Immunology
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Lecture - 46
Cytokines: Introduction (Contd.)

Welcome back to our lecture on the cytokines that we started in the last lecture.

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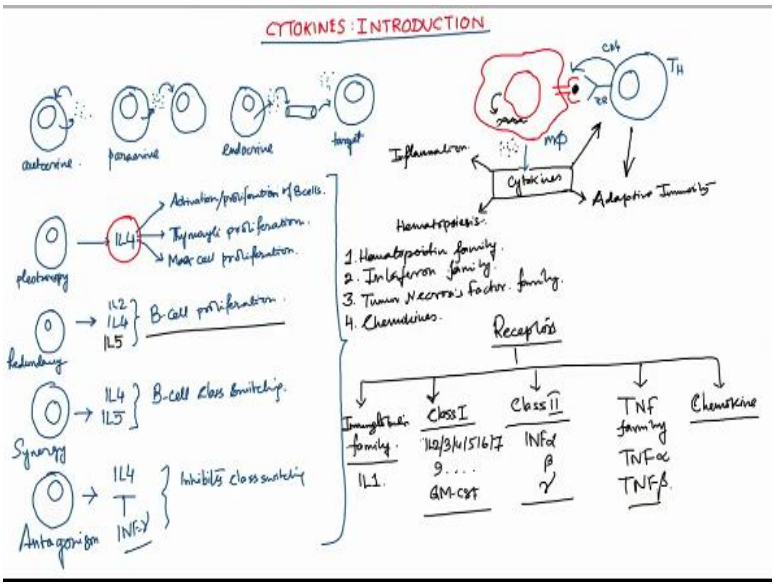

So what we have discussed in our last lecture that the cytokines they are primarily mediating their actions via different types of receptors. And we have discussed at least five different classes of cytokine receptors, the immunoglobulin class, the class one receptors, the class two receptors, the TNF family receptors and the chemokine receptors.

Now looking into the receptors as I told that this receptor structures have been very well characterized, and from the characterized receptor structures, we can have a fair idea of how this cytokines they mediate their action and how they probably exhibits different types of activities like synergy, redundancy or antagonism. So to get an understanding of how these actions are being mediated, let us look into the structures of the different cytokine receptors.

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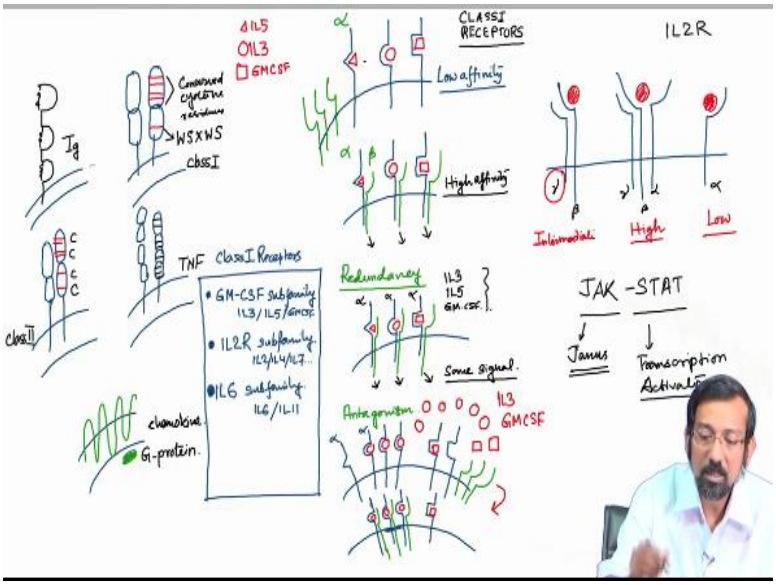

So these are the five broad five classes of cytokine receptors that I have drawn here. So this is an immunoglobulin family receptor, which has this kind of disulfide linkages. So this is the IG class or the IG family of receptors. This is the class I receptors, the class I receptors. These are the class II receptors, the class II or the interferon receptors. And then we have the TNF family of receptors.

And these are the chemokine family of receptors. As I told that these family of receptors are associated usually with G-protein. So these are G-protein coupled receptors. So if you look into the structure of the basic structure of these all these receptors, these receptors, they share a similarity in the structures. Whereas, the chemokine receptors they differ a little bit.

So these receptors have a very common thing amongst them is that they have certain conserved cysteine residues. These are the conserved marked in red, these are the conserved cysteine residues. And they also have a very conserved motif, there is a WSXWS that is a tryptophan serine, tryptophan serine repeat with any amino acid in between. So this is a very common repeat that is found in case of the class I receptors.

