COMPLEMENT SYSTIM

Site : serum and all tissue fluids except urine and CSF

Synthesis : in liver – appear in fetal circulation during 1st 13 W

Function : Responsible for certain aspects of immune response and inflammatory response

Activation : antigen-antibody complex or endotoxin, capsule series of proteins activated sequentially

Inactivation: inhibitors in plasma (short lived)

Biological effects: either beneficial or harmful to host

Complement pathway

:A) Classical pathway

Complement is activated by antigen –antibody -Complex (IgG-IgM)

Fc portion of the antibody form a binding site for C1q -

The numerical sequence of the complement factors in - the classic pathway is :-

C1q,r,s , C4, C2, C3, C5, C6, C7, C8, C9

A) Classical Pathway

The reaction sequence divided into three stages:

1) Recognition stage:

- C1q act as the recognition element

- It binds to Fc portion of IgM or IgG

- The activated C1 molecule can cleave many C4 molec.

2) Activation stage:

The complement components C4, C2, C3, C5, C6, C7,

C8, C9 participate in that order

3) Membrane attack stage:

Complement components C5, C6, C7, C8, C9 participate

where cell membrane damage and cell lysis occur

B) Alternative pathway

This pathway is initiated by:

* Bacterial endotoxin, polysaccharide capsule,

aggregates of IgE and properdin

* It starts at C3 then C5, C6, C7, C8, C9

* The complement compon. C1, C4, C2 are by-passed

* Antibodies are not required to initiate activation of

this pathway

* This pathway provides a means of non-specific

Resistance

Classic Pathway	Alternative pathway
* Specific acquired immunity * * Initiated by antibody * * Interaction of all * * Properdin system not	Non-specific innate immunity Bacterial endotoxin, capsule C1, C4, C2 are components by-passed

involved	* Properdin system is involved

C- **Mannose-Binding Protein Pathway (Lectin pathway)**

A third way of activating the complement system involves mannosebinding protein

. This ,is like the alternative complement pathway, is a

part of innate immune system. When macrophages ingest bactria or other foreign material they are stimulated

to secrete IL-1, IL-6 and TNF- α (Tumor Necroting Facter).

These three cytokines act on hepatocytes,

stimulating them to secrecte acute-phase proteins, among which is mannose-binding protein.

Mannose is a major component of bacterial and fungal cell walls

. It has strucyural similarities to C1q and forms a

multimolecular complex with two serum proteases designated MASP-1

and MASP-2. This complex cleaves C2 and C4 so activates the classical pathway

Function of Complement

1-Cytolysis: activated complement proteins polymerize on cell

surfaces of bacteria or erythrocyte to form pores in its membrane (killing by osmotic lysis)

2-Opsonization:

-binding of complement proteins opsonin (C3b) to surfaces of foreign organisms or particles

-Phagocytic cells express specific receptors for opsonins, so promote phagocytosis

3-Inflammatory response :

Small fragments released during complement activation have several inflammatory actions:

a-C5a is chemotactic and attract neutrophiles and macrophages C5a activate phagocytes and neutrophils

b-C3a,C4a and C5a are anaphylatoxins Cause degranulation of mast cells and release of histamine and other inflammatory mediators

COMPLEMENT FIXATION TEST

The classical Complement Fixation (CF) assay has long been widely used as a simple and sensitive way to detect and quantitate a wide variety of antigens and antibodies.

