# بسم الله الرحمن الرحيم

# Immunology lecture 7

Last time we studied the B cell receptor which is IgM & IgD.

Today we will talk about the T Cell Receptor which is the **TCR**…

#TCR: it is the receptor that belongs to T Cells >> it is always cell bound & there is no secreted form of this molecule

TCR is made of two polypeptide chains: Alpha chain and Beta chain …

-Each chain has two domains: 1) Domain 1 which is the outer domain and it's the variable domain (alpha domain 1 and beta domain 1 are the variable domains)

2) Domain 2 which is nearer to the cell membrane is the constant domain (alpha domain 2 and beta domain 2 are the constant domains)…

#TCR has "half a ring with disulfide bond" structure which is a domain structure >> so TCR is a member of the super immunoglobulin family …

#the antigen recognizing site of TCR is on the outer variable domain which is equivalent to paratopes present on immunoglobulins.

#The development and production of diversity is exactly similar to what occurs in immunoglobulins >> Beta chain is equivalent to the heavy chain, so during the gene rearrangement which drives the activation you will find that we have V ,D,J rearrangement, and then the variable domain will attach to the constant domain forming the Beta chain of the TCR, just like the heavy chain in Igs … but here we have one constant domain coded by 1 gene, not 3 or 4 constant domains as in Igs.

#Alpha chain is equivalent to the light chain of the immunoglobulin; here we have only v & j rearrangement in the variable domain, and then it will connect to the constant domain forming the Alpha chain of the TCR …

So to **summarize** ... here instead of heavy chain we have the **Beta chain ,**,, and instead of the light chain we have the **Alpha chain** …

TCR has extracellular part (the variable & constant domains forming beta sheets structures), trans-membranous part & intra-cytoplasmic part.

#Activation of T Cells is done through signaling,,, actually TCR is not the only responsible molecule for activation of T Cells; since TCR is unable to send messages to the inside of the cell or gives very little amount of signaling upon recognizing the antigen ... activation occurs mostly by accessory molecules which are always in association with the TCR and they are adjacent to it >> CD3, Zeta & Eta molecules

#These molecules are members of the super immunoglobulin family and TCR also belongs to this family...

#**CD3**: a tri-molecular compound (it has 3 polypeptide chains: gamma, delta and epsilon)… a cluster of differentiation protein that is always associated with the TCR... It is called CD3 not because it has 3 molecules but because it is the third one to be included/discovered in the CD nomenclature … it is involved in the signaling pathway for activation of T Cells (upon recognition of the antigen, CD3 transmits signals to the inside of the cell)

In order to produce more signaling for more activation there are further accessory molecules that are also included >> these molecules are called **Zeta and Eta**,, these molecules can transmit signals to the inside of the cell better, as you can see from their
- ( زيطاو عيطا)tails.

SO, recognition occurs through TCR itself, but signaling is mainly achieved by the adjacent molecules… All together form the TCR complex.

#The doctor drew a picture for a naïve cell to show the B Cell receptor >> on its surface there is IgM –serving as a recognizing unit- and also 2 adjacent accessory molecules: Ig-alpha and Ig-beta which are associated with IgM and they transmit the signals to inside of the cell for activation… so the B.cell receptor is actually the IgM which is associated with two polypeptide chains: alpha and beta …

## The complement system

is a collection of proteins (35-40 proteins) which are present in the body in the **inactive form** except one which we will study later,,, some of them are soluble & some are cell bound, and they always activate one another in sequence forming a cascade,,, these protein are activated very very quickly then they are inactivated very very quickly (period from activation to inactivation/ half life takes about milliseconds) this is because we want to have the results of their activation but we don't want to have a continuous activation which is associated with many problems … since the complement system is a destructive system that if left uncontrolled will damage the tissues.