And the class II receptors also has this kind of conserved cysteine residues and these are the TNF family of receptors. They also are characterized by presence of lot of this conserved cysteine residues. So these are the broad classification of the different types of cytokine receptors to which the cytokines they can bind. Now how this cytokines they mediate the action by binding to these receptors.

So binding to these receptors is kind of structural binding, let us say. So there is a kind of an lock and key system of binding. So a receptor usually has a ligand binding area or region where this ligand, in this case cytokine is the ligand, the cytokine molecule is the ligand, it can bind. If we look into one of the very most studied family of the cytokine receptors, which is none other than the class I receptors.

Now this class I receptors, if you look into the class I receptors, the class I receptors can again be sub classified. We can sub classify the class I receptors into at least three different, so if I call this a class I cytokine receptors, this class I receptors can be again, sub classified, and they can be classified into three families. One, the GM-CSF, that is the granulocyte macrophage colony stimulating factor, it is also a cytokine.

And it binds to this kind of cytokines, it can bind to GM-CSF, as well as to IL-3 and IL-4. So this class of receptor, they are called the GM-CSF receptor subfamily. So this subfamily is the subfamily of receptor, remember. So this falls under the classification of class I receptor. A second class of receptor is IL2R receptor subfamily. They are named after a particular cytokine they bind to, but they can bind other cytokines as well.

For example, this class IL2R subfamily, it can bind to interleukin-2, interleukin-4, interleukin-7, interleukin-9, all of them. But they are named after the interleukin-2. So it is the IL-2I R4 receptor, so interleukin-2 receptor subfamily. And also the IL-6 receptor, the interleukin-6 subfamily of receptors. And they can also bind to interleukin-6 or interleukin-11.

This subfamily it can bind to interleukin-2, interleukin-4, IL-7. So it can bind to many. This can bind to IL-3, IL-5 as well as GM-CSF. Now these receptors are usually characterized by presence of more than one subunit. Now it is a multi-subunit receptor. If you look into the pictures that I have drawn here in this part, or in this section, you see that at least there are two different subunits.

And we call them the alpha subunit and the beta subunit. Now among these subunits, try to understand carefully. So among these subunits of the receptors, one of the

subunit is primarily responsible for recognition in binding of the ligand that is the cytokine. And the other receptor or the other subunit binds only when there is a ligand binding by the other receptor counterpart.

So that means, the alpha subunit here for example, this alpha subunit can recognize the specific ligand for example, interleukin-5 or interleukin-3 or GM-CSF. As soon as alpha subunit binds, but this binding is called a low affinity binding. And hence, the receptor is also called, this alpha receptor is called the low affinity receptor. Because, the binding is complete only in presence of the other receptor counterpart that is the beta.

Now when there is a low affinity binding by the alpha subunit, then only the beta subunit comes in. Now then the beta subunit comes in and there is a high affinity binding. So now this is a high affinity receptor. Because, now you have both the alpha and the beta subunits. Beta subunit is primarily responsible for the signal transduction. So this is where from the downstream signal is transduced.

So it is the binding or the receptor binding to the ligand is complete when and when only both the alpha and the beta subunits bind. And this binding offers is offered primarily by the alpha subunit where the alpha subunit has more specificity for the ligand. But, until and unless the beta subunit binds, the binding is not completed. And hence, it is called a low affinity binding.

Once there is by binding by the beta subunit, then only it is a high affinity binding and it binds with high affinity to the ligand. Now once this high affinity binding is completed, the beta subunit is responsible for the transduction of the signal downstream. Now let us see how this kind of receptors or the receptor ligand binding actually mediates the phenomenon like redundancy or antagonism in case of the cytokines.

Now one most important thing that we need to remember in this case is that the alpha subunits have if you look into the structure here, the alpha subunits are specific, ligand structure specific. That means, they offer specific structures for specific

binding sites for the specific ligands or the specific cytokines. But the beta subunits are non-selective. So they are common.

The beta subunit in case of this class I receptor or to be precise, in case of this GM-CSF subfamily the beta subunit is common to all. Alpha and beta subunits, they do not remain together, bound together. So they normally, they do not dimerize. They remain separately. So for example, on this, if this is the cell surface of the cell membrane, you will be finding beta subunits which are lying independent of the alpha subunits.

