Essentials in Immunology

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Lecture No. # 31

Cytokines – Part2

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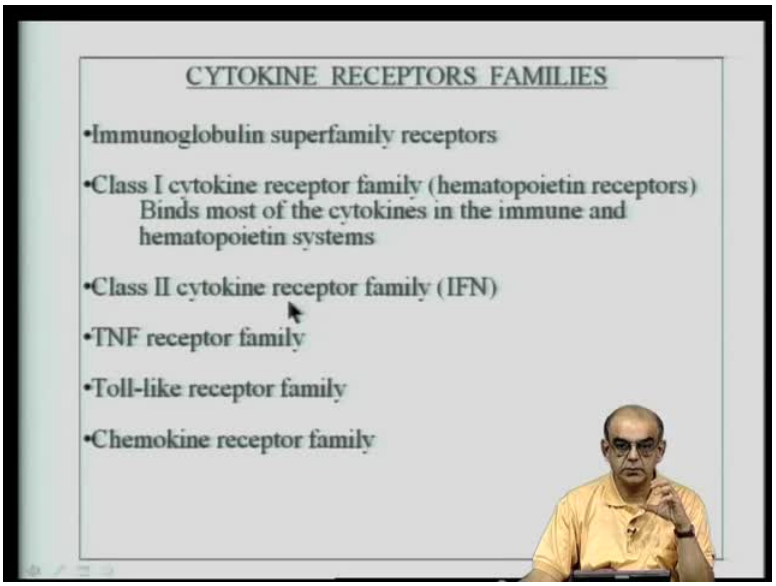

Hello, and welcome to this cytokine lecture number 2; let us first look into what we covered in the previous lecture, and in the previous lecture, one of the important topics that were covered, was to look at the families of the cytokine receptors; so, broadly these receptors were classified into the following families called as a immunoglobulin super family of receptors, class 1 cytokine family of receptors, basically otherwise known as hematopoietin receptors, which are the major class of receptors in the immune system, class 2 cytokine receptor family which binds to interferon, then you have the TNF receptor family, the toll like receptor family, and the chemokine receptor family.

So, what we will do in this lecture is to cover some aspects about the function of these receptors, and how they are regulated, then look into some of the aspects of how cellular subsets are regulated by these receptors or cytokines.

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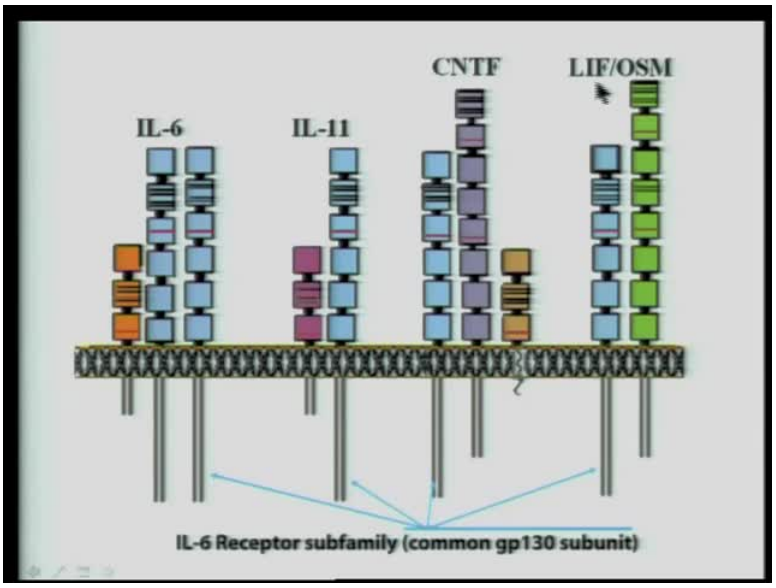

So, basically just to cover little bit of the previous lecture, we had the IL-6 receptor subfamily, which shared a common subunit called as the gp130 subunit, as shown again in this slide; this particular subunit is shared between four different kinds of receptors, which are specific for IL-6, IL-7, ciliary neurotrophic factor, and leukemia inhibitory factor or ocho cytrine m factor.

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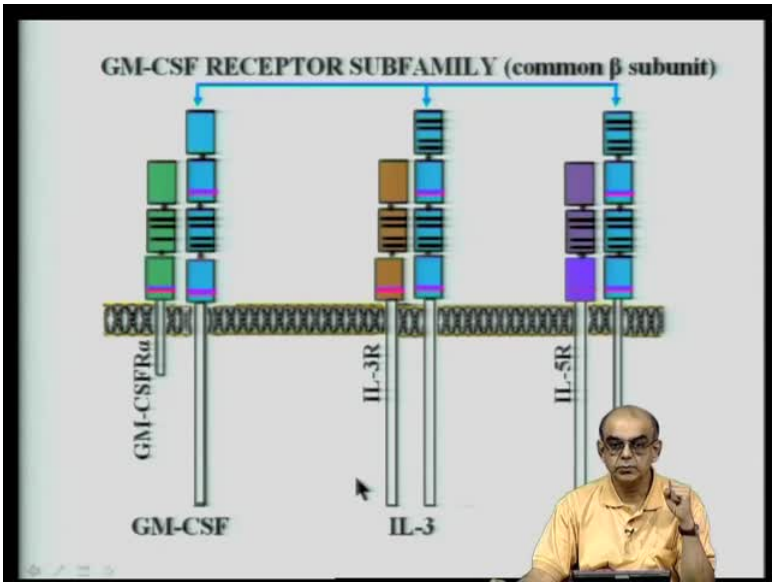

Going on looking at the GM-CSF receptor subfamily of the class 1, family of cytokine receptors, they have a common beta subunit; this common beta subunit is evident over

here, which is the longer subunit in this particular receptor, and the alpha subunit making up the component with GM-CSF alpha subunit, then you have the receptors for IL-3, and IL-5, having a common beta subunit by but another subunit, that binds specifically 2 cognates or specific cytokine in question.

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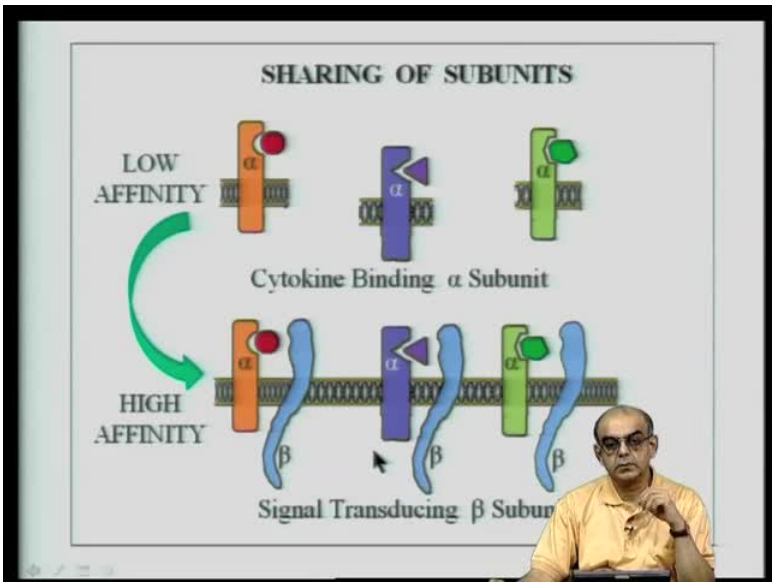

Now, why do these different receptors have common subunits, what is the meaning of this, and what is the function of sharing of these different kinds of subunits; so, happens that many of these or all of these cytokine receptors, especially the class 1 receptors, who have different subunits, many of these subunits bind to their cytokines specifically, for example, they have an alpha subunit or an alpha chain, that can bind to it is specific receptor by itself, but this binding as you can see over here in these three particular cases, signifying the same three receptors, that you see over here in the previous slide for the GM-CSF, IL-3 and IL-5.

So, which have been designated here in different symbols or different objects circle, triangle, and a kind of a trapezium; so, you have the alpha subunit, which is anchored into the membrane, and this binds to it is specific subunit, but this binding is one of a low affinity; in other words, it is not very tightly bound, the k d values are less dissociation constants are less, once this binding occurs, this is another subunit called as the beta subunit which is seen in the previous subunit that has a longer intracytoplasmic tail

which is meant for transducing the signaling events upon binding to which appropriates cytokine ligand.