**#Nomenclature of the complement system:**

Some of the proteins are called **complement components** (they are referred to with the letter "C" ) >> They are 9 in number " C1-C9"

Some other proteins are called **Factors** "some of them are very similar to the complement components group" >> examples of the main factors: factor D, factor B, factor I, factor H, factor J …

Some other proteins are named according to their function,,,

Example: C4BP "C4 binding protein" ,,, CR1=CD35 "complement receptor 1, what does it mean? It's a protein found on a cell that receives complement",,, CR2=CD21,,, CR3, "C means that they are complement components",,, etc.

#all of them are present in the inactive form except one >> for example if it is written:

**IC3** it is no active. $\overline{\mathbf{C3}}$ it is in the active form.

#Activation of the complement components involves an enzymatic activity by **serine esterase** >> which will break/cleave the complement component into 2 parts: a big part (given the suffix b) and a small part (given the suffix a) >> so by this the active site of the complement protein is exposed & it will activate another molecule and so on"

#example: **C3** is a component of the complement system, once it is activated very quickly it will break into 2 parts: **C3a** "the smaller one " and **C3b** "the bigger one" and each one has its own action,,, **C3b** is in the active form but it must be inactivated immediately "very quickly " and when it is inactivated we write it: **iC3b** .. " **i** " means that it is inactive.

## ## Functions of the complement system:

1) Opsonization

2) Cell lysis

3) Process of inflammation

4) Prevention of immune precipitation,,, in which it prevents the precipitation of soluble immune complexes "remember the lattes formation by cross linking which causes precipitation and agglutination "…

5) This system causes solubilization of immune complexes in which it can disturb the lattes formation somewhere in the body and separate them from each other because the binding of the antigen to the antibody is reversible and can be disturbed by this system to prevent their binding and precipitation.

## ## Pathway for activation of the complement system:

**1)the classical pathway** : it is called classical because it is the first one to be discovered ,,, classical also means old but really this pathway is new while other pathways are old .
-It is present in all vertebrates but it is not present in non vertebrates like worms.
-it is activated by the immune complexes in which binding of the antigen to the antibody will activate this pathway …
**2) Alternative pathway**: it was discovered by Pillemer who found that there is another pathway of activation but nobody believed him and after he died they discovered that his findings were true …
-this pathway is driven by foreign things: invading bacteria, viruses, foreign cells, dead cells or even aggregates of foreign proteins in the body.
**3) Mannan or mannose - lectin pathway:** called mannose pathway because of the mannose binding protein (which is an acute phase protein) which is included in this pathway.
-the complement system is activated when the mannose binding protein binds to mannan/mannose which is present on bacteria … and the mechanism of activation of the complement system here is similar to the classical pathway of activation.

# Classical pathway of activation:

#More advanced than the alternative & belongs to the acquired immune response.

#As a rule, it is activated by immune complexes, but can be sometimes activated by cells or viruses.

#the first protein to be involved in the activation of the classical pathway is called **C1 (a complement component)**

#C1 structure: (the doctor drew a picture of it):

It is multimolecular; it is composed of 5 molecules, c1q, a pair of c1r, a pair of c1s.

>> C1q is the largest & is made of 6 strands that are stuck together (similar in shape to a bouquet of tulips) forming a stem part that has a collagenous nature and a globular part which is the head part which recognizes/ combines with the activating element.

-embedded in this bouquet of tulips we have 2 pairs of molecules: a pair of **C1r** and a pair of **C1s**, these molecules are inactive , how to be activated? First, we must have an immune complex i.e. an antibody attached to the epitopes of an antigen,,, the CH2 domain present on the heavy chain of the immunoglobulin is hidden/ not available when the Ig is free, but upon forming an immune complex, there will be conformational change that will expose this CH2 domain on then Fc fragment of the Ig, and only then one of the globular heads of C1q will combine with the CH2 domain, **and we need 2 engagements** i.e. one globular head isn't enough for the activation of the component.

##Why C1q doesn't attach to the Fc fragment of the immunoglobulins that are free??

