Essentials in Immunology

Prof. R. Manjunath

Department of Biochemistry

Indian Institute of Science, Bangalore

Module No. # 16

Lecture No. # 30

Cytokines

Hello, and welcome to this lecture on cytokines– lecture number one of the cytokines. Now, we have seen in previous classes that many of the reactions of the immune system are dependent upon recognition of antigen. Now, just like hormones in the field of endocrinology are secreted in order to adapt to newer situations or changed situations, hormones are released, which enable the body to react and establish a new equilibrium with the new set of conditions. Basically, hormones alter the way different kinds of cells metabolize and react to the new situations by coming up with newer and adaptive responses.

Similarly, the immune system faces newer and altered situations when it is faced with incoming infections, and apart from the specificity of the immune system, that is mediated by the molecules such as the T cell receptor the B cell receptor molecules of the adaptive system, adaptive in adaptive in innate immune system, the cytokines are the ones that are similar to hormones in endocrinology, that help the cells of the immune system to adapt to these changed situations.

Basically, then, what are cytokines, and how would they function? Early on, after it was realized that reactions, such as the mixed lymphocyte reactions, in which two populations of lymphocytes recognize each other as non-self, which result in the proliferation of cells, immunologists knew that this was the end result of the activation of lymphocytes, but they did not know what mediated this activation of proliferation, that usually resulted in such allo responses.

Now, we know of course, the first signal comes through the T cell receptor. Then, of course, you have the co-stimulatory signal; then you have… then you have to have subsequent signals, which have to allow the T cells to proliferate, which means, changes in the phases of the cell cycle. Normally, T cells or naïve T cells or unexposed T cells that are unexposed antigen and not active– they are normally in the T 0 or the G 0 phase, which means that they are restive– in the restive state.

In such situations, lymphocytes, or cytokines, or growth factors, cannot act on these lymphocytes, because they would not have, or they are not properly receptive to the availability of these lymphokines. It is only after the exposure to the antigen and the signals that ensure through the T cell receptor and the co stimulatory signals, do the T lymphocytes put out on their surface, receptors that could engage the cytokines that are available in the media. So, these cytokines were first discovered in, as I was mentioning, in allo reactive MLR situations, where they found that the supernatant of allo mixed lymphocyte reactions had, in their supernatant, growth factors that enabled these lymphocytes to proliferate in response to the availability of those lymphocytes.

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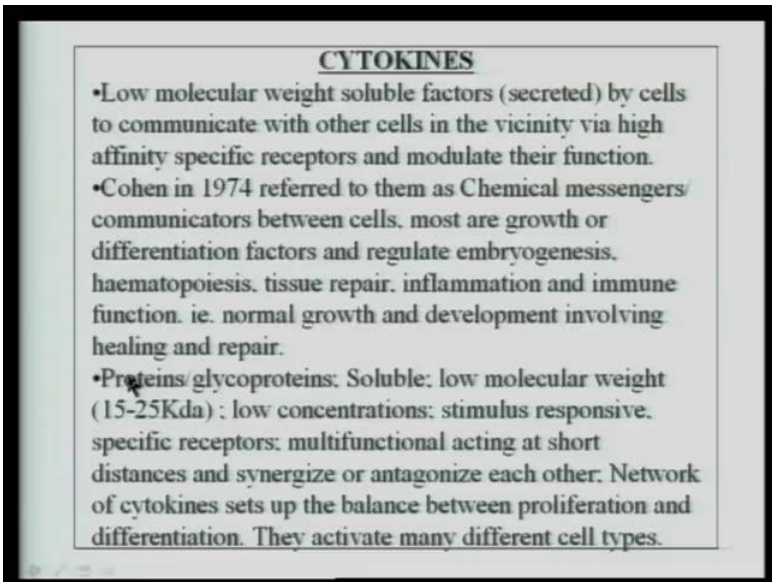

As shown in this slide, you find that cytokines may be looked upon, or now known to be of low molecular weight soluble factors, which are secreted by cells in order to communicate with other cells in the vicinity– mostly, in the vicinity. Some cytokines can function similar to hormones, and at, at on cells in a, in a distinct, in a distinct location.

Now, this ability to communicate with other cells in the in the vicinity, normally, or or always, occurs by binding to high affinity specific receptors. In other words, most of the cytokines always bind to high affinity specific receptors, and the end result is the modulation of the function of these T cells.

So, looking at the history of allo responses, it was first discovered that the supernatant obtained from an allo mixed lymphocyte reaction or responding lymphocytes– in other words, when you mix two strains of lymphocytes together and allow them to be cultured for a period of three to five days, these lymphocytes recognize each other as non-self, and they get activated. This process of activation releases certain growth factors into the supernatant.

So, what was done was to remove the cells by spinning down the cells, and take the self supernatant and add it to normal cells that have been exposed to the antigen, and it was found that this supernatant allow the cells to undergo proliferation in a much better way. So, they had these– the supernatant had the ability to support the proliferation of T cells that had been activated.

So, this was, initially, called as the T cell growth factor, which is, of course, today's IL-2. So, looking at such factors, one needed to culture large amounts of large numbers of lymphocytes, and to purify large numbers of allosupernatant obtained from alloreactive MLR reactions.

Of course, later on, the ability to clone DNA and recombinant DNA technology enabled the cloning of these genes, and now, of course, many of these cytokines are available as recombinant DNA or recombinant DNA that can be put into various kind of vector systems– expression vectors– to make them express and to purify these different lymphokines in large amounts.