So, that is shown over here, and this is the beta subunit having the longer intracytoplasmic chain; so, there are two subunits, now these two subunits bind to each other; the, once the cytokine binds to the alpha subunit, the beta binds to this alpha subunit making this affinity stronger; so, a low affinity binding is converted into high affinity binding, and has the ability now to transduced a membrane signaling events, that ensued you to the cytokine binding of this receptor.

So, that is basically, how these various cytokines function which have common subunits; so, you have a ligand binding subunit, which is specific for it is ligand, but it cannot transduce the signal but upon binding to the transducing subunit, signal transducing subunit, the affinity of the receptor becomes very strong, and this affinity then results in the cascade of events that follow on the downstream events, that follow beneath a membrane will come to that in a little while.

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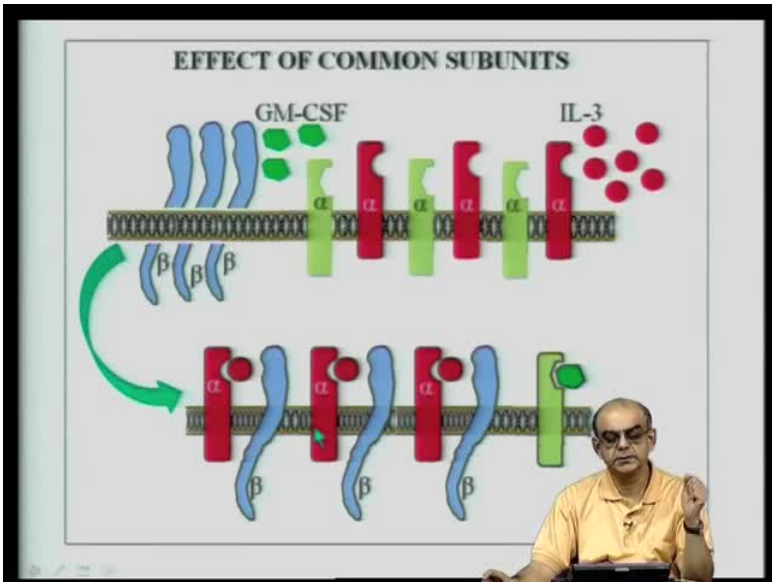

So, what is the function or the effect of these common subunits; the effect of sharing these common subunits, actually has to do with how cytokine function can be modulated, for example, here is shown in this slide, you have three alpha subunits, and this alpha subunit can bind to it is specific ligand like IL-3, and you have three other alpha subunits in green light green, that can bind to it is own specific ligand, the GM-CSF ligand, so on.

The membrane there are equal, let us say, there are equal pop, there is a hypothetical situation, in order to explain the effect or the functional consequence of such sharing of these common subunits.

The membrane you have equal concentrations, let us say of these alpha subunits to two different lymphokines on another location or on the on the cell on a different part of the membrane, you have these beta subunits, sitting there, let us say, there are three of them, now at any given situation, because the entire immune function is driven by the concentrations of lymphokines, that are available locally, let us say in this particular situation, there is more IL-3 molecules than the GM-CSF molecules; so, you have six of these compared to three of these.

So, what happen there, because of the higher concentrations of IL-3, they will bind all their subunits, because this binding is concentration dependent, however the lower concentrations of GM-CSF will ensure, will only lead to the binding of probably one of these or a fraction of the subunits, that are available on the surface the ligand binding subunits.

Now, when these three are bound by the cytokine, the available number of transducing signal, transducing subunit or the beta subunit, then binds to these alpha, but they cannot bind to the unbound alpha subunits of GM-CSF, they can only bind to those that are bound to the GM-CSF and make it a higher affinity interaction.

So, at a given situation, when IL-3 concentrations are higher, you will find that the full functional subunit, that is capable of transducing membrane signaling events are actually the ones that are bound to IL-3, because IL-3 concentrations are higher. So, in other words the higher the concentration of a cytokine, it sequesters the beta subunit, and therefore, makes the availability of the beta subunit lower, and lower for other cytokines subunits. So, the trans membrane signaling or the downstream signaling consequences are taken over or there is a majority of events that are transduced by this particular IL-3 cytokine; therefore, you will see that depending upon the concentration of the lymphokine, one can see how the effects of the final functional consequences of that lymphokine will predominate.

So, therefore, the cytokine actions of any at any given point, on any given type of cell will, then depend upon all the different kinds of cytokines that are present in that local environment, and therefore, one refers to it as a network kind of function.

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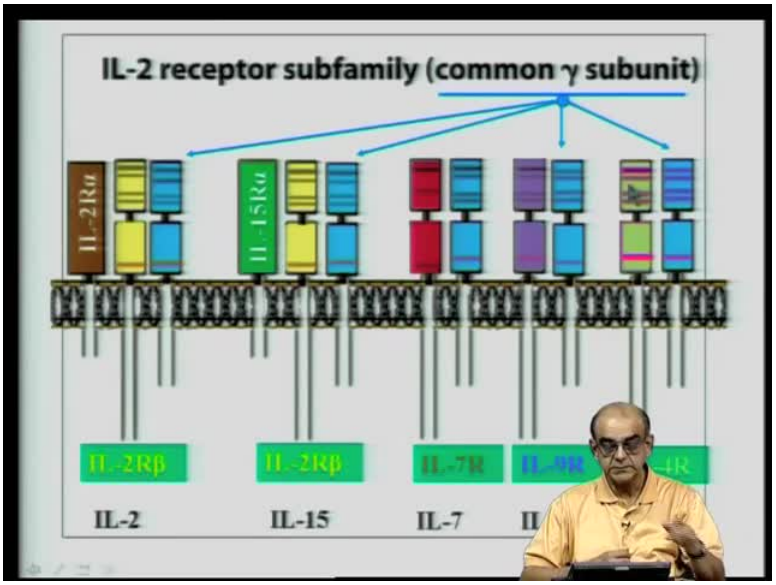

So, this is the IL-2 receptors subfamily which shares a common gamma subunit, which was shown to you in the last lecture; so, the IL-2, IL-15, IL-7, IL-9 and IL-4 are the members of this IL-2 receptor subfamily, and they share these blue subunits, which is the gamma subunit, and the IL-2 receptor alpha is shared, the IL-2 receptor beta is shared between IL-2 and IL-15, but the alpha subunit is the one which is binding to the ligand; in fact, the cytokine signaling via the IL-2 receptor has got a very unique property, and that is how the IL-2 receptor or the IL-2 action is modulated on a given cell.

Once the availability of IL-2 is taken for granted, it is the receptor that go up and down in order to modulate the amount of IL-2, because of inflammatory situations or proinflammatory situations in variety of diseases, a particular lymphokine may predominant, for example, when there is a lot of IL-2, that should, that would actually lead to hyper proliferation of many cell types, which the cell would not be able to tolerate, and therefore, in such situations as in other fields of biology, you have receptor modulation. So, if the receptor goes down, then even if there is a lot of ligand present in the local environment, they will not be able to function or give out their functional

consequences via trans membrane signaling events, and there essentially what happens with IL-2.

Now, when you look at IL-2 signaling, you look at the T cell growth factor acting on T cell, you have a T cell, that is normally present which is naïve, and therefore, as I told you in the last class, it is not receptive to the presence of IL-2 in the in the medium, but once the IL-2 receptor comes is there is available on the cell surface, then that receptor is going to bind to it is IL-2 and bring about proliferation.