Once there is ligand binding like this, once there is an interleukin binding to its corresponding alpha subunit, then only this beta subunit comes and completes this binding. Then only the one of the beta subunits will be available and binding to this alpha subunit bound to the corresponding cytokine. So let us say these are the three different alpha subunits that are present on the membrane corresponding to the three different cytokines like IL-5, IL-3 and GM-CSF.

If you look I have written the legends, for IL-5 is the triangle, the circle denotes the IL-3 and the square denotes the GM-CSF. So now this kind of beta subunits they are floating around, they are not bound to the corresponding alpha subunits. Now what happens, whenever there is a ligand receptor binding, there is the alpha subunit is binding to the corresponding cytokine the beta subunit will come and join and it will produce the high affinity receptor.

Now what happens in case of redundant action? In case of redundancy, we see something very similar. So now if there is presence of all the cytokines, if there is a presence of all of the cytokines surrounding then what will happen? That means, all the three cytokines are available, be it the IL-5 or the IL-3 or the GM-CSF. Then actually, they will saturate all the three types of alpha subunit that are present.

So these are the alpha subunits, they will saturate all the three types of alpha, corresponding alpha subunits and the beta subunits will bind to them. And since the beta subunit is common to all of them, they will transduce the same signal. It means

downstream signaling, the same signal will be transduced and that is why we call that these three so IL-3, IL-5 and GM-CSF they can exhibit redundancy.

So because it transduces the same signal or the redundant signal. Then what is how do they exhibit antagonism? As I told, normally what happens, normally on the surface of the cell, or on the surface, we have this alpha, the alpha for, let us say these are the alpha subunits that are present for IL-3. And also you have the alpha subunits present for the GM-CSF and IL-5.

Now in the vicinity, there is a lot of IL-3 molecules. And also a little bit of the GM-CSF molecules. So what will happen in this situation that if more of this stoichiometrically if the alpha, if the IL-3 molecules are more than they will quickly go and saturate the alpha subunits of the IL-3. So the IL-3 alpha subunits will be quickly saturated. And only a very few of the GM-CSF family or the GM-CSF subunits alpha subunits will be saturated.

So now these are the alpha subunits. The main problem is with the beta subunit. Now the beta subunit, they are limited. You do not have a lot of beta subunit. So there is a limitation of the beta subunits. So let us say you have only this many beta subunits. So if the ligand, in this case it is IL-3, if it competes out the other ligand in binding to its corresponding alpha subunit.

That means if a lot of IL-3 goes and initially binds to the alpha subunits of the IL-3 receptors, then this IL-3 receptors will be saturated. So now they will immediately bind to, they will occupy all the beta subunits that are present. And there would not be beta subunit left out that can go and bind to this alpha subunit of the GM-CSF. So the situation will be something like this.

That all these alpha subunits, the situation will look something like this. So now this beta subunits will go and immediately bind to this alpha subunits, which are already bound to the IL-3 ligand. And there would not be any beta subunit available to bind to the alpha subunit of the GM-CSF. So this and as I told, if there is no beta subunit binding, if there is no beta subunit binding, then the high affinity receptors will not be produced.

So it will remain as low affinity receptor, and it will not transduce any signal. So basically, the here in this case, we see an example of interleukin-3 and GM-CSF competing with each other, they are competing with each other for the alpha subunits and the IL-3 since it is more as compared to the GM-CSF, it will go and immediately saturate all the alpha subunits or the IL-3 alpha sub units.

And since the beta subunit is common, and of course the number of beta subunits is limited that will go and bind to the alpha subunits that are already saturated with IL-3 and beta subunits will not be available to bind to the alpha subunit, which are filled with or bound to the GM-CSF. So naturally, there would not be any signal transduction.

There would not be any downstream signaling, because of unavailability of the beta subunits that can bind to the GM-CSF. So basically, we see an example of how IL-3 can antagonize the GM-CSF. Looking into the another class of the receptor, that is what we call as the interleukin-2 receptors or the IL-2 receptors, this is this also belongs to the class I receptors. Remember, this is still the class I receptor.

Do not confuse it with the class II receptors. It is the IL2R, interleukin-2 receptor type, it is not class II, it is class I. Class II receptors are primarily meant for the interferons. So this is still the class I receptors. They are named after the type of interleukins or cytokines they bind to and that is interleukin-2. These receptors are characterized by a common gamma subunit. If you see they have a common gamma subunit.