At that time, basically, the limitation in the number of cells that one could utilize in antigen-specific mediated immune responses made it impossible for one to purify the large number of lymphokines that are known today, which, then, of course, became possible because of recombinant DNA cloning. It was Cohen, in 1974, who referred to them as chemical messengers or communicators between cells. Most were growth or differentiation factors, and they would regulate embryogenesis, haematopoiesis, tissue repair, inflammation, and immune function– that is, processes that are involved with

normal growth and development involving healing and repair. So, all this was supposed to encompass all the different reactions not only of the immune system, but of various other kinds of processes in the body.

They were subsequently discovered– the cytokines was subsequently discovered to be proteins– more so, glycoproteins. They were soluble in nature, low in molecular weight– about 15 to 25 kilo Daltons– and they would be available only in very, very low concentrations, and they were stimulus responsive stimulus responsive, meaning that when you stimulate a T cell or a leukocyte, this would end up secreting kinds of lymphokines.

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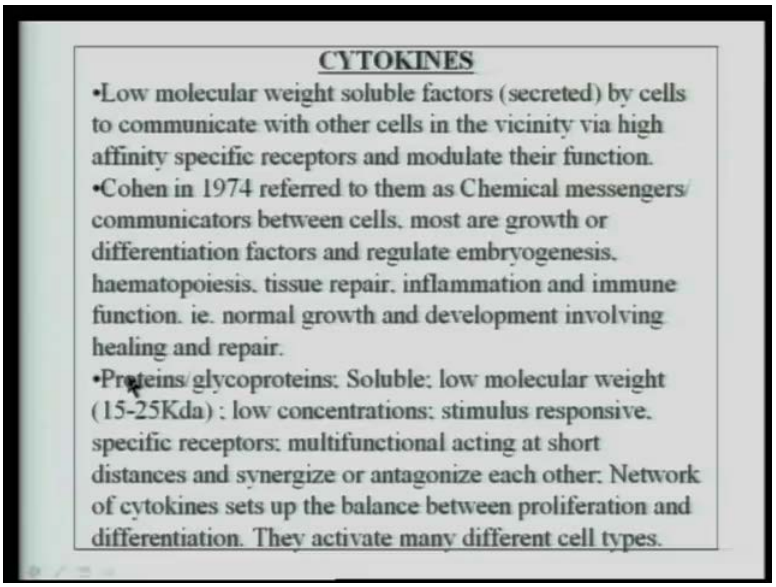

In other words, an inactive or unactivated, or cells that were not exposed to different kinds of stimuli, would not secrete these lymphokines. So, therefore, these lymphokines the secretion of these lymphokines had to be inducible. In other words, these were not constitutive. Except for a few cytokines, most of the cytokines are inducible in nature– inducible meaning that they were stimulus responsive.

The availability of specific receptors made the action of these cytokines specific in nature, and many of these cytokines were found to be multifunctional, and they were acting at short distances. In other words, this was something of a, of a feature that would regulate the action of these lymphokines, whose action would be very, very potent, otherwise, if they were, could, could stick around for long periods of time.

One of the features that enables specificity of action of these lymphocytes, of lymphokines, I am sorry, is they are short half life. For example, a lymphokine known as tumor necrosis factor has a very short half life of about of about 15, 15 minutes, in vivo.

Secondly, these lymphokines are usually secreted in the vicinity of their interacting lymphocytes. So, all of us now know that T cells recognize antigen in association with the MHC complex that is presented or available on the surface of antigen presenting cells. Hence, and cell to cell contact is a must in many of these reactions. The cell to cell contact enables the formation of minute microscopic pores or so-called antigen bridges, through which the lymphokines can get across to the… the cell that had to be stimulated, and therefore, the… the proximity of location made the, made the possibility of a strong active cytokine in that location. They did not have to diffuse for long distances, and these are essentially unstable molecules subject to degradation over long, long distances.

These cytokines have the ability to not, not only to act by themselves, but they also can synergize and they can antagonize each other's function. In other words, this synergy or antagonism is what sets up the balance of the immune system that we, finally, see as being inflammatory responses, or over, over reactive TH T H responses, that end up in auto immunity, or to little response that ends up in immune, immune separation.

Most of the cytokines act together. In other words, in an immune response, it is just not one cytokine that plays a role during orchestration of immune responses. Multitude of different cell types play a role, and therefore, because there are a number of cell types involved, each one of the cell types may respond by secreting, or respond to by binding to the cytokines. By binding them, there has to be a multitude or a network of cytokines that sets up this balance. This balance is, actually, between proliferation of cells or allowing T cells– naïve T cells– that have been activated, to proliferate. Therefore, you have the specificity of action towards a particular antigen. In other words, antigen mediated proliferation of T cell responses to orchestrate T cell help, and B cell proliferation to a particular, particular epitope, helps it to make antibodies to that particular epitope.

In order to make antibodies, these B cells have to differentiate into plasma cells, and more the number of plasma cells, the more, more the number of antibody molecules in the circulation. Therefore, these lymphocytes are the ones that are, actually, managing all

these kinds of different kind proliferative responses, as well as differentiate responses. For example, the differentiation of an active B cell to become a plasma cell that spews out or secretes large quantities of the antibodies that it should. So, they activate, as I told you earlier, many, many different kinds of cell types.