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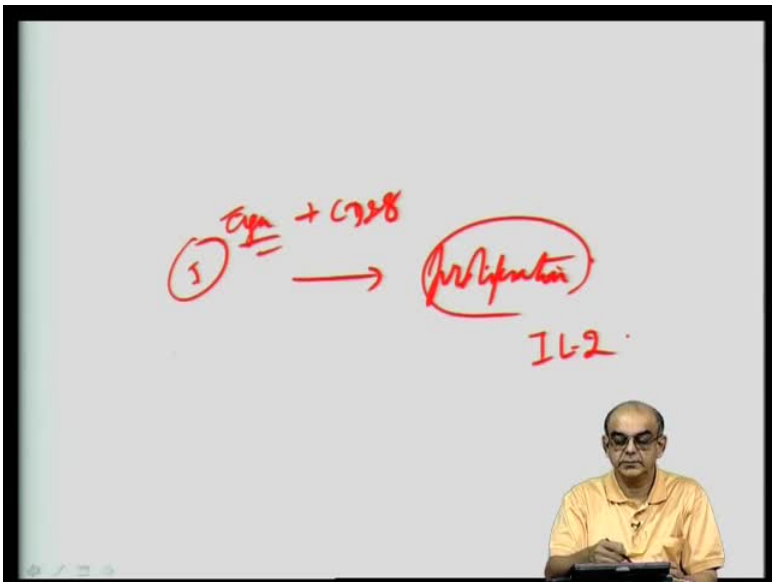

So, once a naive T cell is activated, so you have a T cell, and if it is activated by antigen which is the first signal, so the antigen plus the co stimulatory molecule via the CD20 8 signaling leads to proliferation, and secretion of IL-2, so, that is the basic the preliminary event, which leads to T cell proliferation or the end result of T cell activation.

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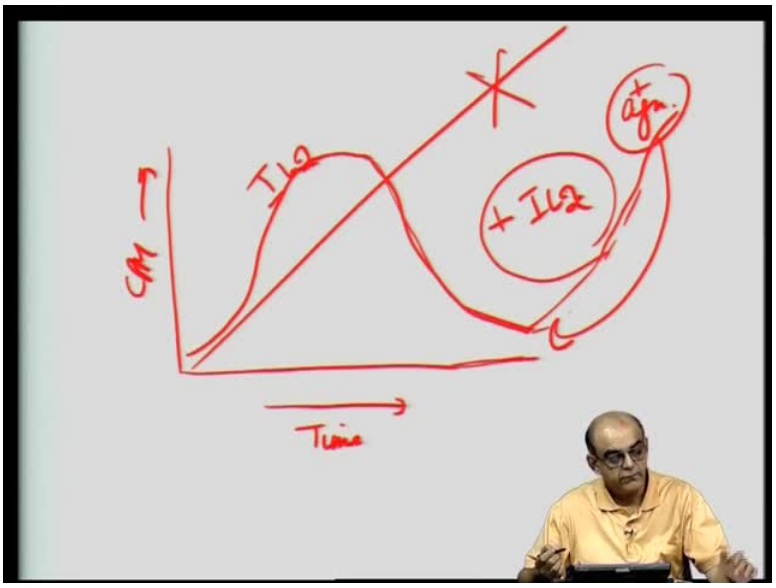

So, if you were to assume that T cells, once they have, they start to proliferate, if you go on providing exogenous IL-2, which has now which is now available by cloning recombinant DNA cloning methods, you can take this IL-2 or buy this IL-2, and keep on giving it to these T cell culture, and a very interesting phenomenon is seen in the, in such a situation, if you plot the number of CPM or of tritiated thymidine incorporated basically, because you are looking at the proliferation of cells, which will lead to the replication of DNA, and therefore, tritiated thymidine specifically gets incorporation by incorporated into this macro molecule, macro molecular DNA, and this is what is measured by filtration, so you have an acid perceptible sting or you catch them on glass fibre filters, which is then counted in the counter; so, you get increasing CPM incorporated into the DNA, which is a measure of the proliferation of that population of T cells.

So, if you look at time, after antigen activation provided you have a lot IL-2 added to the culture; you will see that the CPM incorporation will go up in the beginning stages, but after a while, it will Plato, and subsequently it comes down; therefore, if you take antigen activated cells, and you provide them with the continuous supply of IL-2, it does not result in continuous proliferation over a period of time which not happen, however it goes up in the beginning stages, and then, it comes down, what is the reason for this?

The reason for this is that, although IL-2 is there, initially more of IL-2 is bound brings about the signaling events, which leads to activation of several types of genes including IL-2 gene induction, which leads to the secretion of more of IL-2; therefore, more IL-2 accumulates in the medium, however after a while the IL-2, the very IL-2 receptor that binds to this IL-2 starts to modulate it is function. In other words, it cannot bind as much of IL-2 or it cannot lead same strength of functional consequences that was happening in the initial stage after antigen activation.

This can, this curve can be restored by again providing with antigen; so, if you provide again with antigen to the, and the antigen presenting cell, again you will see that the incorporation goes up, what is the reason and what is the mechanism such a phenomenon.

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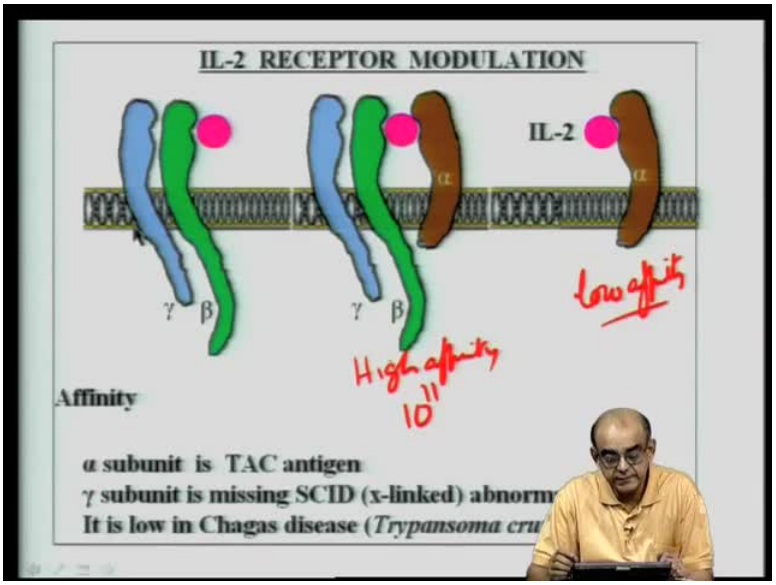

So, you will see that most cells, for example, in T cells, you will have there are three subunits to this IL-2 receptor, which is called as, you saw in the previous transparency it is called as alpha subunit; this alpha subunit is also known as the T cell activation antigen, TAC antigen, the reason being that this alpha subunit is what predominates on activated T cell population, and initially, when they immunize these T cells, in order to identify the structure on the cell surface, that would bound, that would actually bind to the IL-2, they found that all the monoclonal antibodies, that were derived from such an immunization with activated T cells actually bound to an antigen, which is now

characterize as the alpha subunit of the IL-2 receptor, and therefore, because they predominated on activated T cells, this was called as a T cell activation antigen or the TAC antigen for short.

So, when you, when you refer to these, they call it as the TAC antigen or the anti TAC antibody, but surprisingly actually, this alpha subunit is not the one, that is responsible for the consequences of T cell proliferation or the downstream events, it so happens, that the IL-2 transducing subunits are actually other subunits called as the beta and gamma subunits; so, this beta and gamma subunits are the ones that actually transduced a signal as evident in this drawing by the longer intracytoplasmic tail, so what happens is that, on a normal naive T cell, you do not have as much of the alpha subunit, but it has the beta subunit and the gamma subunit at very low concentrations.

So, what happens is that, this, this particular beta and gamma subunits are able to bind to the IL-2 ligand with what you call as an intermediate affinity; so, this particular subunit combination is of intermediate affinity having a k d value of approximately 10 to the minus 9 molar; so, you will see that, when you look at this intermediate affinity, the IL-2 binds to it is receptor, and after this binding is over, the alpha subunit starts to gets induced on the cell surface, in other words, there is a trans membrane transducing event which then induces this particular gene on the cell surface, and more of alpha subunit comes out on the cell surface, which binds to this beta gamma subunit, making this making this particular complex, a high affinity receptor, which has something like 10 to the 11 molar k d.