Unlike in case of the GM-CSF family, where we have seen they have a common beta subunit, these receptors are usually characterized by the presence of a common gamma subunit. And there is also, they can also exist in the three forms that is the low affinity form, the intermediate affinity form, and the high affinity form. So the low infinity form is only the alpha subunit. The intermediate form is the beta gamma subunit.

And the high affinity form is when all the alpha, beta and the gamma, all the three can bind to the ligand together. So the gamma chain is kind of expressed constitutively. So it is always expressed in most of the cell types and the alpha and the beta chains, these alpha and the beta chains, they are restricted to activation. So alpha beta chains are not always expressed.

The alpha and the beta chains are kind of their expression is restricted, and they are restricted to the activation and that is why that ensures that only when there is an antigen binding to for example, for the T-cells, the CD4+ and the CD8+ T-cells. So activation of these T-cells, it ensures that only when there is an antigen activation, this CD4 and the CD8 cells they express the high affinity receptors. So the high affinity IL2Rs.

It is not expressed under normal conditions. So that is why you have three different receptor subunits, the alpha, the beta and the gamma. So the alpha and the beta subunit expressions of this receptor expression is mostly coordinated with the activation. So once there is an activation of the cell or the T-cell for example, then only the alpha and the beta subunits will be expressed.

And that would lead to formation of the high affinity receptor. That high affinity receptor is the effective form of the receptor. That can only transduce the signal. The low affinity receptors cannot transduce a signal, remember. So the low affinity form or the intermediate affinity form can never transduce the signal. They have lower affinity for the corresponding ligand.

And the high affinity signal, a high affinity receptor is the receptor type, which can actually transduce the signal downstream. And it is formed only in presence of alpha, beta, gamma the three subunits together in this case. And as I told that the gamma subunit is constitutively expressed and the alpha and the beta subunit expression is primarily linked to the activation.

And this phenomenon basically ensures that the T-cell can express the high affinity receptor, only the high affinity receptors, only when there is an activation, not always. So they are not always and not always binding to IL-2 always, they are not. So T-cells

are not always they are secreting IL-2 or binding to the IL-2, IL-2 receptors, the high affinity receptors are not always present.

So let us see how or what exactly happens downstream of the ligand binding or downstream of the binding of cytokines. So downstream means, after there is a high affinity receptor formation and high affinity receptor binding, what happens next. So what is happening next? That means, how the signal is transduced. We have understood the receptor types, the different receptor types.

We have kind of a fair idea about the different receptor subunits and how they work. And what are the low high and intermediate affinity receptors. But now we need to know that how these receptors they actually function, they actually work. So most of these receptors, they are linked to what we or what is known as a JAK-STAT pathway. What is this JAK-STAT pathway? What is this JAK?

The JAK is named after the Janus. It is named after the Janus. And it is the Janus Kinase and the STAT which is, the STAT is also known as the signal transducer and activator of transcription. So this is an activator of transcription, transcription activator. And this is the Janus Kinase. It is sometimes we also call it like a just another kinase.

It is just a joke to say the JAK is because inside the cell, we have so many kinases in so many signal transduction processes that are going on and usually all this signal transduction processes, they are mediated via these different types of kinases. And so we sometimes call them as just another kinase. But it is not really the fact. So it is named after this Janus, which is a Greek God.

And so it is the JAK Kinase, which actually activates the STAT or the and the transcriptional activator or the STAT.

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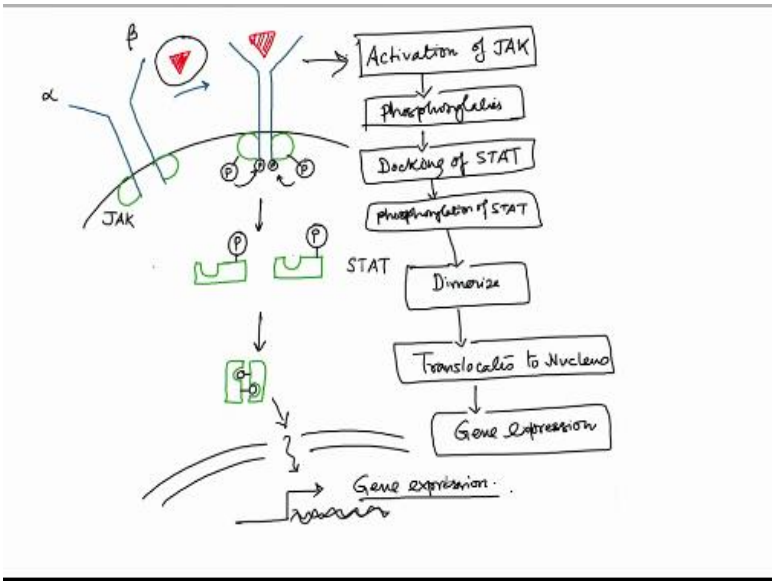

So let us see how this works, how this JAK-STAT pathway, it actually works. So most of these receptors of the cytokine family, they are linked to a one of these type of tyrosine, receptor tyrosine kinases like this JAK, which is also a receptor tyrosine kinase and this JAK is associated with these receptors with this let us say this is the alpha and the beta subunits of the receptors.