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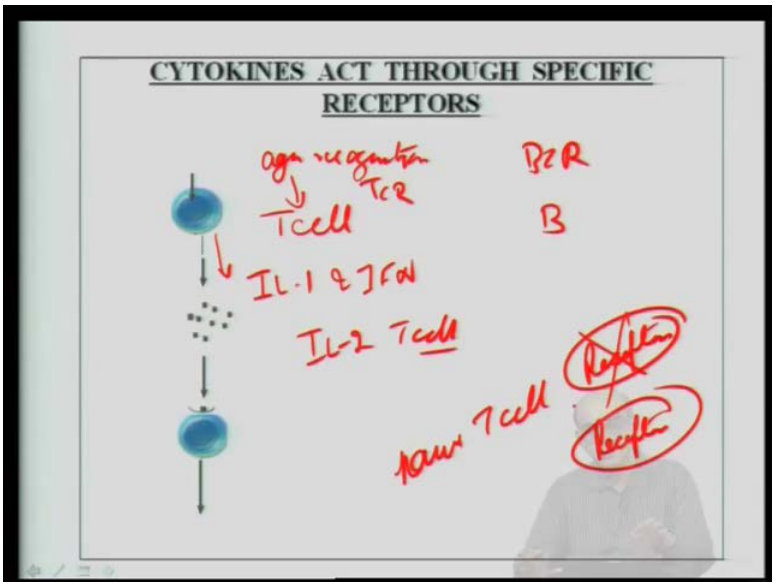

So, going on, as I told you, basically, then the specificity of lymphocytic response or an immune response is endowed by the T cells or the cells that are going to make the cytokines Basically, then if it is a T cell, you have the incoming stimulus, which is the antigen recognition, which in the case of a B cell, it is through the BCR or the B cell immunoglobulin receptor, and in the case of the T cell, it is because of the T cell receptor mediated antigen recognition. This T cell then goes on to further phases of the cell cycle, which then allows it to respond to various kinds of stimuli, or for that matter, cytokines, and then spews out different kinds of cytokines by itself.

So, if it is an antigen presenting cell–a macrophage– it would respond to TLR activation, or for that matter, activation through various kinds of cytokine. They, themselves, will secrete various kinds of cytokines such as IL-1, or for that matter, interferon. These cytokines that are then coming out along the vicinity of the activated cells, they denoted here by these black spots, will go and then bind to the target cell.

The target cells also can be of different cell types. So, for example, IL-2 acts mostly on T cells, although it can act on other, other cell types, as well. So, this cytokine binds to a

receptor that is expressed on these T cells which are expressing a T cell specific receptor, which can bind to the IL-2 with high, high affinity.

Now, this modulated… this– this response– can of course, be modulated, by modulating the number of molecules of this receptor, as well as the affinity of this, of, of this receptor. That we will come to a little later on.

So, how does one… how do the cytokines avoid nonspecific activation of T cells in an immune system? Basically, a naïve T cell or a naïve cell type that needs to respond does not have receptors if it is not activated. So, there are no receptors on the cell surface in an inactive or inactive T cell or a naïve T cell. So, once the T cell is activated, the receptors are expressed on the cell surface and start to bind the relevant cytokines.

So, you can see that the non-specificity of different kinds of lymphokines is avoided, because inactive or naïve T cells, or naïve cell types, do not express a particular receptor on the cell surface, and they are able to respond to these lymphokines only after activation and expression of the receptor on the cell surface.

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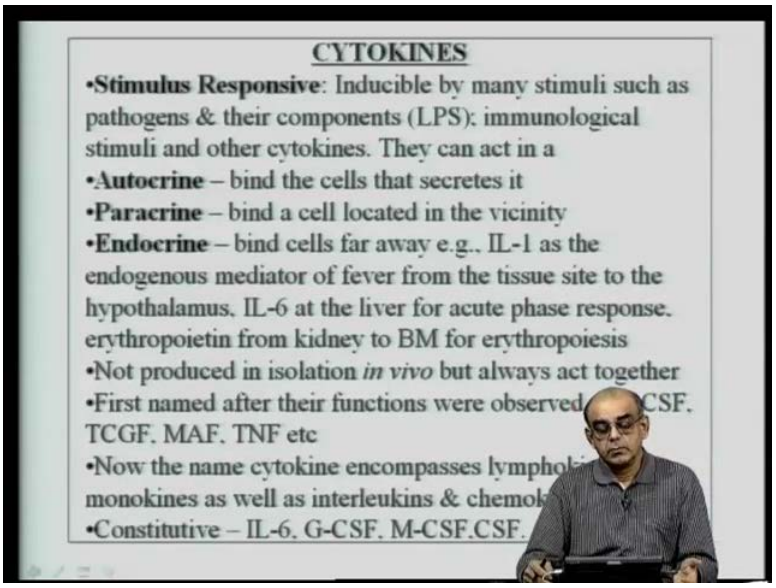

Going on, further, the nature– what are the properties of cytokines– is given in the slide. As I told you earlier, they are stimulus responsive– stimulus responsive, meaning that they are inducible by many stimuli, including the antigen via the antigen receptors. Other stimuli include those such as, as I told you, pathogens through the TLRs, or their components such as lipopolysaccharide, again, through the TLRs, immunological stimuli, and other kinds of cytokines.

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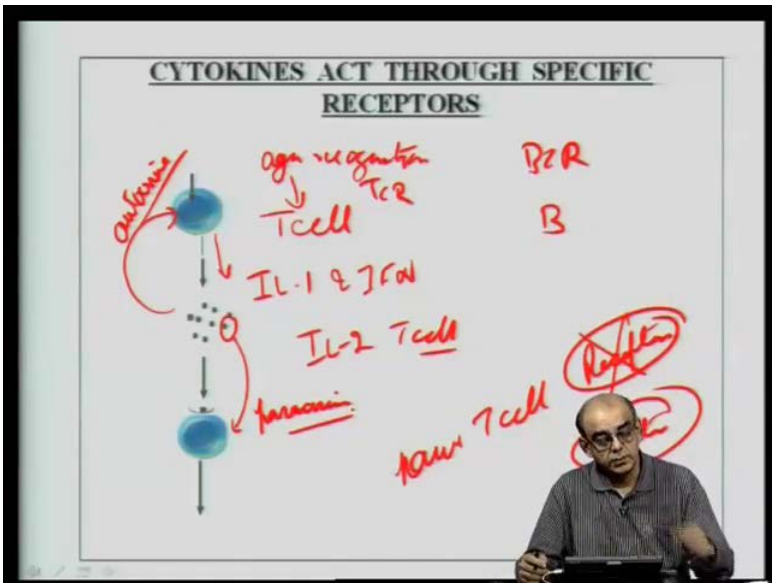

Now, these cytokines act in three different kinds of ways. They can be autocrine, meaning that in a autocrine situation, the cell that is secreting this lymphokine has the lymphokine acting on itself. So, these lymphokines act on the same cell that secretes it. So, this is called as autocrine. So, a cell– a T cell– that secretes IL-2, can bind the IL-2 that it is secreted immediately, and respond to it by activating various kinds of genes.