So, you will see that from the low affinity, you have the convergent by the binding of this TAC antigen converted to the high affinity receptor on the cell surface. This alpha subunit has got a short intracytoplasmic segment, and therefore is the low affinity subunit. So, what happens is that, as the cell gets activated and starts to proliferate make more IL-2, as time goes by the concentration of this TAC antigen starts to increase on the cell surface, when it increases on the cell surface, it starts to bind more and more or so to say, scavenge the IL-2 that is there in the medium, but once it binds to more and more of IL-2, it is unable to transduce the signal, and therefore, the cell becomes non-responsive, although IL-2 is being bound by the subunit.

So, this gamma subunit is actually the one, that is present to a certain extent, constitutively in the beginning, but is missing in SCID by patients, severe combined immunodeficient patients, which are, which is x linked disorder, they have an abnormal thymus with the abnormal T cell numbers in the thymus, and they are prone to a lot of infections. This gamma subunit is also very low in Chagas disease, which is caused by Trypansoma cruzi, in fact, they have formed by infecting in vitro with trypansoma cruzi, they have found that this subunit gamma subunit starts to IL-2 receptor starts to decrease on the cell surface in such situations, and therefore, there is no IL-2 mediated signaling, and that leads to lack of T cell proliferation, and therefore, problems with T cell responses.

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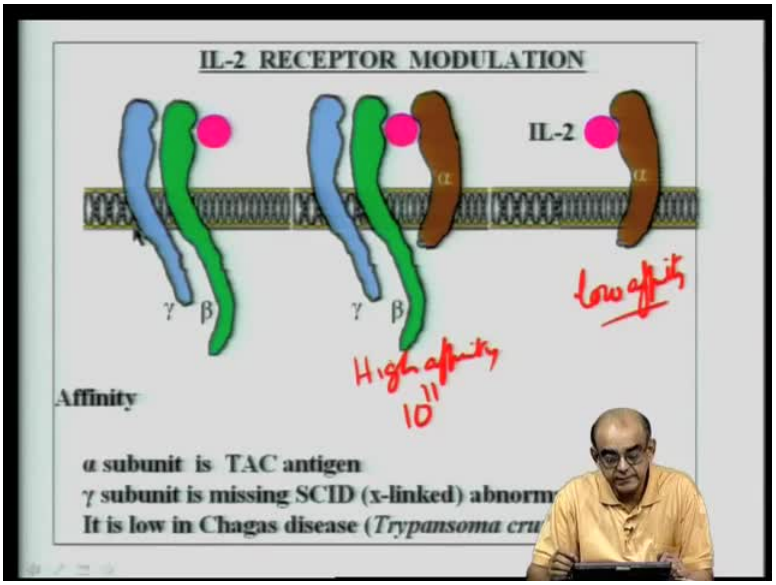

So, this is basically how a T cell response is actually regulated, and is responsible for the modulating effect on the proliferation, that I just explained in the previous slide. So, going on further, you see that apart from this sort of signaling events that are regulated by the availability of different kinds of subunits on the cell surface. There are other places, where there could be regulation, and that is the trans membrane signaling event itself.

There are several events that can be regulated and to look at the cytokine or the class 1 cytokine receptor family in general as well as interferon family in general, you find that these kind of receptors are actually going through what are called as Janus activated

kinases as well as stats or signal transducer and activator of transcription, so this is called as the JAK STAT pathway.

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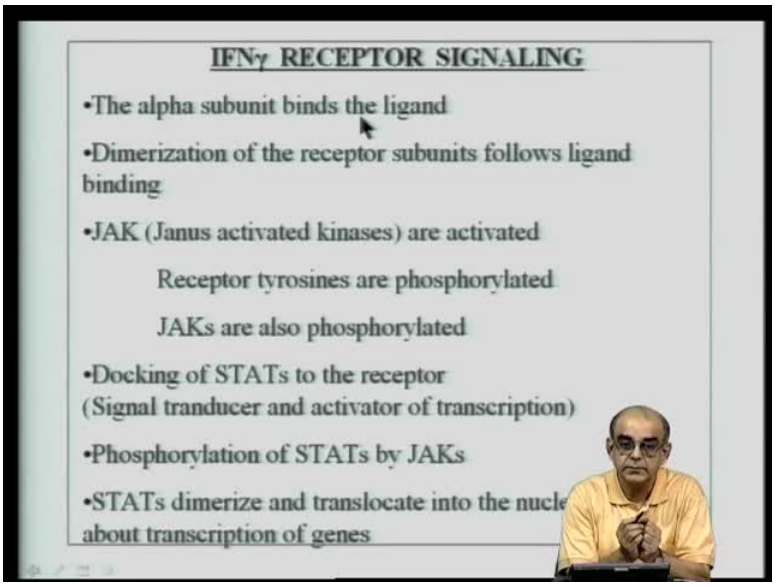

So, what happens generally in these receptors is that, alpha subunit first binds the ligand by the alpha subunit actually changes this alpha subunit, in such a way that it dimerize is with another subunit, so the alpha and beta subunits come together, and this coming together actually binds the ligand more tightly, which we saw in the in the previous slide, where we looked at how the alpha and beta subunit come together to form the high affinity binding receptor.

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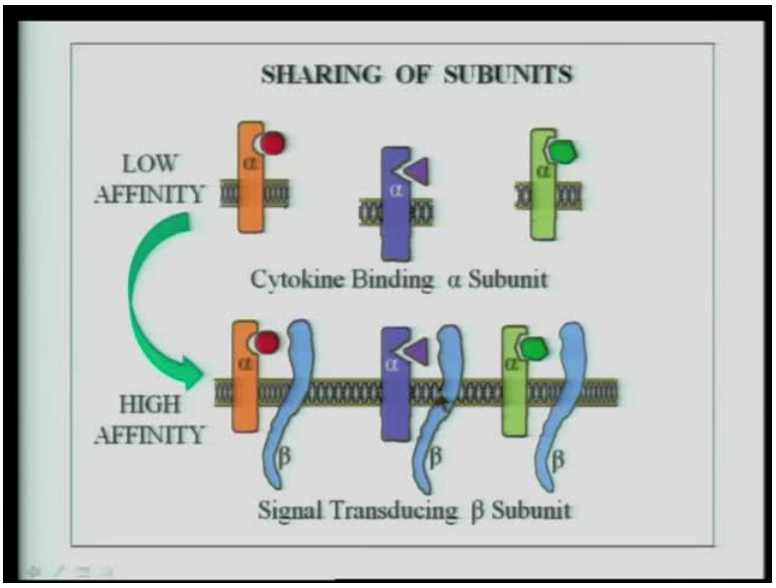

So, this dimerization of the receptor follows this ligand binding, and this dimerization, then leads to downstream consequences beneath the membrane, which are basically phosphorylation events, mediated by kinases; so, these kinases are nothing but the Janus activated kinases. This is a situation or a step, where tyrosines that are present in the receptor of the cytokine, in this case a gamma interferon receptor is phosphorylated, but a multiple residue, multiple tyrosine residues.

In the whole event the kinase itself auto phosphorylates, this phosphorylation, then leads to another step, which is characterized by the docking of this particular protein called as the stats will see that in the next, in the next figure.

This docking of the STAT to the receptor brings it in close proximity to the tyrosine kinase or the kinase activity of the, of the Janus activated kinase, which starts to phosphorylate the stats. Once the stats are phosphorylated, they are now available to dissociate, and bind to themselves causing a dimerization of the STAT protein itself STAT subunits; this dimerization, then allows these dimers to get into the translocate into the nucleus, and subsequently, it binds to the upstream promoter elements of various promoters, that are promoter elements, that are present in upstream of various genes, that are activated by the cytokines.

