Volume 2, Issue 10, October 2024 ISSN (E): 2938-3765

UNIQUE PRINCIPLES OF COMPLEMENT BINDING REACTION

Umarova Tamila Abdufattoevna
Assistant of the Department of Clinical Laboratory Diagnostics with a Course of Clinical and Laboratory Diagnostics PGD;

Kudratova Zebo Erkinovna
PhD, Associate Professor of the Department of Clinical Laboratory Diagnostics with a Course of Clinical and Laboratory Diagnostics PGD;

Xodjimetova Ruxshona
Cadet (Laboratory Doctor) of the Department of Clinical Laboratory Diagnostics with a Course of Clinical and Laboratory Diagnostics PGD;

Raxmatova Madina
Cadet (Laboratory Doctor) of the Department of Clinical Laboratory Diagnostics with a Course of Clinical and Laboratory Diagnostics PGD;
Samarkand State Medical University, Samarkand, Uzbekistan

Abstract
The unique ability of complement to bind specifically to antigen-antibody complexes of different nature has been utilised in the complement binding reaction (CBR) proposed in 1901 by J. Borde and O. Jangou. The particular advantage of the complement binding reaction is that the nature of the antigen involved (corpuscular or soluble) is irrelevant, since complement binds to the Fc-fragment of any IgG and IgM antibody, irrespective of its antigenic specificity.

Keywords: antibodies, antigens, specificity, immune complex, complement system.

Introduction

IgM and IgG antibodies as part of the immune complex acquire the ability to bind Clq, a subcomponent of the first complement component, which causes sequential activation of the other proteins of the complement system along the so-called classical pathway. The consequences of complement system activation are determined by the nature of the constituent antigen. Assembly of the membrane-attacking complex (C5b - C9) accounts for haemolysis and lysis of some Gram-negative bacteria - bacteriolysis. Binding of complement by immune complexes in which the antigen is represented by soluble compounds, as well as by Gram-positive bacteria, has no visible consequences. However, as a result of binding to the immune complex, complement proteins undergo irreversible changes and complement lyses the ability to lysate sensitised erythrocytes. The complement binding reaction is based on these patterns of complement activation. Since the

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5

process of complement binding is not visually manifested, J. Borde and O. Zhangu proposed to use a haemolytic system (sheep's erythrocytes + haemolytic serum) as an indicator, which shows whether complement is fixed by the “antigen-antibody” complex [1,3,5,7,9].

If no immune complex is formed, complement remains free and binds to the second system, causing haemolysis, accompanied by visible changes. The complement binding reaction is a complex serological reaction. It involves complement and two “antigen-antibody systems”. In essence, it is two serological reactions. If no immune complex is formed, complement remains free and binds to the second system, causing haemolysis, accompanied by visible changes. The complement binding reaction is a complex serological reaction. It involves complement and two “antigen-antibody systems”. In essence, it is two serological reactions [1.8,9].

There are 5 main ingredients needed to perform an CBR:

- antigen (usually lysate, extract, hapten; less frequently, suspension);
- antibody (test serum);
- complement;
- antigen (sheep's erythrocytes);
- antibody (haemolysin to ram's erythrocytes).

First system, diagnostic Second system, indicative The first system is diagnostic, comprising antigen and antibody (one component is known, the other is not). A certain amount of complement is added and the mixture is incubated for some time to complete the reaction. If the antigen and antibodies of this system are matched during incubation, the complex “antigen + antibody + complement” is formed [4,6,7,8,9].

In the absence of a detectable immunoreagent, immune complexes are not formed and the complement remains free. Externally, the formation of immune complexes is not manifested by any phenomena [5].

The binding of complement by the immune complex is recognised by a second system, the indicator system. It consists of sheep's erythrocytes (antigen) and their corresponding haemolytic serum (antibodies), i.e. a ready-made immune complex. Specific antibodies that cause cell lysis are called lysins, and in the case of erythrocytes, haemolysins. Haemolysins are only able to exert their lysing effect on erythrocytes in the presence of complement. If complement binding occurs in the first phase of the reaction, the sensitised erythrocytes added to the mixture in the second phase of the reaction are not lysed. Absence of haemolysis is registered as a positive result of CBR. If no immune complex is formed, the remaining free complement causes lysis of sensitised erythrocytes. Haemolysis in the second phase of the reaction indicates the absence of the antigens or antibodies sought. For control purposes, reactions should be performed simultaneously under exactly the same conditions with normal serum and serum with a known positive result. The antigen for the complement binding reaction can be cultures of various killed microbes, their lysates, bacterial components, extracts obtained from pathologically altered and normal organs and tissues. Because all microbial antigens adsorb some amount of complement, before titration they are tested for anticomplement properties by determining the solubilizing dose of complement, that is, the largest amount of antigen that does not cause delayed hemolysis [1,2,3].

