Lecture 8

Last time we talked about the complement system. Today we will complete it and discuss its regulation :)

This sheet will include the following :

1- Mannan pathway of complement activation

2- Functions of complement proteins

3- Regulation of complement proteins

4- Serology

1. Mannan/Mannose/Lectin Pathway of Complement Activation

How does it work?

- We have *mannose binding protein* which is produced non-specifically and is combined with mannose molecules on the bacteria. This will activate certain enzymes which are: **MASP 1** and **MASP 2.**

  MASP= manual associated serine protease

- MASP 1replaces CR1 and MASP2 replaces C1S.

So in the Mannan pathway of activation we don't need the C1, it is bypassed. MASP1 and MASP2 will do the job of C1R and C1S.

- Once this is happened the *MASP 2 will activate C4* and it will carry on just like the classical pathway.

- Once we get to C5 it is cleaved by C5 convertase into C5b and C5a.

- The C5b attaches to cell membranes and after binding to the membrane there is no longer a need for the convertases, after this step there is no more cleavage/ no more enzymatic activity.

- And then C6 and C7 will bind to the membrane (you know have C5b, C6 and C7). Once this happens, the state of the whole complex changes from hydrophilic to hydrophobic, now it can be inserted into the membrane and has effects there.

- After insertion, it will bind C8 and C9, the complex can bind more than one C9 they can reach up to 18 molecules that polymerize together and form a hole or a tube.

- This hole in the middle of the tube is going to allow water and different molecules going inside to the cell freely, the integrity of cell membrane is comprised, and the cell will swell and undrgo lysis.

- This complex C3b6789n is known as MAC

n= C9 number and it can reach up to 18

MAC = membrane attack complex

2. Functions of complement proteins:

i. **Cell lysis**: Via MAC; once it occurs on the cell surface it lyses.
ii. **Opsonization**:

- When we talked about the classical and the alternative pathways, we mentioned how the C3b and C4b molecules are deposited on the surface of the activating agent which could be a bacteria, an infected cell, an immune complex…etc

- These C3b and C4b have receptors CR1 (complement receptor 1) present on phagocytic cells as well as CR3 and CR4 receptors → therefore *anything with C3b and C4b on it* will adhere to a phagocytic cell and will then be opsonized and phagocytosed (the same as how the imunoglobulins adhere to the FC receptor and finally phagocytosis will occurs).
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iii. **Inflammation**: mediate inflammation through anaphalatoxins: **C3a , C4a , C5a**. Remember that whenever you cleave C3, C4, or C5 a small portion is produced which is discharged into the environment.

C5a is the strongest of them all, C3a is moderate and C4a is the weakest.

- They work by: They have a chemotactic function and can attract and activate the phagocytic cells to perform phagocytosis and produce respiratory bursts.
- They also work on mast cells and cause degranulation.

iv. **Prevention of immune precipitation and solublisation of immune complexes:**

- We mentioned before the lattice formation which is the cross linking of antigens by means of antibodies. This interaction between the antigens also depends on *Fc – Fc interactions*. Fc fragments attract one another and help make the immune complexes bigger.

- When you have activation of the complement, the **C3b** produced interferes with the interactions; hence you can disrupt the immune complexes by inserting C3b between them. It is believed that the classical pathway prevents the precipitation by producing C3b, and if you already have precipitation you can activate the alternative pathway (aggregates can activate it non-specifically) and this produces C3b which will break them apart.

So the classical pathway protects against precipitation, but if it has already occurred, the alternative pathway is activated.

3. Regulation of complement proteins:

- Regulation is important because many of autoimmune diseases are due to the prolonged activation of the complement proteins. Therefore we have strict regulate that either prevent their activation or prevent their prolonged activity.

- All of them are negative regulators except the **properdin which is a positive one**.

- **C1 inhibitor**: it inhibits the activation of C1 either by separating the components of C1 or by removing it from the activating surface. Therefore it prevents useless activation of C1 and also stops it.

- **C4b binding protein**: it acts on C4b which is part of the C3 convertase **in the classical pathway.**

It alsoaccelerate the decay of C3 convertase

- **Factor I:** C3b and C4b are inactivated by factor I which cleaves them into further break down products. C4b will be cleaved to C4c and C4d and C3b will be cleaved into C3d and C3e. These further break down products of the complement have their own specific receptors: CR3 and CR4. Keep in mind that C3b and C4b are recognized by CR1 receptor.

- **Factor H**: Works on C3b of the convertase **in the alternative pathway**. They are soluble regulators and work on the convertases.

- Those regulation factors control the early stage of activation, but then other factors will take over when it reaches the MAC phase. We have other proteins known as**1- S protein** or vetronectin protein. This protein allows the aggregation of complement components to carry on but it prevents the insertion into the plasma membrane, it stays in the solution and is hence useless. There is another protein similar to it called **Clustrin:** it's a soluble protein which has the same effect as S protein.

- **Anaphalatoxins inactivators**: will inactivate the C3a , C4a , C5a
- **Membrane bound regulators:** MAC might cause a bystander effect; for example when we have bacteria the MAC will be activated and it might attack a body cell that is near the bacteria and harm our cells.