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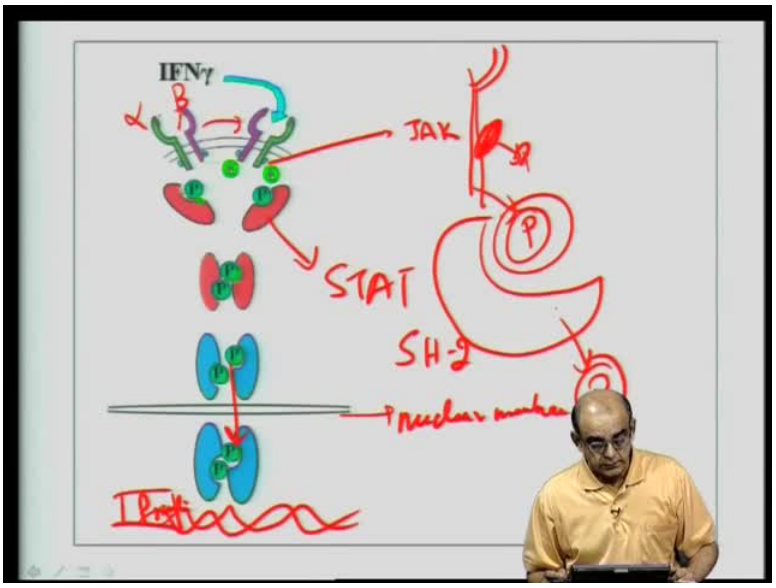

So, looking at this in a little graphically, you find that in the case of interferon, as you can see here, this is the alpha subunit, and this is the beta subunit; so, initially these are not bound by interferon, so one of these subunit starts to bind interferon, and this binding of interferon by the subunits starts to allow these dimerization to occur or in other words, alpha and beta come together with binding of interferon.

This binding of interferon as I told you in the previous slide, activates this what you call as Janus activated kinase, which you see as a button there; so, you have the receptor over here, this is the Janus activated kinase, so this binding event activates, this kinase the function of a kinase is phosphorylation. So, this once the, once the enzyme is activated, it is starts to phosphorylate, what does it phosphorylates, the receptor subunit, and it phosphorylates itself, so there is octo phosphorylation as well as phosphorylation of these receptor subunits.

Now, these are the stats which I told you in the last slide; these have got in them SH2 domains or these are called SH2 proteins, these have the ability to bind to these phosphorylated tyrosine residues, which is shown over here; therefore, these subunits come and bind to these subunits, in the whole process, they are brought in proximity, you see the membrane is such a large space of the cell, so all these phosphorylation events are actually mediating the movement of the STAT towards these tyrosine residue, so they are become proximal with the tyrosine kinase.

Once there is proximity the stats gets phosphorylated on the tyrosine residues, and this is what is seen over here, and these phosphorylated stats subunits, now start to come together, they come out from there, and they come together to dimerize; this dimerization event unlocks certain sequences, which allow them to go into the nucleus, this being the nuclear membrane.

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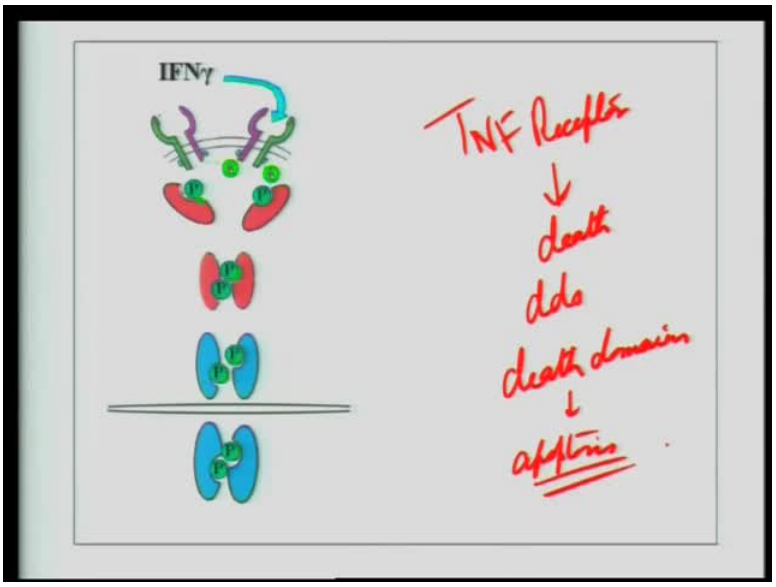

Once they go inside, they start to bind to various kinds of DNA promoter elements which are upstream, enabling the transcription of that gene, so this is how the general cascade of events occurs for the cytokine receptors, especially for the class 1 and class 2 family; ofcourse, when you look at, when you look at the TNF family of receptors, they have their own receptors, basically these are bringing about death, and therefore, downstream signaling events have subunits, which are composing of what are called as d d's or death domains, because these are proteins that mediate the final function of apoptosis; so, you have a the details of this cascade need not be covered in this class, because this is not a signaling class, but basically leading to apoptosis, that signaling pathway is different from, that what you see for interferon and IL-2.

Basically the end result of the TNF pathway is the activation of the transcriptional factor NF kappa b, which gets into the nucleus trans locates into the nucleus, again after being phosphorylated and the removal or disengaging from the I kappa b subunits, which are degraded by the lubricant pathway; so, this then trans locates to the nucleus, just like

what happens with the stats, and the activated NF kappa b binds to it is cognate elements on NF kappa b inducible genes.

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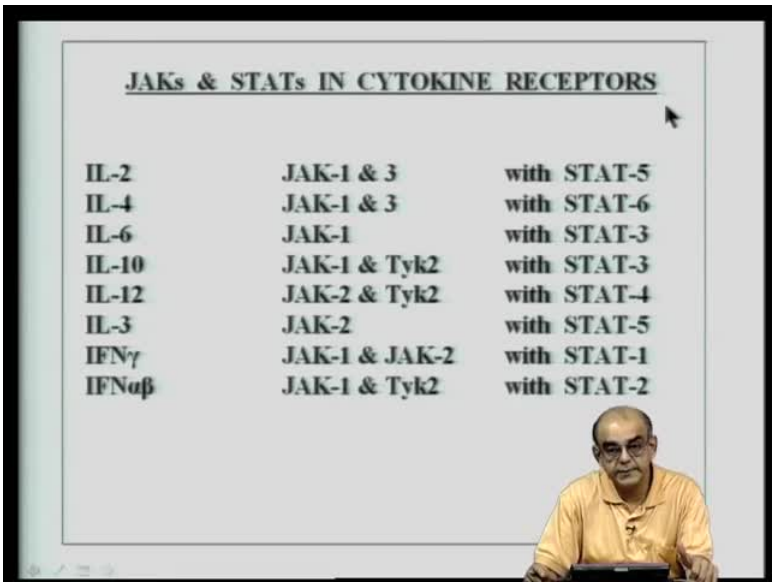

So, if you were to look at what sort, what are the different kinds of Janus activated kinases or stats that are involved for the different kinds of cytokines, you find the list given over here, for IL-2, it is JAK-1 and JAK-3, which are associated with the receptor subunits, and downstream events include STAT-5, IL-4, which basically IL-2 and IL-4 have many overlapping function, because they share their subunits, it has JAK-1 and JAK-3, but STAT-6 is the one that performs the downstream signaling and translocation into the nucleus.

IL-6 uses JAK-1 in some situation, it uses other, but basically it uses JAK-1 along with STAT-3; IL-10 on the other hand JAK-1, and another tyrosine kinase called as TYK-2, which is coordinated downstream by STAT-3. IL-12 another very important cytokine IL-10 being characterize as a kind of an immunosuppressive cytokine, but IL-12 being a proinflammatory cytokine, IL-10 being an anti-inflammatory cytokine, IL-12 activates JAK-2 and TYK-2 along with STAT-4 downstream signaling event; IL-3 utilizes JAK-2 with STAT-5, gamma interferon utilizes JAK-1 and JAK-2 along with STAT-1; IFN alpha beta utilizes JAK-1 TYK-2 along with STAT-2.

So, these are the different kinds of cytokine receptor associated proteins that you find with the, these are the, with the different cytokines, that are listed over here, and the pathway that were described in the last two last couple of slides.