Because free immunoglobulins are not bound to the antigen so the binding site on the CH2 domain on the immunoglobulins is hidden so activation of the complement system will not occur and we don't need activation when there is no antigen ,, but once the antigen is present ,it combines the free immunoglobulin , so the binding site on the CH2 domain of the immunoglobulin will be exposed for binding to a globular head of C1q >> finally the complement system is activated only when the antigen is present and attached to the immunoglobulin and the binding site of CH2 domain is exposed for C1q globular heads to attach …

-for activation you need at least 2 globular heads from C1q to be attached to 2 adjacent Fc fragments of the immunoglobulin on the same antigen.
-WHEN ACTIVATION OF C1q occurs (2 globular heads are attached to 2 separate Ig's Fc fragments) ,there will be conformational change in C1 which will activate C1r i.e. expose the active site of C1r >> C1r is a serine protease enzyme ,its active site is usually hidden ,but when the conformational change occurs the active site of this enzyme will be exposed >> then C1r will go and activate/ cleave C1s into 2 pieces >> now C1s which is also a serine protease/esterase enzyme is also activated.

To summarize: first, C1q which is in the C1 complex will bind to the immune complex "immune complex =antigen +antibody " so is activated then it leads to the activation of C1r which then activates C1s .. " when these molecules are in the active form we write the name of the molecule with aline above it which means that it is an active molecule ex: **C1s .**

#The second protein to be activated in this pathway is the **C4** " it is the fourth molecule to be discovered but it is the second one to be activated in this system in which they discovered C1 then C2 then C3 then C4 but when they discovered C2 and C3 they found that there is a molecule that is activated before both C2 and C3 which was hidden then they discovered it and called it C4"…

#any C4 molecule that happens to be passing by, will be bound by this complex (immune complex + C1)

#after C4 is bound, C1s will activate it and cleave it - by an enzymatic action - into: **C4a** "the smaller " and **C4b** "the bigger" >> C4b will bind to the activating surface of the immune complex itself or to the cell that is attached to Ig, waiting for the next component (C2) to be passing by to grab it and activate it, while C4a will go away in the serum since it is a hydro-toxin and it is not needed.

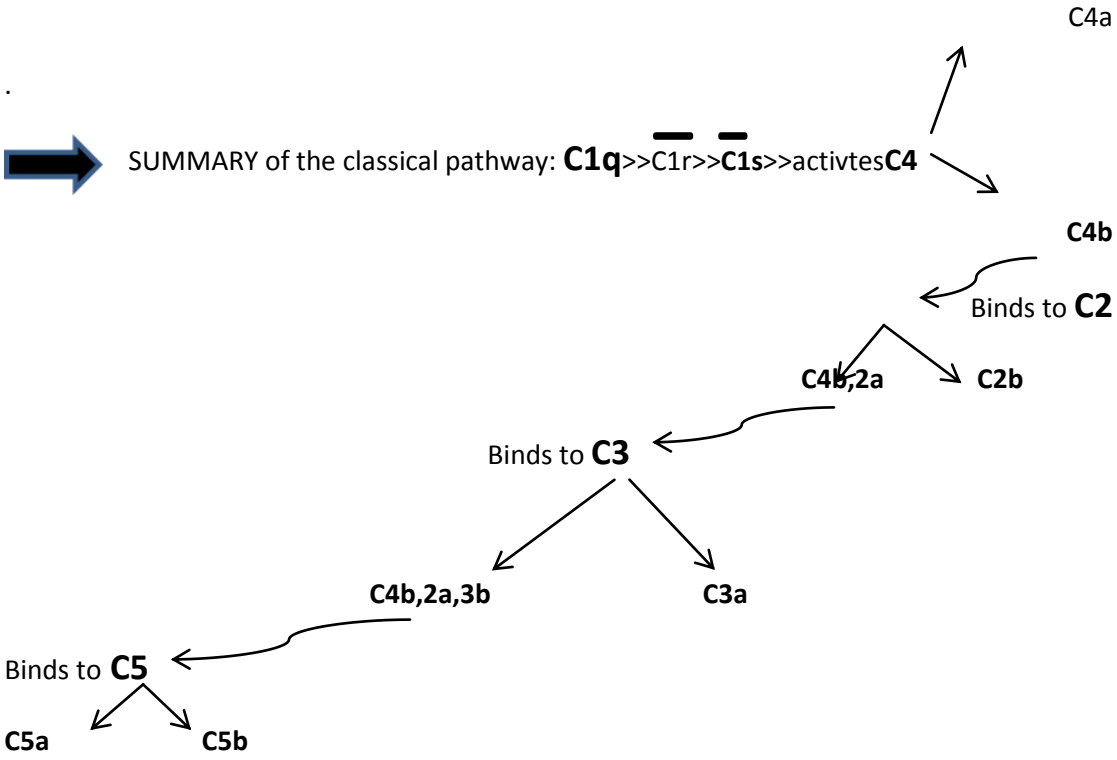
 Now we end up with an immune complex which is the antibody attached to an antigen + C1 + C4b which is attached to the complex and is sitting on the activating surface of it.