- Thisusually doesn't happen because we have a regulators in our cells that prevents the MAC from doing that such as:

  - *The Membrane cofactor protein (MCP)* it works as a co factor for the activity of factor I which breaks c3b and c4b, occasionally it needs a cofactor such as MCP.
  - *Decayaccelerate factor (DAF or CD55):* another membrane-anchored protein, it accelerate the Decay of convertase for both classical and alternative pathways, so if there convertase settle on our cells it will be dissociated and inactivated by thisprotein.
  - *C8 binding protein*: another name for it is homologus restriction factor. It is present almost in all our cells**, it will inhibit the attachment of C8 and C9,** so the MAC system will not be completed.

- CD59 or protectin (MIRL: membrane inhibitor of reactive lysis): protects our cells by preventing the C9 polymerization

  — If we have deficiency in these regulators we might have diseases. Such as **paroxysmal nocturnal hemoglobinuria**:

- It might be caused by a deficiency in DAF and CD59
- Paroxysmal= seizures نوبات
- Nocturnal= active/occurs at night
- Hemoglobinuria: hemoglobin is found in abnormally high concentrations in the urine

- pH of the blood becomes more acidic at night since you breathe less and accumulate CO2, this makes the membrane more prone to lysis.

4. Serology

- **Serology**: is the use of immunological reaction (AB and AG reaction), for diagnosis (i.e ; to detect the presence or absence of a certain antigen or antibody)

- What is unique about antigen antibody interaction? **Specificity.**
- Ag + AB = interaction phenomenon:

For example, if we mix an antigen and an antibody we will have precipitation which will indicate the presence of a specific antigen or a specific antibody.

The phenomenon could be observed easily as in the appearance of *precipitation*, or using other methods such as *agglutination, immuno fluorescence, enzymes, radiation*...etc

1) **Precipitation**: we mixed antigen + antibody →cross linkages between them → the immune complex will get bigger and bigger → they will precipitate in the lower half of the solution. In this case the antigen is soluble.

- When do we get the maximum precipitation?

When we have an optimum concentration of the antigen and antibody.

If we have too many antibodies you will find that all the epitopes are blocked by the antibody and there is a little chance for the antigen to cross link with the antibody.

Therefore, excess antibody amounts will not lead to a precipitation. On the other hand if we have excess antigen also there will be no cross link so there is no precipitation.

- In general:
  - If there is balance between antigen and antibodies we called it **a zone of equivalence**
  - Too many antibodies this is known as **pro-zone** → no precipitation no agglutination
  - Too many antigens **post-zone** → also no effect

- If we perform this, the precipitation will not be seen by the naked eye, so we need special instruments to detect the interaction. How do these instruments work?

We direct a ray of light through the solution; this light has a receiver which measures its intensity. The more immuno-complexes there are the less the light that will pass through. By measuring how much light was transmitted you can work out the concentration of the antigen or the antibody. This is known as **Turbidimetry**.

Instead of measuring the transmitted light we could measure the scattered light which has a certain angle (some of the light is deflected sideways after hitting the immune complexes, this is received and measured). This is known as **Nephelometry**.

So, you can measure either the incident light which is going through or the scattered light sideways.

The instruments used are expensive.

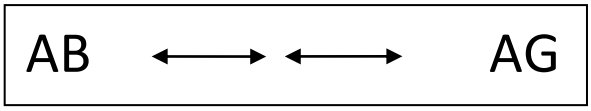
- You can instead use **<u>precipitation in a semisolid media</u>** because precipitation in semisolid media will produce a line of precipitation that can be seen by the naked eye so there is no need for the expensive special instrument.

An example of a semisolid media is agar: a powder that becomes jelly-like once we add hot water; agar actually comes from sea weeds.

In bacteriology we pour it onto Petri dishes but in immunology we pour it on a glass slide plate. We make holes in the agar.

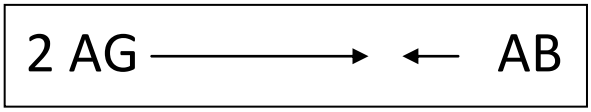
- Let's assume we put and 1k AB and 1k Ag in the plate and left them overnight, what will happen?

The Ag and the AB will diffuse in all directions, and they will meet finally at a point halfway between the two, which will produce a line called *line ofprecipitation*. The line will appear in the zone of equivalence.

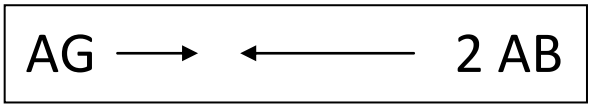


- If we repeat the experiment but added 2k Ag and 1k AB, what will happen?

The antigen will need to travel longer to be diluted and to reach the zone of equivalence of the antibody.



- If we add 2k AB and 1k Ag: the antibody will diffuse toward the antigen.



- This is double diffusion: you place the Ag and the AB in these holes and you leave them overnight and they diffuse toward each other.

- If you have a line that means that the antigen is stuck to the antibody (this is qualitative, if we have AB X and it stuck it means the antigen must be X), but we only have a small inclination on how much X antigen is there which is by the width of the line and hence it is semi-quantitative only.

- The use of agar and other semi-solid media can be helpful in diagnosing serious diseases such as meningitis. You can add an electric current which makes the movement of the Ag and AB toward each other much faster, this is knows as Counter **Current Immuno Electrophoresis**.

- This is important since the patient may die if not given rapid treatment and we want to know what organism is causing the meningitis. We take the CSF sample of the patient which will have microorganisms (menigicoccus, H.inf…etc) and we add AB against these bacteria into the plate (we use multiple plates and Abs), we perform the electrophoresis. This will give us the results and the diagnosis in a few hours rather than taking a full day.

Success consists of going from failure to failure without loss of enthusiasm.

Winston Churchill

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