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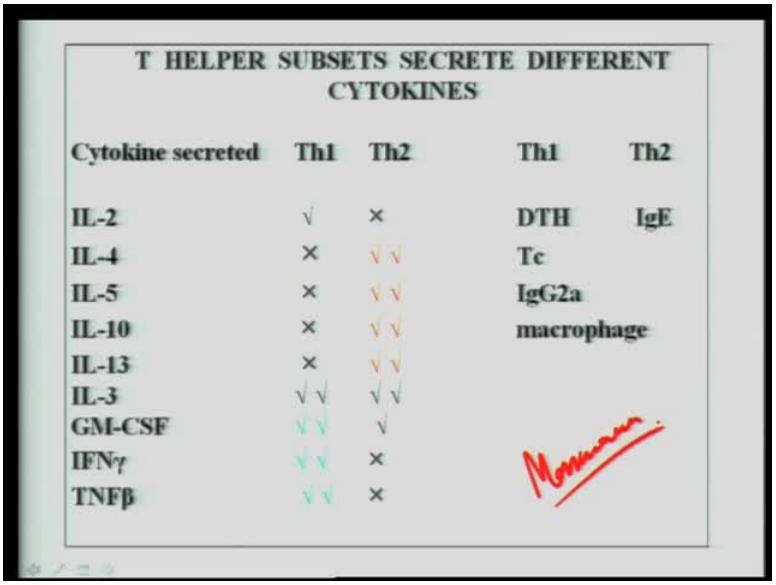

So, going on further a very important aspect of T cell immune responses and cytokines is one of classification of different kinds of T helper cells; so, once you, once you look at the CD4 positive cell, we call them as a T helper cell, but you see there is so much of heterogeneity even within a certain kind of cell; so, one can classify peoples or students belonging to particular class, let us say, they are studying in, in the fifth standard, but then when you go and look at the peoples themselves, you can even classify them further as very hardworking, and being in the first 10 percent of the class, as to others who do not study or are not able to pass the exam; so, there is heterogeneity even within the class of peoples.

Similarly, here with this T cell populations, you have heterogeneity, and they were actually initially characterized by Mossman, who classified or who observed that T cells actually secrete different kinds of cytokines, based upon the secretion pattern of these cytokines, one can classify or separate or purify T cells, which are of different types, and it so happens that, in this particular slide, you have two different kinds of T helper cells called as Th1 and Th2. These two different kinds of T helpers have a very important bearing and consequence of the on the immune system.

So, if you look at Th2, and you see the red marks or the red ticks over here, you will see that, so these are the cytokines that are secreted by Th2, IL-4, IL-5, IL-10, and IL 13; on the other hand, these cytokines are not produced or secreted by the other type of T helper cells; in other words, there is a dichotomy in the type of cytokine that are secreted by these 2 T helper subsets.

So, if you look at these cytokines IL-3, GM-CSF, gamma interferon, and TNF beta, you will find that these are secreted by the Th1 subset, so you also have TNF alpha on the TNF that also being secreted in such situations of inflammatory situations, so you see that, these are peculiar to the Th1 subset, let us these are peculiar to the Th2 subset.

Now, as we went into the functions of all of these lymphokines, you will find that all of these would help T cells, whereas gamma interferon would help in IgG blocking of switching to IgE, and therefore, it would block these, when there is a abounding of these cytokines or T helper, one predominance, it would not allow the B cells to switch to IgE responses.

So, these are some of the situations, where you see that macrophages are activated by Th1 cells, because of the presence of gamma interferon cytotoxic T cells are activated by Th1 response; remember, cytotoxic T cells are actually CD8 positive, but these TH1and Th2 are CD4 positive cells.

Now, these cytotoxic T cells or for that matter CD8, bearing cells also have cytokine secretion patterns, but in a major way, these are the ones that have been defined very clearly for the CD4 subsets; so, the DTH or delayed type hyper sensitivity reactions are actually facilitated by a Th1 response. In a TH2 response, you have more of IgE, because you have IL-4 and IL-5 which would help in the switching differentiation of B cells class, switching of B cells that would give IgE; so, therefore total antibody production is facilitated by Th2 cells.

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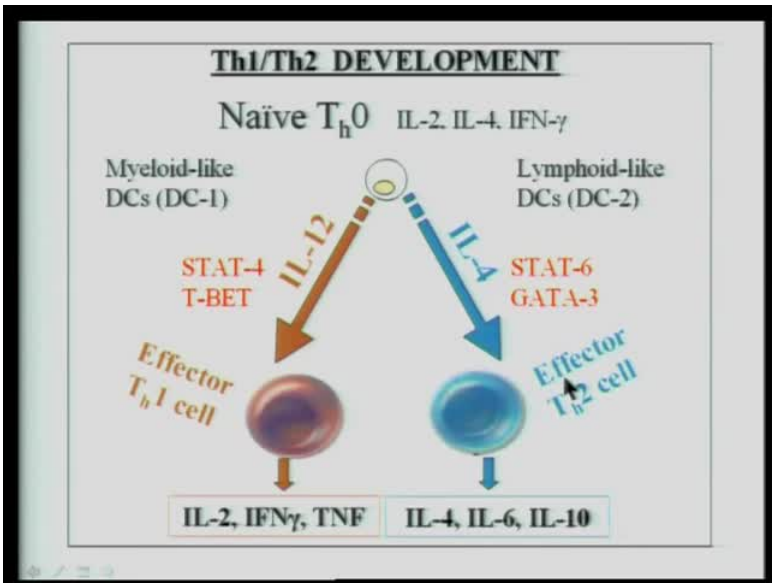

So, you see there are two different kinds of Th2, and this having a very important bearing on the outcome of immune responses after antigen activation; so, what is the nature of this particular regulation of these T helper subsets, so you will see that, you have what is called as a naive Th0 cell, which normally would secrete IL-2, IL-4 and gamma interferon, and these are actually differentiated to form effector T helper 1 or so-called effectors T helper 2 cells which actually secrete the final lymphokine, they have to secrete.

Now, there are two types of dendritic cells, which are basically antigen presenting cells which drive the development of the Th0 cells or the precursor T cells to finally the effectors T helper cells; so, you have these Th0 cells which develop to become the effected Th1 cells in the presence of IL-12; so, more the IL-12 in the medium, there is a differentiation or development of the effectors Th1 cell.

Now, for this IL-12 to act, you need the transcriptional factor called as T bet, and the participation of the STAT-4 molecule; this is facilitated by antigen presenting cells which are myeloid like, and they are called as DC-1 as oppose to DC-2 which are lymphoid like, but they influence or help in the differentiation of this Th0 to form effected Th2 T cell types

Now, this is facilitated in the presence of IL-4 so if the IL-4 predominates in the medium, then you will see the differentiation of this Th0 to become effected Th2 cells.

Now, the Th2 cell differs from the Th1 cell, as we saw in the last slide, in that they secrete IL-4, IL-6 and IL-10 as predominant cytokines, and IL, the Th1 secreting IL-2, gamma interferon and TNF as a predominant cytokines.

So, these lymphokines actually lead to predominantly inflamished, and these actually are called as anti-inflammatory, because they further antibody, total antibody responses or humeral antibody synthesis; so, how is this pathway regulate, this STAT-6 and GATA-3 are the one GATA-3 is transcriptional factor; so when GATA-3 is active, then you have Th2 formations, when T BET is active you have Th1 formation, and STAT-6 is predominantly active for this pathway of Th2 effective cell differentiation.

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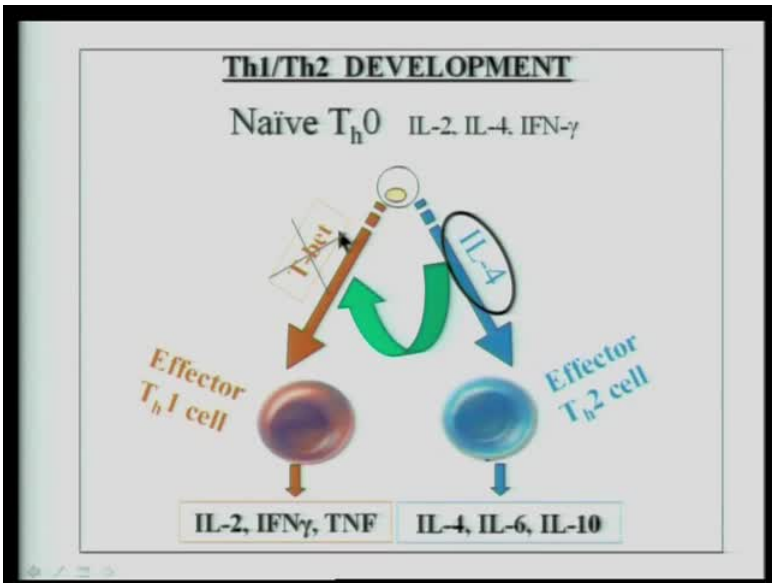

So, if you look at this, it so happens that, the lymphokines that are responsible for this kind of differentiation into Th1 and Th2 cells are actually cross inhibiting the two pathways; in other words, they are mutually trying to inhibit each other. So, this IL-4 which was responsible for the differentiation of effective Th2 cells, you find that the same IL-4 is able to inhibit the differentiation of Th1 cells; so, there is more of IL-4 in the medium, you have a of differentiation of Th0 into Th1 (( )).