They are associated with this alpha, beta subunits of the receptors. Whenever there is a binding of a cytokine or a ligand to this receptor, this receptor is bound to this cytokine, this JAK is activated. So that leads to active, the first step is activation of JAK. So now JAK is activated. And now once JAK is activated, the JAK what it can do is it can phosphorylate.

Now it starts to phosphorylate, when JAK starts to phosphorylate the receptor subunits, so now it phosphorylates at specific sites on this, these are all tyrosine kinase receptors and so they are phosphorylated at specific sites. So they are at specific tyrosine residues on the receptor subunits. Now when this tyrosine residues on the receptor subunit are being phosphorylated that basically offers a site for this STAT.

Now this STAT normally is roaming around. And now that leads to, that brings the STAT to this site or that helps the STAT to dock on to the receptor. Now the STAT goes and docks on, docking of the STAT. So the next step is docking of STAT. So

now STAT, which is normally moving around, it goes and docks on the receptor or binds to the receptor. So normally the stack cannot bind.

The STAT can bind only when there is phosphorylation of this tyrosine residues on the receptor by the JAK. JAK is activated. Activation of JAK leads to phosphorylation of specific tyrosine residues on the receptors, on the receptor subunits. And that brings the stat there at that place. Now once the stat is docked on the receptor or there is binding of this STAT to the receptor, then immediately this JAK it phosphorylates STAT.

Now STAT is then phosphorylated. There is phosphorylation of the STAT and this STAT phosphorylation leads to that leads to the dimerization of the STAT. So this is the phosphorylated, the two subunits of the STAT and this is the phosphorylated STAT. So this docking of the STAT and then STAT phosphorylates, phosphorylation of STAT.

Now STAT is phosphorylated and as soon as it is phosphorylated this phosphorylated STAT now translocates to the nuclei. It enters the nuclei and so now it dimerizes. Who dimerizes? The STAT dimerizes and this dimer then it translocates to it translocates to the nucleus. So that leads to translocation to the nucleus and finally gene expression. So that leads to expression of, so that leads to gene expression or transcription of specific genes that mediates the function.

So this is the overview of the way how the cytokine receptor, downstream of cytokine binding actually works. So let us consider this is the cytokine. The cytokine binds to the receptor. So the alpha and the beta subunits they normally remain apart. When there is cytokine binding they come together. So there is receptor dimerization.

And when this receptor dimerization occurs the receptor dimerizes or they comes together then there is activation of JAK. This is the first event. Then receptor dimerization leading to the activation of JAK. When there is activation of JAK, as a told JAK is a tyrosine kinase receptor. So it immediately phosphorylates specific target tyrosine residues in the receptor subunits.

Now this tyrosine residues which gets phosphorylated in the receptor, they now can offer site, they can offer a site for binding of the STAT that is docking of the STAT. Now the STAT then moves and comes to the receptor and docks on the receptor or binds to the receptor. The docking of the STAT is basically ensures or it is required for phosphorylation of the STAT.

Now the phosphorylation of STAT occurs by the same Janus Kinase or the JAK. So JAK now phosphorylate STAT and this phosphorylated STAT, normally the STAT is not phosphorylated. When it is docked to the receptor, it gets phosphorylated by the JAK and then as soon as it gets phosphorylated it dimerizes and dimerization so this is the dimeric STAT. This is the STAT dimer.

That STAT dimer then translocates to the nucleus, and then it codes and binds to the corresponding DNA sequence it has to bind to and then it activates gene expression or it activates transcription of some specific target genes. And that is how the whole signaling process works. So I think we have been able to, I have told about the different forms of types of receptors, receptor binding and a very basic overview of the receptor subtypes and a very basic overview of the mode of functioning of the receptor.

So we will stop here for today for this lecture. And we will discuss about, we will keep discussing about the cytokines and the different actions of the cytokines in our upcoming lecture. Thank you.