As opposed to autocrine, paracrine is a situation where the cell that secretes the cytokine is not activated by the cytokine itself, but the cytokine goes and binds to another cell in the vicinity, as in this case, which is called as paracrine. So, depending upon the stability of the lymphokine, you have cells in the vicinity, or at various distances from the secreting cell, act as target cells.

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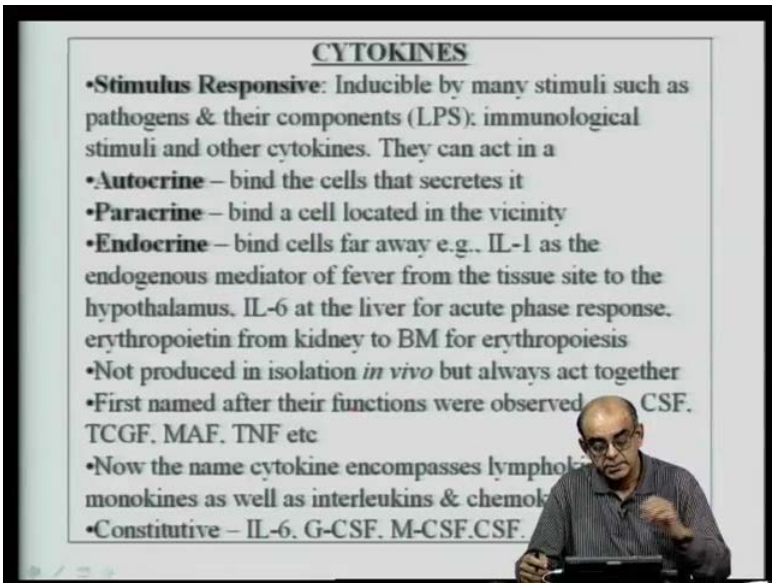

In addition to paracrine, of course, you have certain lymphokines that may act in an endocrine manner, which means that they bind like hormones. They bind; they are secreted in one location or made in one location, and then, they go and bind to cells and bring about their action at distances away from the cell that it, actually, that was that , actually, secretes the hormone. So, here, the cytokines– they act that act in a, in a, in a endocrine manner, they bind to cells that are far away. For example, IL-1 is the endogenous mediator of fever. So, when you have fever, IL-1 is active. How does it do that? It is… it comes from the tissue site where it is secreted, goes to the hypothalamus to regulate the body temperature.

IL-6 acts at the liver, although it is secreted by different kinds of cells, which are at different locations apart from the liver. They act on the liver to stimulate the acute phase response, in response to different kinds of stresses. It also acts on erythropoietin, secretion of erythropoietin from the kidney. Now, erythropoiesis from the bone marrow is also stimulated by this sort of endocrine action of…, of these kinds of cytokines.

Cytokines are not produced in isolation, in vivo. In vitro, of course, you can have cells producing certain kinds of cytokines, but in vivo, they always act as a network. If they are not produced in isolation and they are always acting together, it is the sum total of a variety of cytokines that produces a function.

First, during the history, they were named after their functions. So, they knew what the functions of these various cytokines and they were named after that, for example, colony stimulating factor, which stimulates the… stimulates the total number of colonies for the purpose of haematopoiesis; T cell growth factor, because this factor was purified from a mixture of T cells that were undergoing alloreactive responses; macrophage activating factor, because you can have cytokines activating macrophages activating the process of phagocytosis or opsonization.

Now, the name cytokine encompasses the other terms such as lymphokines, which were given to those kinds of proteins or polypeptides that were secreted from lymphocytes, or monokines, for those kinds of polypeptides that were secreted from activated macrophages and monocytes. So, all these different kinds of terms came under the name on now, refer to as, generally, as cytokines, including those called as chemokines.

As I told you earlier, most of them are inducible in nature, but some cytokines such as IL-6, granulocyte colony stimulating factor, and the other kinds of colony stimulating factors, are mostly constitutive in nature, because they need to support haematopoiesis.

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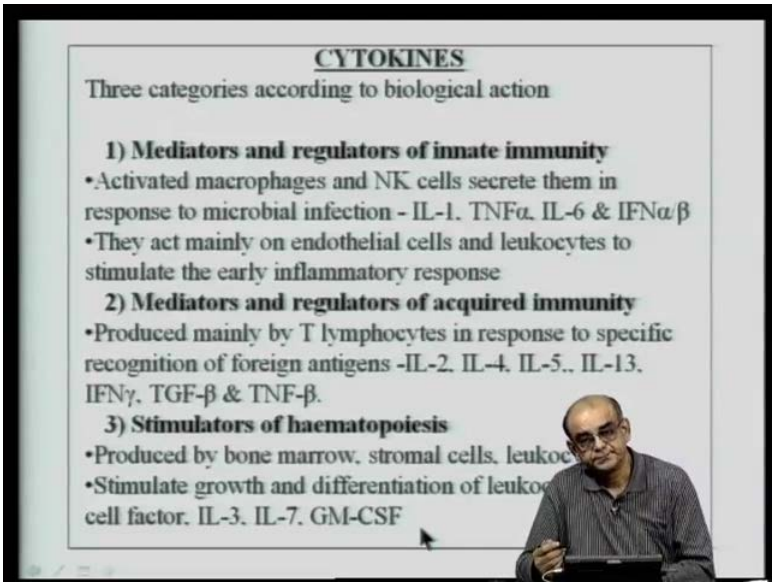

Going on, further, as they were discovered– the functions were discovered– cytokines can also be classified based upon their biological action, for example, mediators and regulators of innate immunity, there are cytokines that take part in innate immunity, those that take part in adaptive immunity, and those that they may have… there might be an overlapping action between innate and acquired immunity.

