the thesis) 6

PART II - HISTORICAL SURVEY 7 - 26
The Early Studies of Immuno-haematology 7
The Early Experiments on Erythropagocytosis 8
The Concept of a Haemolytic Anaemia and the Donath-Landsteiner Antibody 9
Erythropagocytosis 10
Erythropagocytosis in vivo 11
The Mechanism of Phagocytosis 12
Erythropagocytosis due to the Antibodies of the ABO Blood Group System 15
Attempts to Determine the Opsonic Potency of Antibodies and Various Sera 15
The Antibodies of Acquired Haemolytic Anaemia 17
The Characteristics of the Antibodies of Acquired Haemolytic Anaemia 19
A. The Cold Auto-antibodies of Acquired Haemolytic Anaemia 20
B. The Warm Auto-antibodies of Acquired Haemolytic Anaemia 23
C. The Donath-Landsteiner Type of Antibodies 24

PART III - THE DEVELOPMENT OF A TECHNIQUE FOR THE COMBINED STUDY OF AGGLUTINATION, HAEMOLYSIS AND ERYTHROPHAGOCYTOSIS 27 - 43
The Preparation of a Satisfactory Leucocyte Suspension 28
CONTENTS

Experiment 1. A Comparison of Various Methods of Preparing Leucocyte Suspensions 30
Experiment 2. The Effects of Anticoagulants and 'Dextran' on Phagocytic Activity 32
Experiment 3. The Effects of the Time of Sensitisation of Erythrocytes by Antibody, and the Time of Incubation of Sensitised Erythrocytes with Leucocytes, on the Degree of Erythrophagocytosis 34
The Method of the Combined Titration of Agglutination, Haemolysis and Erythrophagocytosis 37
The Determination and Recording of Erythropagocytosis 39
The Determination and Recording of Agglutination 41
The Determination and Recording of Haemolysis 41
Summary of Part III 41

PART IV - THE MECHANISM OF PHAGOCYTOSIS AND SUPRAVITAL OBSERVATIONS. 44 - 53

The Mechanism of Phagocytosis 44
Supravital Observations 47
Summary of Part IV 52

PART V - THE APPLICATION OF THE COMBINED STUDY OF AGGLUTINATION, HAEMOLYSIS AND ERYTHROPHAGOCYTOSIS TO THE ANTIBODIES OF ACQUIRED HAEMOLYTIC ANAEMIA. 54 - 81

Materials and Standard Methods 54
Sera 54
Erythrocytes 54
Leucocytes 55
Complement 55
Antiglobulin Reactions (Coombs' Tests) 57
A. Observations with Normal Sera 58
B. The Cold Auto-antibodies of Acquired Haemolytic Anaemia 59

Experiment 4. The Demonstration of Agglutination, Haemolysis and Erythrophagocytosis produced by a Cold Auto-antibody 59
Experiment 5. The Influence of pH on the Ability of a Cold Auto-antibody to produce Agglutination, Haemolysis and Erythrophagocytosis 61
Experiment 6. The Combined Titration of Agglutination, Haemolysis and Erythrophagocytosis produced by a Cold Auto-antibody 62
Experiment 7. The Effect of Thermolabile Serum Components on Haemolysis and Erythrophagocytosis produced by a Cold Auto-antibody 64

C. The Cold Auto-haemolysins of Paroxysmal Cold Haemoglobinuria (The Donath-Landsteiner Type of Antibodies) 66
CONTENTS

Experiment 8. Agglutination, Haemolysis and Erythrophagocytosis produced by an Antibody of the Donath-Landsteiner Type; the Effect of Acidification; and the Necessity for Sensitisation in the Cold

Experiment 9. The Combined Titration of Agglutination, Haemolysis and Erythrophagocytosis produced by a Donath-Landsteiner Type of Antibody

Experiment 10. The Effect of the Presence and Absence of the Thermolabile Components of Fresh Normal Serum on the Haemolytic and Opsonic Activities of a Donath-Landsteiner Type of Antibody

D. An Unusual Variant of the Donath-Landsteiner Type of Antibody

Experiment 11. The Titration of Cold Agglutinins present in the Serum of Patient L.J., and the Determination of their Thermal Amplitude

Experiment 12. The Effect of pH on Haemolysis, Erythrophagocytosis and Sensitisation to Anti-globulin Serum produced by the Serum of Patient L.J.

Experiment 13. The Determination of the Thermal Amplitude of the Agglutinins, Haemolysins and 'Incomplete or Sensitising' Antibodies Contained in the Serum of Patient L.J.

Experiment 14. The Combined Titration of Haemolysis and Erythrophagocytosis produced by the Antibody present in the Serum of Patient L.J.