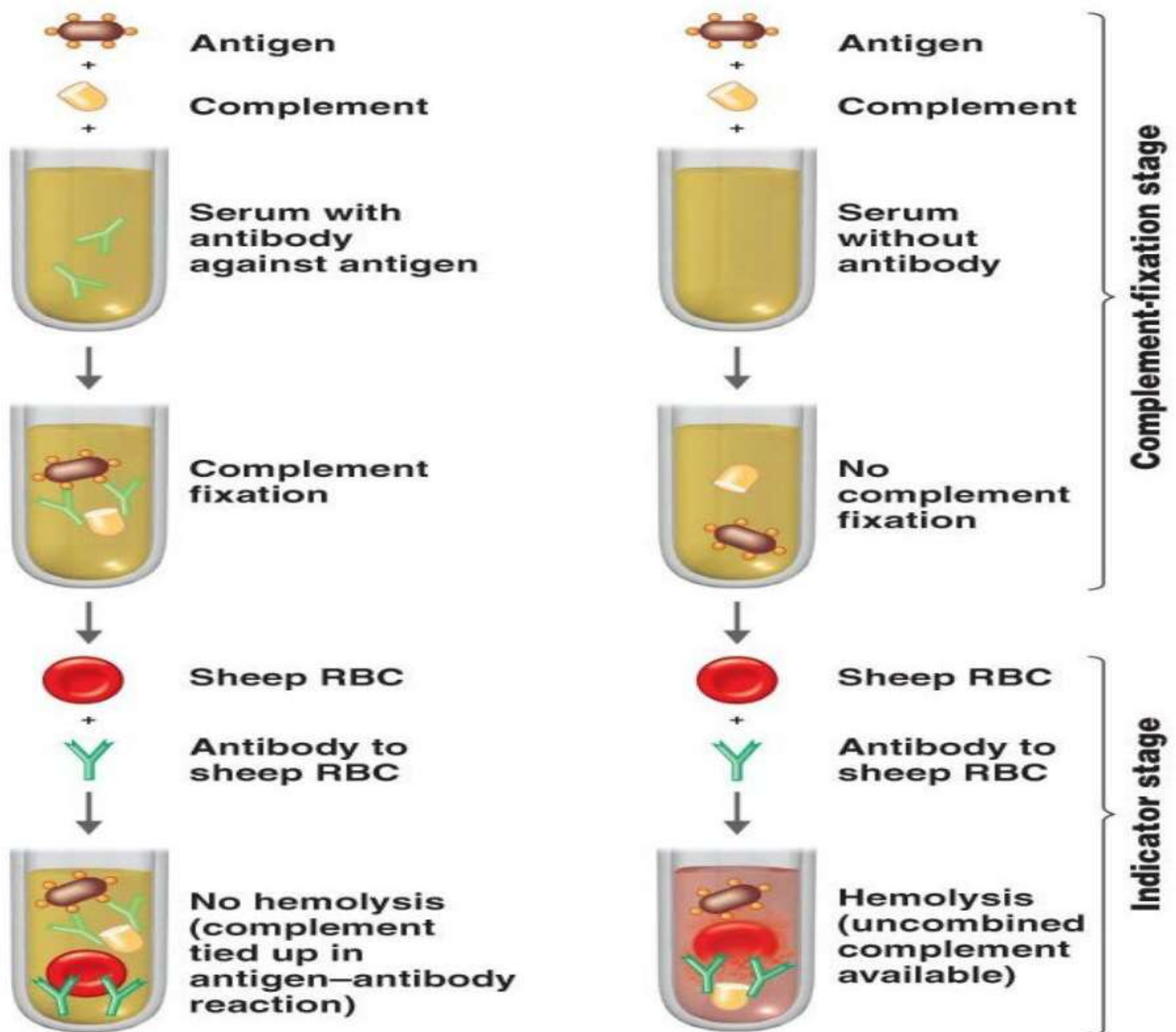
- Principle:

  It is the nature of complement not to react with an antigen or an antibody alone, but to enter into combination with antigen-antibody complexes. The lack of specificity of complement allows it to react with almost any antigen-antibody complex.

  The indicator system used in CF is sensitized sheep red blood cells (RBCs).

  In a *positive or reactive test*, the complement is bound to an antigen-antibody complex, and is not free to interact with sensitized RBCs so they remain unlysed and settle to the bottom of the well to form a button.

  On the other hand, in a *negative or nonreactive test*, the complement remains free since there is no antigen-antibody complex for it to bind to, and it interacts with the sensitized RBCs causing them to lyse.