So, in innate immunity, activated macrophages and natural killer cells– NK standing for natural killer cells– secrete these cytokines in response to microbial infection, for example, IL-1, TNF alpha– TNF standing for tumor necrosis factor. There are two types of TNF– alpha and beta– TNF alpha, IL-6, and interferon alpha and beta. There are many different types of interferons– alpha and beta interferons are, are called as type one interferons, as opposed to immune interferon or gamma interferon, which is the interferon that is that results due to activation of, of lymphocytes, which then has different kinds of actions on, on, on the immune cell.

These these cytokines they act mainly on endothelial cells, apart from other kinds of cells, endothelial cells, and leukocytes, to stimulate the early inflammatory responses. So, those cytokines that are liberated or secreted in response to TLR TLR stimulation, such as TNF and other other cytokines, they have action on endothelial cells causing alterations and adhesion. Therefore, different kinds of lymphokines are trafficking into the different locations by altering the adhesion molecules, which is VCAM-1, ICAM, and so on so forth.

The mediators and regulators of acquired immunity are produced mainly by T lymphocytes in response to specific recognition of foreign antigens via, of course, the antigen presentation machinery. So, these include IL-2, IL-4, IL-5, IL-13, and so on, gamma interferon, TGF beta, and TNF beta.

Then, of course, depending on the function, as I told you, because they are constitutive in nature, there could be stimulators of haematopoiesis. These are produced by the bone marrow stromal cells as well as leukocytes. They end up stimulating the growth and differentiation of leukocytes, such as the stem cell factor, IL-3, IL-7, and GM-CSF.

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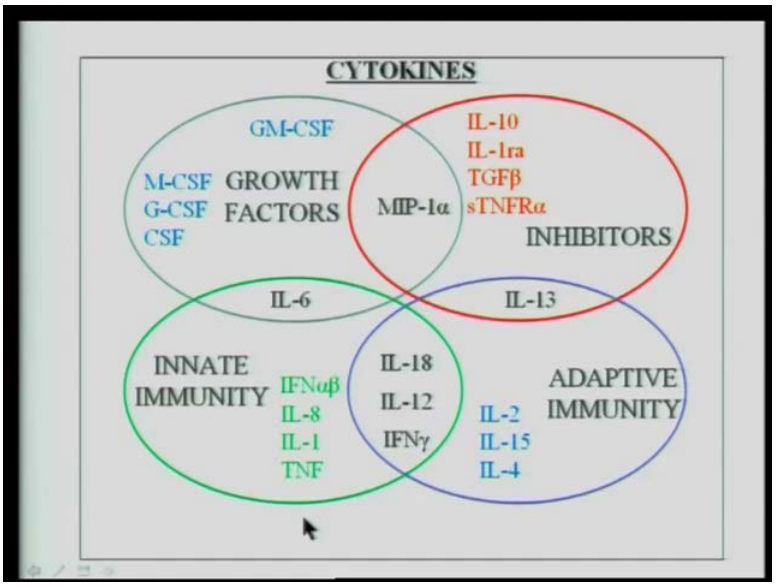

So, if you were to look at all these different kinds of cytokines, you can see that there is some overlap of MIP 1 alpha between the growth factors and inhibitors, and between, if you look at adaptive and innate immunity, you find that IL-18, IL-12, and gamma interferon have are in the overlap between adaptive immunity and innate immune cytokines.

These, of course, in the green, are those that helps in innate immunity; the ones in blue, here, are helping the adaptive immunity, and there are various inhibitors to regulate the action of these various kinds of cytokines– IL-10 is mainly immunosuppressive cytokine, IL-1 receptor antagonist, TGF beta, as well as soluble TNF receptor alpha. So, you have these cytokines which are constitutive, which are… they act like growth factors.

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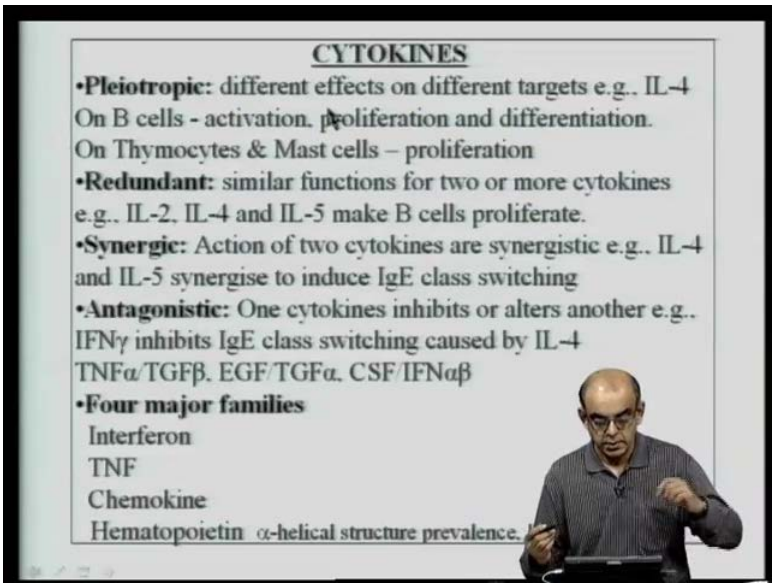