Thus, the following prepared ingredients are required for RSC staging:

1) serum to be tested, inactivated at 56 °C for 30 min in a water bath;

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5

2) an antigen (a suspension of bacteria or an extract from them) that does not independently cause either hemolysis or its retention in the dose established by titration;
3) complement at a working dose determined by titration;
4) hemolytic serum in a dose corresponding to its triple titer;
5) 3% suspension of mutton erythrocytes;
6) physiologic solution.

Recording of RSC results. It is carried out according to the 4+ system

(++++) - 100% binding of antigen by antibodies and formation of complexes "antigen + antibody + complement". Complete delay of hemolysis: after centrifugation in the tube a precipitate of erythrocytes is formed, the supernatant is transparent, colorless.
(+++) - 75% binding of the antigen by antibodies. A certain part of the complement remains free and is activated by the hemolytic system. Clearly delayed hemolysis: after centrifugation, a red blood cell precipitate is visible at the bottom of the tube, the supernatant has a slightly yellowish color due to lysis of part of the red blood cells.
(++) - 50% binding of the antigen by antibodies. Half of the complement is in free form and causes hemolysis of almost half of the erythrocytes of the indicator system. Partial delay of hemolysis: after centrifugation, a sediment of erythrocytes is visible at the bottom of the tube, the supernatant is intensely colored red-orange.
(+) - doubtful result. Very weak delay of hemolysis: after centrifugation at the bottom of the tube - an insignificant sediment of erythrocytes, the supernatant is stained the same as in negative RCB.
(-) - negative result of RGC. Complete hemolysis: after centrifugation there is no precipitate at the bottom of the tube, and the entire content is a transparent red-orange solution - "varnish blood".

Application of RCB

The complement binding reaction, which is highly sensitive, has acquired great diagnostic value. It can be used to record the formation of an immune complex in cases where there are no visible manifestations of the antigen-antibody reaction, such as precipitation, agglutination or flocculation. Both soluble and insoluble antigens can be used in RCB. Due to its high sensitivity, 100-200 times higher than that of the precipitation reaction, RCB analyzes systems with low antigen and antibody content. RCB is mainly used for the diagnosis of infectious diseases. For diagnostic purposes, antigens can be determined in the tested sera using diagnostic serum [11,12]. However, it is more common to detect specific antibodies in the serum using antigen diagnostics.

References

1. Kudratova Z. E. et al. Current modern etiology of anemia //Open Access Repository. – 2023. – Т. 10. – №. 10. – С. 1-4.
2. Burxanova D. S., Umarova T. A., Kudratova Z. E. Acute myocarditis linked to the administration of the COVID 19 vaccine //Центральноазиатский журнал образования и инноваций. – 2023. – Т. 2. – №. 11. – С. 23-26.

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5

3. Кудратова З. Э. и др. Атипик микрофлора этиологияли ўткир обструктив бронхитларининг ў зига хос клиник кечиши //Research Focus. - 2022. - Т. 1. - №. 4. - С. 23-32.
4. Kudratova Z. E, Normurodov S. Etiological structure of acute obstructive bronchitis in children at the present stage - Thematics Journal of Microbiology, 2023. P.3-12.
5. Kudratova Z. E., Tuychiyeva S. K. Atipik mikroflora etiologiyali o'tkir obstruktiv bronxitlar etiopatogenezining zamonaviy jixatlari. Research Focus, 2023, B. 589-593.
6. Kudratova Z. E., Karimova L. A. Age-related features of the respiratory system. Research Focus,Tom 2, P. 586-588.
7. Исомадинова Л. К., Даминов Ф. А. Современная лабораторная диагностика хронического пиелонефрита у детей //Journal of new century innovations. – 2024. – Т. 49. – №. 2. – С. 112-116.
8. Isomadinova L. K., Daminov F. A. Glomerulonefrit kasalligida sitokinlar ahamiyati //Journal of new century innovations. – 2024. – Т. 49. – №. 2. – С. 117-120.
9. Isomadinova L. K., Qudratova Z. E., Shamsiddinova D. K. Samarqand viloyatida urotiliaz kasalligi klinik-kechishining o'ziga xos xususiyatlari //Центральноазиатский журнал образования и инноваций. – 2023. – Т. 2. – №. 10. – С. 51-53.
10. Isomadinova L. K., Qudratova Z. E., Sh B. F. Virusli gepatit b fonida Covid-19 ning klinik laborator kechish xususiyatlari //Journal of new century innovations. – 2023. – Т. 30. – №. 3. – С. 60-65.
11. Isomadinova L. K., Yulayeva I. A. Buyraklar kasalliklarning zamonaviy diagnostikasi //Центральноазиатский журнал образования и инноваций. – 2023. – Т. 2. – №. 10 Part 3. – С. 36-39
12. Kudratova Zebo Erkinovna, Tamila Abdufattoevna Umarova, & Sirojeddiova Sanobar. (2024). Modern types of immunoenzyme analysis methods old problems. Web of Discoveries: Journal of Analysis and Inventions, 2(6), 67–70.

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5