#C1s cleaves C4 & C2.

#The third molecule to be activated in the system is the **C2**>> C2 will bind to the immune complex, then acted upon by C1s, then it will be broken down into: **C2a** "a little bit bigger & has a good enzymatic activity" and **C2b** "a little bit smaller and it will go to the solution, it has some activity but it is not a hydro-toxin" >> C2a will bind to C4b " some books write it as" **C4b,2a**" since they are stuck together & other books consider that C2b is the one that attaches to C4b as in "C4b,2b", **but we'll consider "C4b,2a".**

#C4b, 2a which is sitting now on the activating surface will bind to C3 which is the fourth one to be activated in the system >> then C3 will be broken down by C2a enzymatic activity into C3a "the smaller, goes to the serum " and C3b which will stick to the complex on the activating surface giving a new molecule/complex (C4b, 2a, 3b)… (C4b, 2a) which converts C3 into 2 pieces is called **"the C3 convertase of the classical pathway of activation".**

# (C4b, 2a, 3b) complex then will act on C5 and convert it into 2 pieces: C5a and C5b **"the C5 convertase of the classical pathway**".




# Alternative pathway of activation

#Is very primitive & belongs to the innate immune response & was discovered later after the classical pathway.

-**C3** is the most abundant one in the serum and it is the most important component or protein in the complement activation,, it is present in the serum in the inactive form,, but during its presence in the serum a little bit of it is being continuously activated – spontaneously-, some people say it is due to hydrolysis of this molecule by water while others say that it is due to some enzymes that break it down, but hydrolysis is more likely to believe & accept than enzymatic cleavage …

-once it is hydrolyzed it becomes active but it stays as it is,it will not be broken down to C3a and C3b that is why it is more probable to be caused by hydrolysis not by enzymatic cleavage >> so it is written " **C3* or C3-H2O** " which means it is active ,, when it is activated it behaves like C3b which is the active form of C3 in the classical pathway .

# but C3* will immediately be inactivated by many factors (complement regulatory proteins) like factor B and factor I which are regulators of the complement activation which will inactivate it very quickly ,,, this occurs continuously in our bodies in which C3 will be activated very quickly then immediately it is inactivated also very quickly like a car that its engine is working but the car is not moving ,this is known as " **Tick over mechanism** " which produces a small amount of C3* then it is immediately inactivated and then there is no further activation of the complement activation pathway ,, but when there is foreign body like bacteria or virus,C3* will stick to the foreign surface and it will be protected from immediate inactivation by the complement regulators , so it will have a longer half life and it is fixed and stable in the active form ,it is now able to initiate the activation of the alternative pathway of the complement system.

# when C3*/C3b is fixed, it binds **factor B** which is a protein that is similar to C2 in the classical pathway, it binds to the active surface of the fixed C3* then we will get the arrival of an enzymatic protein which is present in the serum **in its active form** which is **factor D** -it is the only one of the complement proteins to be present in its active form in the serum- but it will not act on factor B unless factor B is bound to C3*/C3b.