So, these– the action of the cytokines– further, can be described as being pleiotropic– pleiotropic meaning that they can engage in different effects on different kinds of targets. So, a single cytokine has different actions on different targets, for example, IL-4, on B cells, can have the action of activation, proliferation, as well as differentiation. So, not only do they make the B cells proliferate, they also allow them to differentiate and allow class switching, meaning that class switching from one class of immunoglobulin molecules, they go on, after switching, to secrete a different class of immunoglobulin molecules, basically, because of the gene rearrangement system that is already, that you have already learnt about. On thymocytes and mast cells, they can act as a proliferative cytokine. So, you see, they can, they can differentiate B cells; on thymocytes and mast cells, they act they, they, they increase proliferation.

The actions of many of the cytokines are redundant in nature; that means, their functions overlap. This overlap is, basically, because of the property or the structural features of their specific receptors, and the subunits that they… that they encompass. They can share various kinds of subunits, therefore, you have some of these functions overlapping between different kinds of cytokines, which we will learn in, in the next class.

They have similar functions, example, IL-2, IL-4, and IL-5 make B cells proliferate. So, they all have proliferative actions. Another example is that IL-4 and IL-2– they can make certain kinds of cells called as CTL-L2 to proliferate. This CTL-L2 was actually used to

measure in a bioassay for the presence of IL-2 or IL-4, and basically, it is a cytotoxic T lymphocyte that has retained its specificity, for the lymphokine lost his specificity for the stimulating antigen.

These lymphokines can synergic in action. In other words, the action of two cytokines could be synergistic; that means, more than additive. So, if the mechanisms are different, one would say that certain concentration– equal concentrations– of two different cytokines will give you a doubling of its action, but many of the cytokine actions are, actually, synergistic. So, more than additive in nature. So, IL-4 and IL-5 synergize to induce IGE class switching. So, in other words, IL-4 and IL-5 on their own would be stimulating a certain amount of IGE class switching, but when they come together, it goes up many, many fold.

Cytokines can be antagonistic; one cytokine can inhibit and alter the action of the other, which we learnt learn much more when we look at T-helper 1 and T-helper 2 actions and how they differentiate. Gamma interferon, in another example, inhibits IgE class switching that is caused by IL-4 in in activated B cells. So, B cells that class switch to IGE, because IL-4 has bound to its receptor, can be inhibited from doing so by the binding of gamma interferon to its specific receptor.

TNF and TGF beta– they are antagonistic; EGF and TGF alpha antagonistic, CSF and IFN type one interferon alpha beta are referred to as type one interferons; gamma interferon is referred to as immune or type 2 interferons. In addition to additional classification into innate immune cytokines and adaptive immune cytokines, there are four major families of cytokines, based upon their structure and other criteria such as interferon, TNF, chemokines, and the hematopoietin family, which have a prevalence of alpha helical structure, and little or no beta pleated sheet.

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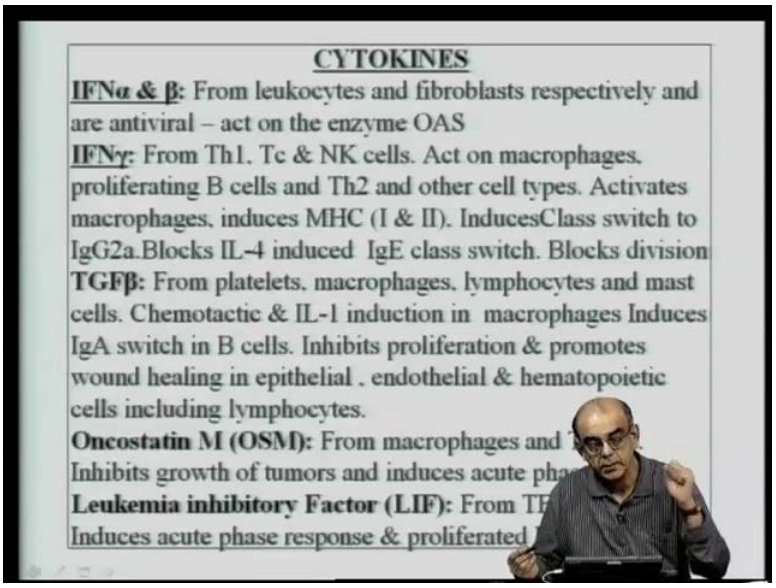

So, if you look at the interferon family, basically, interferon type 1 interferons, they are secreted from leukocytes and fibroblasts, respectively, and they are, basically, antiviral in nature. Virus infection induces enzymes such as oligoadenylate synthetase, and interferons, actually, help in this action.

Interferon gamma, which is the immune interferon, they are secreted from Th 1 cells, T c cells, or cytotoxic T cells, and NK cells. They act on macrophages, proliferating B cells and Th 2 cells, and other cell types; they activate macrophages; they induce MHC– both classes– both MHC 1 and 2, and they induce a class switch to IgG2a in differentiating lymphocytes. They block IL-4-induced IGE class switch, as mentioned earlier, and they block, basically, they are anti proliferative in nature.

TGF beta are produced from platelets, macrophages, lymphocytes, in mast cells. They are, basically, chemotactic in nature, and they induce IL-1 from macrophages. Also, they induce IGA switch in B cells; they inhibit the proliferation and promote wound healing in epithelial cells, endothelial, as well as hematopoietic cells oncostatin M or OSM; they are secreted from macrophages and T cells; they inhibit the growth of tumors and induce the acute phase response.