E. The Warm Auto-antibodies of Acquired Haemolytic Anaemia

Experiment 15. The Ability of the Lytic Warm Auto-antibodies and the failure of the Non-lytic Warm Auto-antibodies to produce Haemolysis and Erythrophagocytosis

Experiment 16. The Combined Titration of Agglutination, Haemolysis and Erythrophagocytosis produced by a Lytic Warm Auto-antibody

Experiment 17. The Effect of the Thermolabile Serum Components upon the Agglutinating, Haemolytic and Opsonic Activities of a Lytic Warm Auto-antibody

Summary of Part V

PART VI - THE APPLICATION OF THE COMBINED STUDY OF AGGLUTINATION, HAEMOLYSIS AND ERYTHROPHAGOCYTOSIS TO CERTAIN SPECIFIC BLOOD GROUPS AND OTHER HAEMOLYTIC SYSTEMS

A. The Specific Blood Group Iso-antibody, Anti-A

Experiment 18. The Demonstration of Agglutination, Haemolysis and Erythrophagocytosis of Group A Erythrocytes under the influence of Two Immune Anti-A Sera and One Virtually Non-lytic Serum, and the Effect of the Thermolabile Serum Components
CONTENTS

Experiment 19. Agglutination, Haemolysis and Erythropagocytosis produced by Group O (Anti-A) Sera of Immune and Naturally-occurring Types whose Complementary Activity had been lost through Storage

E. The Rh Iso-antibodies

C. The Naturally-occurring and Immune Rabbit Anti-human Antibodies


D. The Reversible Agglutinin of Trypsinised Erythrocytes

Experiment 21. Agglutination, Haemolysis and Erythropagocytosis produced by the Reversible Agglutinin of Trypsinised Erythrocytes

Experiment 22. The Effect of Time of Sensitisation by the Reversible Agglutinin of Trypsinised Erythrocytes on Agglutination, Haemolysis and Erythropagocytosis of these Corpuscles

B. Tannic Acid

Experiment 23. Agglutination, Haemolysis and Erythropagocytosis of Tanned Erythrocytes and the Effects of Heat-inactivation

F. Observations on Haemolytic Systems where Haemolysis was not induced by Antibodies

Experiment 24. The Absence of Erythropagocytosis following Haemolysis by Hypotonic Saline Solution

Experiment 25. The Absence of Erythropagocytosis following Haemolysis of P.N.H. Red Cells by the Serum Factor in Normal Acidified Serum

Comment

Conclusions (A declaration of where the thesis has been considered to advance medical knowledge)

Summary

References

Page
85
87
89
91
92
92
94
95
96
96
98
99
100
104
109
PART I - INTRODUCTION

An Outline of the Study Undertaken.

The phenomenon of erythrophagocytosis, as the name implies, refers to the ingestion of red blood corpuscles by living cells endowed with this ability. It is known that the spleen is abundantly supplied with phagocytic cells of the reticulo-endothelial system, but how great a part is played by erythrophagocytosis in the destruction of aged normal red cells is unknown. Exactly what changes occur in erythrocytes as they approach the end of their normal life span of 120 days, and what is the actual mode of their destruction, are problems which still remain unsolved.

In disease, however, certain observations have supplied evidence of the existence of agents in the circulating blood which directly or indirectly lead to red cell destruction. The agents or antibodies with which this thesis deals, exist in several forms which may be classified according to their effects on erythrocytes. Thus, there are agglutinins, haemolysins and incomplete or sensitising antibodies. The last group, although producing no directly detectable effects, sensitise erythrocytes to agglutination by antiglobulin sera.

In the past, evidence has been produced that an alteration of the surface of erythrocytes initiated by the action of antibodies is also one factor responsible for erythrophagocytosis, and the present thesis is concerned with this erythrocyte change, the types of antibodies which will produce it and the mechanism of the process of ingestion. It is particularly concerned with erythrophagocytosis in relation to the antibodies of acquired haemolytic anaemia. Because a comparison has been made between this and the two related phenomena of agglutination and haemolysis, a historical survey has been traced of all three in as far as they are
concerned in this disease. A summary is presented of those properties of
the antibodies of acquired haemolytic anaemia which were known at the time
when the experimental work embodied in the text was completed. Any account
of the clinical aspects of the patients whose sera have been examined is
beyond the scope of this work, but it might be mentioned here that they
were classified by Dacie and de Gruchy (1951) into three main clinico-
pathological syndromes: Group I, idiopathic acquired haemolytic anaemia;
Group II, haemolytic anaemia following virus pneumonia; and Group III,
chronic haemolytic anaemia with haemoglobinuria and Raynaud's phenomena.
Serum from patients suffering from haemolytic anaemia of the Donath-
Landsteiner type (paroxysmal cold haemoglobinuria) were also examined. The
four clinico-pathological groups overlap the three main serological groups
into which the antibodies that can be demonstrated in the sera of these
patients, may be placed. An analogy can be drawn between these classifications
and those of anaemia: (1) according to the more common clinical concept
with regard to the aetiological factors and (2) according to the
morphological and other characteristics of the erythrocytes that are
involved.