Basically (( )), you find that there is more and more of IL-4, more and more of IL-6, and more and more of IL-10, so more the IL-4, you get more of Th2 activity, and an inhibition of the Th1 type; on the other hand, if you look at the Th1 effectors cytokine,

basically gamma interferon which it secretes, there is more or gamma interferon that is synthesize, it has a block on the development of Th2 cells.

On the other hand, more gamma interferon actually accelerates or an or facilitates the differentiation into Th1cells; so, more Th1 more gamma interferon, and therefore, the pathways driven basically towards a Th1 bias. Now, all these events are actually dictating the outcome of disease, and we will come to that in basically in the next few slides.

So, going on further you find that the same gamma interferon also has a blocking on the GATA-3 transcriptional factor; in fact, that block is what blocks this differentiation pathway. On the other hand, this is the, this enhances the IL-12 mediated T BET activity; so, if you go on further, again to show you that IL-4 does the job by inhibiting T BET transcriptional factor; so, these are the ways by which these two cell type are actually mutually inhibiting each other.

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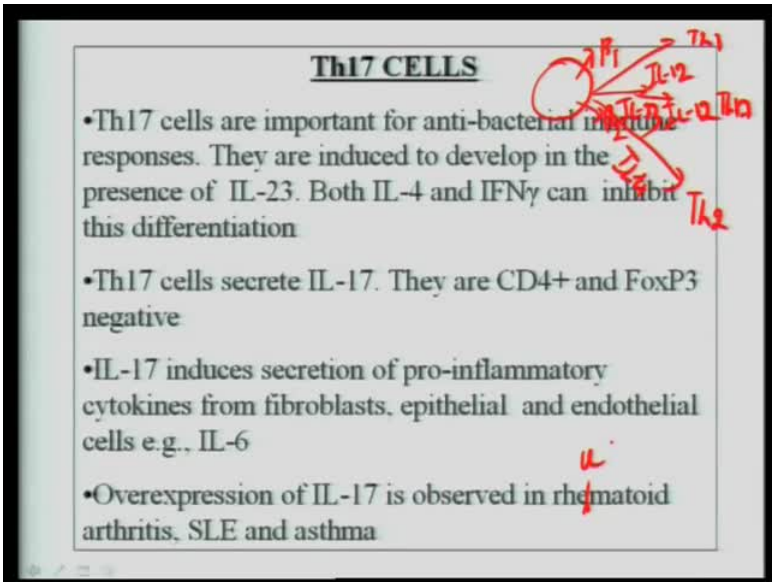

So, going on further in addition to Th1 as well as Th2 cells, there is a very important cell type called as the Th17 cells. Now, these Th17 cells are very important, and they have found it to predominate in actually anti-bacterial immune responses, they are induced to develop in the presence of a cytokine called as IL-23; now, both IL-4 as well as gamma interferon can inhibit this differentiation.

So, if you look at Th17 cell, after they differentiate they secrete amount of IL 17; now, these cells are CD4 positive, and FoxP3 negative, FoxP3 again is a transcriptional factor, that is present on a different cell type, we will come to that in a little in the next slide; so, you see IL 17 induces the secretion of proinflammatory cytokines from fibroblasts epithelial cells as well as endothelial cells, for example IL-6.

So, basically, if you were to look at these the differentiation of IL 17 or T cells look at the differentiation of these different kinds of T helper cells, Th1, Th2 as well as IL 17, you see that, basically a cell, the Th0 is having or the precursor will be having a receptor for IL-12 receptor beta 1, once this differentiates gets activated by antigen, it puts out beta 2 receptor, which is for the IL-12 cytokine; once this happens, this particular cell is able to bind IL-12; so, if it able to bind IL-12, there are different ways, by which it can go for differentiation, if IL-12 predominates over there, it differentiates into Th1; if in the milieu you have IL-4, this differentiation to Th2.

On the other hand, if IL-23 predominates in this situation, then they develop into Th17 cells; so, this is how you have the development of the precursors, which are not yet become effector cells which then differentiate into Th1, Th17, or Th2, based upon the predominant present presence of IL-12, IL-23 or IL-4, for these the presence of these factors are associated with the induction of the appropriate type of transcriptional factor whether this T BET or GATA-3.

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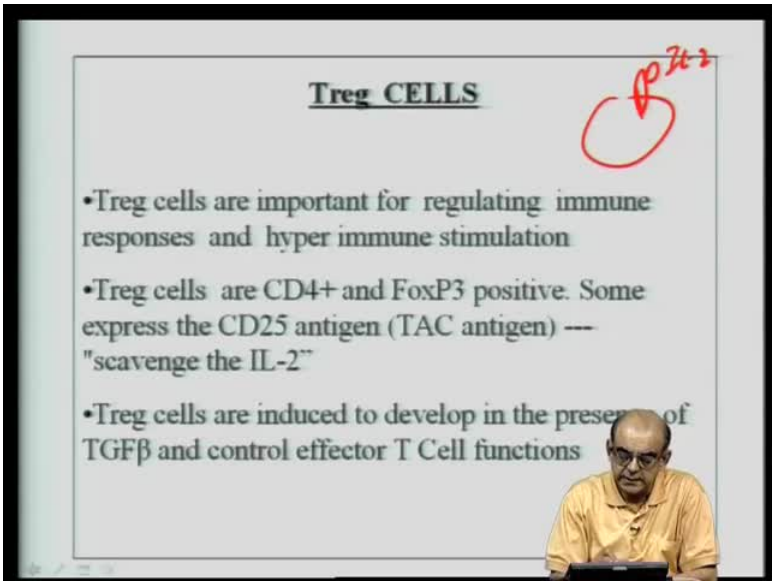

So, going on further the over expression of IL 17 is actually observed in rhematoid arthritis, systemic lupus, erythematous and asthma; so, there is a small, so this has to be rheumatoid arthritis, and then you go on further to look at a different kind of T helper T subset, which is now you had 3 subsets of T cells called as the Th1, Th2 the Th17, now you have the Treg cells, these are regulatory T cells, which are basically meant as a regulator of immune responses; so, if you have over reaction or a hyper immune response, specially in terms of inflammation you have this T reg cells, that play very important role in trying to suppress and one going immune response.

So, they are important for regulating immune responses and hyper immune stimulation states; so, these Treg cells again come in different subsets having different surface markers, but basically they are CD4 positive and FoxP3 positive at transcriptional factor, that is characteristic of these Treg cells, some express the CD25 antigen or the TAC antigen, what is the consequence of expression of this TAC antigen? The consequence of the presence of the TAC antigen of the surface of the cells is that, it binds the IL-2, and therefore, it scavenges the IL-2, and makes it unavailable for the neighboring T cells that are actually proliferating, and therefore, the inflammation be inflammatory situation, where proliferation is occurring is actually comes down, because more of IL-2 is sequestered onto the subunit and basically these cell will proliferate, because this TAC antigen cannot mediate the trans membrane signaling event, and therefore no gene induction.

So, the Treg cells are induced to develop in the presence of TGF beta, and conclude effector T cell functions like what I told you here in addition to other kinds of mechanisms of function. So, therefore, there are four major types of T subsets called as the Th1, the Th2, the Treg cells, and the Th17 cells, so going on further, there is a bias, how is this Th1 and Th2 play a role in disease mediation or disease states.

So, you find that in various disease as well as in clinical situation, one finds a predominant bias towards one cell type or the other, so you find certain diseases actually has a Th1 bias in certain other diseases has actually Th2 bias, and certain other stimuli actually promote the development of Treg cells.