In addition to this, another cytokine that one come to across in different kinds of text books, is the leukemia inhibitory factor, which is secreted from thymic epithelial cells– TEC– thymic epithelial cells, during the differentiation in the thymus as well as bone

marrow stromal cells; they induce the acute phase response and they help embryonic stem cells to proliferate.

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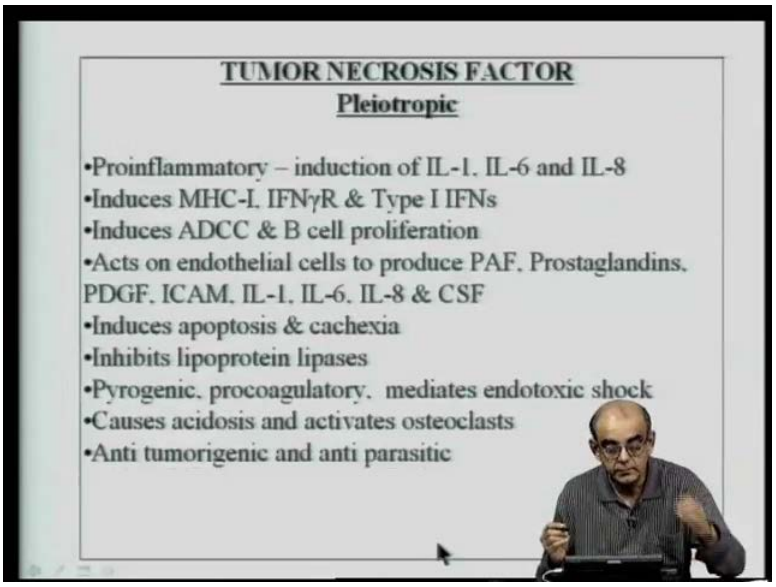

The other major family being the tumor necrosis factor is a very good example of a pleiotropic lymphokine. In fact, this is also referred to as a proinflammatory type of lymphokine, and these sort of TNF lymphokines are bound in different kinds of auto immune inflammatory situations, like arthritis and so on. They are proinflammatory, as I just now mentioned to you, by the induction of IL-1, IL-6, and IL-8. These are the three different kinds of cytokines which are result in the proinflammatory situation, which the TNF induce, induces. They induce MHC 1 on the cell surface of different kinds of cells, gamma interferon receptor, as well as the secretion of type one interferons. They induce what is called as antibody dependent cell mediated cytotoxicity, because they are activating macrophages, therefore, the induction of F c receptors and B cell proliferation.

So, they act on endothelial cells, basically, to increase adhesion molecules, or lymphocyte trafficking can be altered, and they also have to increase to secretion of prostaglandins and the platelet derived growth factors. Basically, and all importantly from the point of view of studying the mechanism of action of different kinds of cytokines, TNF is a very important lymphokine that induces apoptosis in cells and, of course, cachexia, depending upon whether it is TNF alpha or TNF beta, they inhibit

lipoprotein lipases and have various kinds of other kinds of actions, such as being anti-tumorigenic and anti-parasitic in nature.

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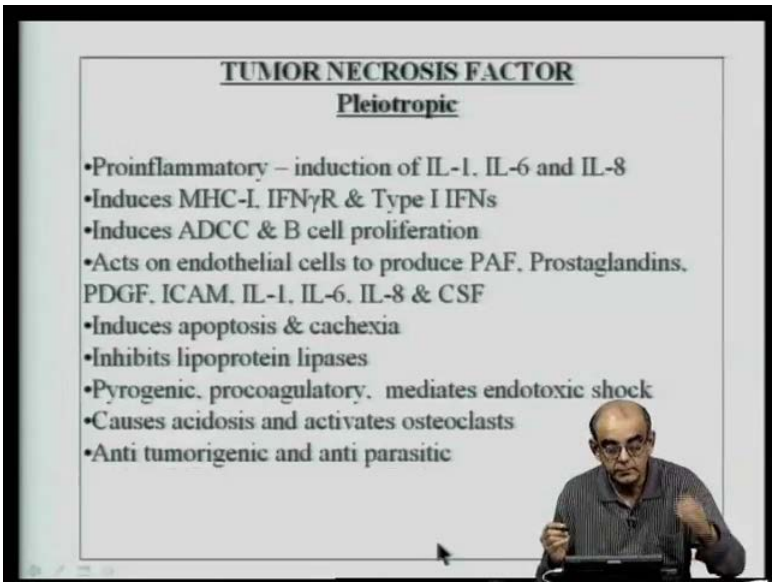

The chemokine family are a very important set of polypeptides, which is a large family. They are small, structurally homologous polypeptides, basically, involved in regulating leukocyte migration and trafficking into different kinds of lymph nodes or into different kinds of locations within the body, like, you know, between malt tissues and other kinds of locations in the body. They are produced from the leukocytes epithelial cells, fibroblast, and endothelial cells, upon stimulation with various kinds of lymphokines, such as TNF, IL-1, or for that matter, TLR-mediated, TLR-mediated stimulation by, by microbes or antigen.

They are usually small in molecular weight– 8, 8 to 12 kilo daltons. They are structure… they are structurally homologous, and basically, they contain two disulphide bounds or two disulphide linkages, or four cysteine, cysteine residues. These two N-terminal cysteine residues that participate in disulphide bonding form the basis of the classification of subfamilies of the… of the… of these chemokines.

So, if these two N-terminal cysteines are are adjacent to each other, they are called as c c chemokines; if they are separated by one aminoacid, they are called c x c chemokines, where x is the is the intervening aminoacid residue which can be any aminoacid residue,

or they are separated by two aminoacids; it is called as the c x x or c c x x c chemokines; they lack two cysteines in lympho tactin.