Moreover, the work is confined to in vitro studies and is not
greatly concerned with the numerous reports of erythrophagocytosis in vivo.
It commences with the development of a technique whereby the agglutination,
haemolysis and erythrophagocytosis which were to be studied, could be
examined in material from the same tubes, and therefore under identical
conditions. The results of titration experiments could then be strictly
correlated. Supravital observations on erythrophagocytosis have been
included and the mechanism of erythrophagocytosis discussed.

The method for the combined study of these three phenomena was
then applied to the investigation of normal sera and to the antibodies of acquired haemolytic anaemia. An unusual antibody combination has been described.

Similar investigations were extended to the following antibodies and antibody systems:

1. The blood group specific antibody, anti-A.  
2. The Rh iso-antibodies, anti-D and anti-e.  
3. The naturally occurring and immune anti-human red cell antibodies of normal and immunised rabbits.  
4. The reversible agglutinin of trypsinised erythrocytes.

Lastly, some other haemolytic systems were investigated in which haemolysis was not induced by antibodies. For these purposes it was produced by:  
1. The tanning of erythrocytes,  
2. The action of hypotonic saline solutions,  
3. The action of normal serum, under the influence of a lowered pH, on the erythrocytes of a patient suffering from paroxysmal nocturnal haemoglobinuria.

In every instance it was determined whether erythrophagocytosis could be produced in vitro, what conditions of pH and which cell types were necessary for its demonstration, and what was the role that complement assumed in the reaction.

The Reasons for the Study

During the early researches in immuno-haematology, there was a close correlation in the development and understanding of the basic mechanisms of agglutination, haemolysis and erythrophagocytosis. In recent years, however, a great deal of knowledge has accumulated on the antibodies of acquired haemolytic anaemia regarding their ability to produce agglutination, haemolysis and sensitisation to antiglobulin serum,
yet the subject of erythrophagocytosis had been relatively uninvestigated.

While on overseas study leave from the Institute of Medical and Veterinary Science, I spent some nine months at the Postgraduate Medical School of London working under Dr. J. V. Dacie in the Department of Haematology. It is largely due to the work of Dr. Dacie that the antibody patterns in acquired haemolytic anaemia have been so adequately described, and it was at his suggestion that Dr. Lawrence L. Schwartz, from Mt. Sinai Hospital, New York, and I commenced the study of erythrophagocytosis in respect of these antibodies.

When this work was almost completed and before it was accepted for publication, a somewhat similar paper appeared in the literature in which different methods were employed, but in which most of our work on the specific blood group systems was described. These authors (Jordan, Frouty, Heinle and Dingle; 1952) also touched on the antibodies of acquired haemolytic anaemia, but their work overlapped the present study only in respect of the antibody of paroxysmal cold haemoglobinuria (or the Donath-Landsteiner type of haemolysin).

At the time when the experiments embodied in this thesis were carried out, they represented an attempt to study a then partially uninvestigated aspect of the properties of many types of iso- and autoantibodies, and a comparison was made between the related phenomena of agglutination, haemolysis and erythrophagocytosis.

Acknowledgements.

Over the last three years Dr. Dacie has collected a large number of pathological sera taken from patients suffering from acquired haemolytic anaemia, and I am deeply indebted to him for making this material available. Indeed, I believe that a similarly complete study
has not been carried out simply because other investigators have lacked access to such a full collection of sera and to so many appropriate patients. I am also grateful to him for allowing me to work in his department, where Dr. Schwartz and I received his continual advice and encouragement, and where I had the opportunity to learn his methods and to become familiar with his work. He also encouraged me to publish this work in the form of a thesis.

I am grateful to Miss Marie Out bush and Dr. P. L. Rollison, of the Blood Transfusion Research Unit of the Medical Research Council, who kindly supplied many of the anti-A and anti-Rh sera.

I would like to sincerely thank Dr. L. I. Schwartz, in intimate association with whom most of the work embodied in this thesis was carried out. Because of their knowledge of German, both he and Dr. S. Klaar, of the Institute of Medical and Veterinary Science, have been of great assistance in the translation of many papers written in that language to which reference has been made in the text. Appended at the beginning of this section is either the original or a copy of a letter from Dr. Schwartz in which he has given his written consent to the use of our joint investigations as a basis for this thesis.

Publications.

By far the great majority of the work was performed in London and an account of the combined studies of Dr. Schwartz and myself has recently been accepted for publication in 'Blood'. Still more recently, I have separately submitted another paper which has been accepted for publication in the same journal. It contains the report of a case of chronic acquired haemolytic anaemia associated with haemoglobinuria and Raynaud's phenomena. This patient's serum contains an auto-antibody
of unusual serological characteristics. Full reference to these findings is made in the text. Should reprints of these papers be available when the thesis is completed, they will be appended at the end.

The remainder of the experimental material has been undertaken in Adelaide since my return from Europe and America. Whether performed in London or Adelaide, I wish to state that the work is entirely original and that I personally conducted or assisted Dr. Schwartz in the performance of every experiment. The actual composition of the thesis is entirely my own. In conclusion, at the end of Part VI, it is stated where the work has been thought to advance medical knowledge.