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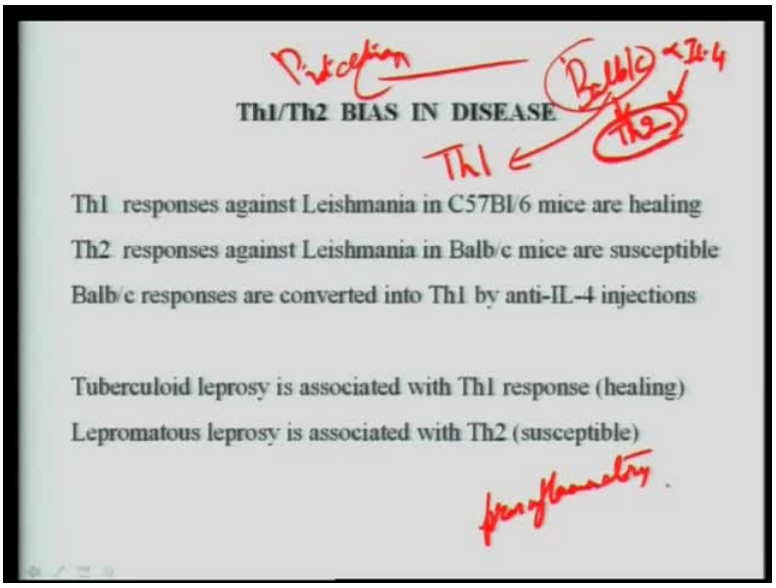

So, all this is actually mediated at the level, there are several steps that is thought to determine this sort of bias, depending upon how the antigen binds in terms of it is affinities, and how strongly it binds to the T cell receptor, and you find, that in these situations examples being in lympho in leishmania, specially in b 6 mice, you find that leishmania infection, actually does not kill the mouse, but they are protected or they do not come down with dead.

On the other hand, another strain of mouse called the balb c mice, when infected with leishmania, they are susceptible to this organism and lead to death; so, these, when you look at these mice you find that their lymphoid cells or T cells are actually having basically a bias towards Th2 type, but the heeling mice which are the b 6 mice have a bias towards Th1 type, as I told you earlier these two cell types are cross inhibitory, so IL-4 inhibits the Th2 type and gamma interferon actually inhibits gamma interferon, inhibits the Th2 type I am, and IL-4 inhibits the Th1type. So, you can actually give anti IL-4 to balb c mice, so if you inject to balb c mouse, now this balb c mice is now having a Th2 bias; so, there is a lot of IL-4 that is influencing or encouraging the formation or differentiation of Th2 cells.

So, if we block this high amount of IL-4 by injecting anti IL-4 antibodies, the IL-4 is neutralized, and therefore, there is a block in Th2 development, and it gets converted to Th1, because of the presence of IL-12, in that in that local medium; remember that all

these situations are of relative degree, so you find in the local environment, it is not as if that IL-12 is not present, but the concentration of IL-4 predominates over that of IL-12; so, it is a question of relative concentrations of these various kinds of cytokines, that are predominating in that particular inflammatory or non-inflammatory situation.

So, once this becomes Th1 type, you finds that the balb c mouse is now protected; so, you have protection ensuing, because you injected IL-4; on the other hand, the opposite situation, where if you inject anti interferon gamma in c 57 black 6, instead of heeling they become susceptible, why, because anti gamma interferon neutralizes the interferon that is, there, that is influencing the formation of Ph1 cells, and allows the IL-4 cells to develop.

So, the other situation that has been found in humans is in Tuberculoid leprosy is of two types in humans called as Tuberculoid leprosy, and lepromatous leprosy; usually, Tuberculoid leprosy leads to a kind of a heeling situation, where the number of organisms actually decrease, whereas in lepromatous leprosy, there is much more nerve damage, because of the organism, and it leads to more deleterious consequences, and it so happens, when one does not than blot or looks at the concentration of mRNA, that is there in these infected tissues from these patients, you find that the Tuberculoid leprosy patients predominate in a Th1response, which is leading to a healing type of response, and a lepromatous leprosy patient has a predominant bias for Th2 response, which leads to a kind of susceptibility.

So, you see that many of these bias is actually associated with the outcome of diseases protection or susceptibility, but it is not to say that, always the Th1 responses are always going to be beneficial; in fact, there are many situations, where Th1response is not beneficial, where too much of Th1 response actually leads to a lot of inflammation; so, it becomes proinflammatory, causing a lot of inflammatory responses, and leads to necrosis or deleterious consequences on the tissues, inflammation is what causes it; so, a more of proinflammatory cytokines leads to damage to the tissues and is not preferable.

So, an immune response is actually a balance between inflammatory responses and the other type of responses, so it is a balance between Th1 and Th2, a different disease situations would be would receive a better prognostic consequence or a better prognostic effect, if one looks at what type of Th bias is required for protection. So, if one can

convert a bias, which is deleterious to a bias which is going to be advantageous would be preferable in the clinical situations, and in fact, that is what is being tried in the clinical situation involving cytokine therapies.

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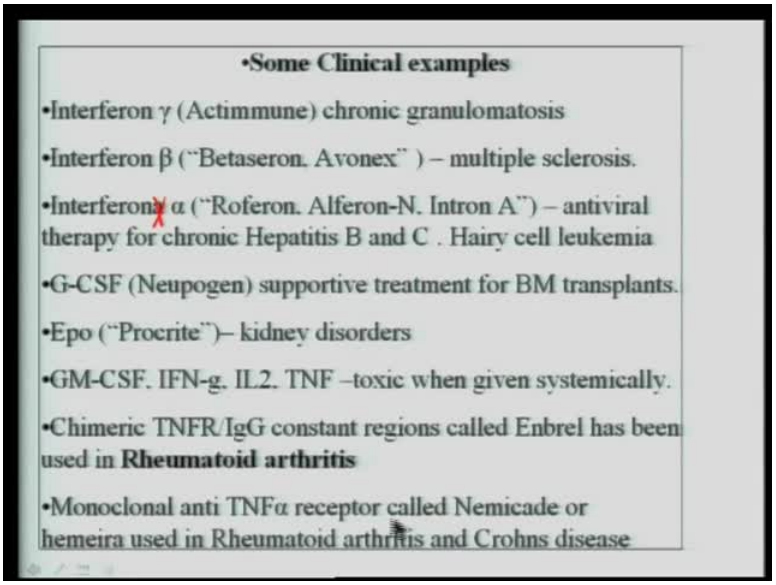

Some of these clinical examples are, for example, you have gamma interferon being tried the name being given to this sort of drugs is called as the act immune, which has been tried in chronic granulomatous disease, where you have problem with the antigen presenting cells, and the macrophages, and you have interferon beta, which is called as Betaseron or Avonex, which is being tried in multiple sclerosis interferon alpha, which has been, so which has been treid in antiviral therapy for chronic hepatitis B and C, as well as in hairy cell leukemia, and the products are called as Roferon Alferon and intron, then you have Neupogen, which is nothing but granulocyte colony stimulating factor as a supportive treatment for bone marrow transplantation, then you have GM-CSF, then you have chimeric TNF receptor, rTNF receptor, which is as a fusion with the IgG constant regions is called as Enbrel has been used in the case of rheumatoid arthritis.

Monoclonal anti TNF alpha receptor has been, has been used in the case of rheumatoid arthritis and crohn's disease was needless to say in many of these cytokines are toxic when given systemically, and therefore, one has to be careful in how it is administered, in what concentration and in what mode.

So, summarizing this class to go into the various kinds of clinical situations, it would be of more detail, and therefore not able to cover in a short space of time.

We have covered in these two lectures of cytokines, we have covered the nature of cytokine their properties, and described some of the salient properties of, some of these major cytokines, and then looked at how these cytokines are classified, and what sort of receptors they bind, and what are the classes of these receptors, and looked into how these receptors act, and what is the meaning of how they share their subunits, and then went on to look at how these different cytokines can actually classified different kinds of T helper cells, and how a T helper cell bias is actually observed in different kinds of disease situations, and how they can be altered, by using the, by using the cytokines themselves to alter the differentiation of these various T subsets. Thank you very much.