(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

- <u>Reagents:</u>

- A hypothetical **viral antigen (VA)** in several unknown serum samples.
- "Sensitized" erythrocytes, which are simply sheep RBC coated with rabbit anti-red blood cell antibodies; we call these ***EA*** *(Erythrocyte + Antibody)*. In the absence of complement these cells remain intact, but they will be lysed if exposed to any source of complement; these EAs are our **indicator cells**.
- A standard source of **Complement**, usually fresh guinea pig serum diluted appropriately. The dilution is such that it contains just enough complement to completely lyse a standard amount of EA.
- A **standardized antibody** to the antigen, by immunizing a rabbit with purified VA. Before use the rabbit antiserum is heat-treated (56° C for 30 min) to remove its inherent complement activity.

- <u>Procedure:</u>

- A series of test samples (for example, heat-treated serum samples from various patients), mix them with a standard amount of our antibody, and incubate the mixture for an hour (allowing the antibodies to react with the antigen, if it is present). To this mixture we then add a standard amount of the guinea pig serum as a source of complement, and after a short time (to allow the AgAb complexes to interact with the complement) we add a standard amount of the sensitized erythrocytes (EA). We incubate the samples at 37° C for a half hour and examine them visually (or spectrophotometrically) for lysis; a suspension of intact RBC is cloudy, but it becomes transparent when the cells are lysed.

Tube No.	Sample					Result	Interpretation
1)	(saline alone	+	C)	+	EA	lysis	neg control
2)	(Ab + saline	+	C)	+	EA	lysis	neg control
3)	(Ab + VA	+	C)	+	EA	no lysis	pos control
4)	(Ab + *unk* No. 1	+	C)	+	EA	lysis	no VA present
5)	(Ab + *unk* No. 2	+	C)	+	EA	no lysis	VA present
6)	(*unk* No. 2	+	C)	+	EA	lysis	neg control, OK
7)	(Ab + *unk* No. 3	+	C)	+	EA	no lysis	VA present??
8)	(*unk* No. 3	+	C)	+	EA	no lysis	anti-complement activity

- Interpretation:

The protocol would be as shown on the table up (where "*unk*" refers to an "unknown" or test sample).

Tube 1 tells us that the erythrocytes have been properly sensitized and that our guinea pig serum (as a source of complement) is effective.

Tube 2 shows us that the antibody alone does not interfere with the complement-dependent lysis of the sensitized RBC.

Tube 3, the antibody will form complexes with the added VA protein, and these complexes will consume ("fix") the complement; therefore, there will not be enough complement left to subsequently lyse the indicator cells.

Repeating this test with two unknown samples in:

Tubes 4 and *5* tell us that sample No. 2 must contain the viral antigen VA, while sample No. 1 does not.

We carry out one additional negative control, by re-testing unk sample number 2 in the absence of specific antibody. As expected (tube #6), no complement is fixed, and the indicator cells are lysed.

However, the last two tubes (7 and 8) illustrated a possible complication in interpreting these results.

Tube 7 shows no lysis, suggesting the presence of viral antigen in sample #3 (as was the case with sample #2). However, when we run this same sample again in the absence of specific Ab, there is still no lysis, although we expect the same result as in our negative controls (tubes 2 and 6). This shows that serum #3 inhibits complement activity by itself, a property referred to as "anti-complementary;" we therefore can say nothing about the presence or absence of viral antigen in this sample unless we find a way to remove this activity.

Knowing of this possibility, it is clear that we must repeat this control test for every positive sample (as we did for sample #2 in tube 6) to confirm that the positive result is not the result of anti-complementary activity. (Such activity might be due to the presence of unrelated immune complexes in the serum, the presence of antibodies which happen to recognize components of the guinea pig complement, or the presence of drugs which inhibit complement.)

- Controls should be used along with the test to ensure that
- Antibody and serum are not anti-complimentary.
- The appropriate amount of complement is used.
- The sheep red blood cells do not undergo autolysis.