#This enzymatic protein (factor D) will cleave factor B into :1)**Ba** "the smaller one ,it goes to the solution but it is not a toxin " 2)**Bb** which has an enzymatic cleaving activity & is equivalent to C2a, will be stuck to the activating surface & we'll end up with (C3b, Bb) which is called "**the C3 convertase of the alternative pathway**", it will cleave a new C3 molecule into C3a"goes to the solution" and C3b, now we have (C3b, Bb,3b) on the activating surface , which is called "**the C5 convertase of the alternative pathway** ", then C5 is taken up and broken down, by the C5 convertase into C5b & C5a (which is an anaphylactic that goes to the serum)… , so now both classical & alternative pathways will end up cleaving C5.

#C3 convertase produces lots of C3b molecules which are not all attached to the enzyme itself, so some of the C3b are going to attach on activating surfaces of the same type in the nearby environment and then they can fix more factor B and start another cycle with more convertase molecules cleaving more C3 molecule, this is a self-amplifying loop that is why we called the alternative pathway as "**the amplification loop**".

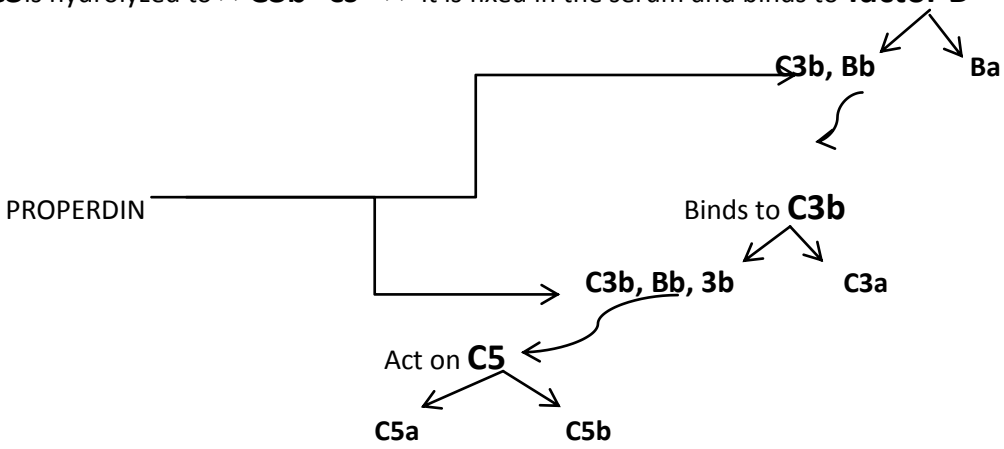
#The alternative pathway can be initiated by the classical pathway, since the classical one produces C3b molecules.

#**Properdin** :it is another protein of the complement pathway , its job is to prolong the half life of the convertases of the alternative pathway only , so it doesn't act in other pathways,, it prolongs the half life by 20 times to give chance for more and more activation …

-Properdin is the only positive regulator of the complement system while all other regulators of this system are actually negative regulators which might break down molecules or do other functions " we will discus them later "…

Summary of the alternative pathway:

**C3**is hydrolyzed to >>**C3b** "**C3***"">> it is fixed in the serum and binds to **factor B**




# Mannan Pathway:

When lectin –mannose binding protein- attaches to a bacteria having mannose on its surface, this will activate the mannan/ mannose, now, mannan activation goes through 2 proteins (enzymes), MASP-1 & MASP-2 (Mannan Associated Serine Protease -1 & 2)… once the combination occurs between lectin & mannose, MASP-1 is activated –it is equivalent to C1r- which then will activate MASP-2 –it is equivalent to C1s- , then C4 will be activated by MASP-2 and then the cascade continues as in the classical pathway... so in the mannose pathway there will be no need for C1 for initiation of the pathway.

PLEASE refer to the transparencies they will help you to understand everything.

Sorry for any mistake.

بالتوفيق للجميع يااااااا رب ولا تنسونا من دعواتكم ☺

تم بحمد الله وفضله☺