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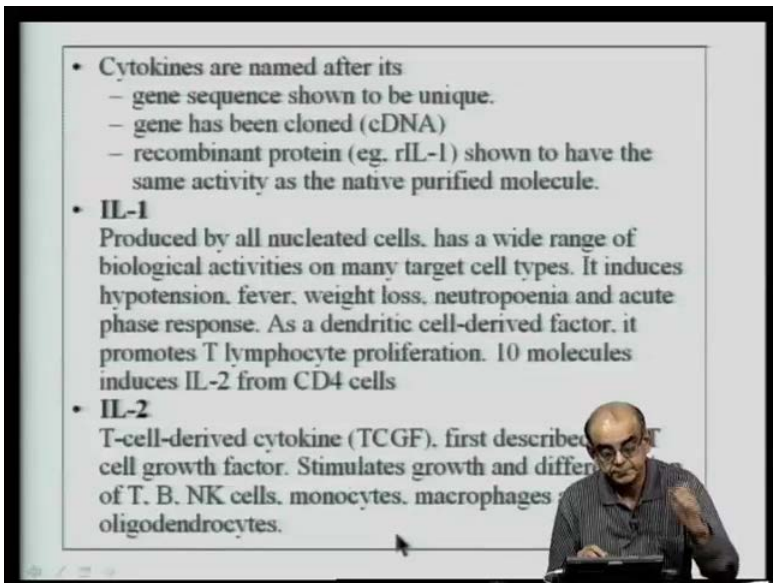

Now, cytokines– the work on cytokines involves looking at gene sequence; it has shown to be unique in order to be classified as a separate lymphokine; the gene has to be cloned, so the cDNA has to be available, and the recombinant proteins must be shown to have the same activity as the native purified molecule, in order to be named as a lymphokine.

There are several different kinds of lymphokines, or they are called as interleukins, and these interleukins number from 1 to 35 and more. So, basically, this lecture will cover some of these lymphokines– some of the important lymphokines– but for a, for a more exacting or a more detailed treatment, one has to go into different kinds of textbooks, because the subject of cytokines or interleukins, itself, can go on for more than or several, several classes.

Now, IL-1– IL-1 is produced by most nucleated cells they have a wide range of activities in many different kinds of many target cell types. They induce hypotension, fever, weight loss, neutropenia, and acute phase responses. IL-1, as I told you, can also act as a, as a hormone in mediating the in the fever response.

As a dendritic cell derived factor, in other words, it is derived from antigen presenting cells; it promotes T cell proliferation. In fact, this IL-1 secretion induces T cells to produce IL-2, which is one of the first response that one sees in T cell mediated responses. As little as ten molecules can induce IL-2 from CD 4 cells. IL-2, of course, is a T cell derived cytokine, first named as TcG f; it stimulates the growth in differentiation of T cells, B cells, NK cells, monocytes, macrophages, and oligodendrocytes. In fact, IL-2 is one of the cytokine that has been worked on very thoroughly by in the, in the, in the immune system.

IL-3 is produced from T-helper cells, natural killer cells, and mast cells. It facilitates histamine secretion from mass cells; it is a haematopoietic growth factor, which stimulates colony formation of erythroid megakaryocyte, neutrophils, eosinophils, basophils, mast cells, and monocyte lineage or type of cells.

IL-4 it is secreted from Th 2 cells, and basically, it acts, it acts on B cells. It can also act on T cells to cause proliferation; it acts on endothelial cell, fibroblasts, and thymocytes; it induces secretion of IgE and IgG 4 by B cells.

IL-5 is a Th 2-derived cytokine; it is also mast cell-derived glycoprotein, stimulates colony formation from eosinophils, and it, importantly, it induces IgA class switch in activated B cells.

IL-6 is multifunctional. It regulates B cell function, haematopoiesis, and the acute phase response. It secreted by lymphoid, non lymphoid monocytes, macrophages, Th 2, and bone marrow stromal cells.

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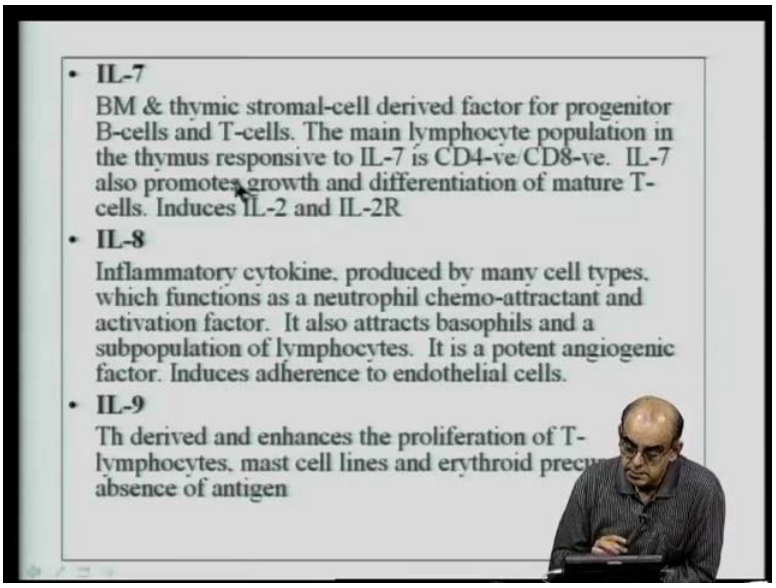

IL-7– IL-7 is secreted by bone marrow and thymic stromal cells. It acts on progenitor B cells and T cells. The main lymphocyte population in the thymus that responds to IL-7 are CD 4-negative and CD 8-negative. IL-7 also promotes growth and differentiation of mature T cells and it induces secretion of IL-2, as well as the, the expression of the IL-2 receptor.

IL-8 is, actually, chemokine. It is an inflammatory cytokine produces… produced by many cell types, which functions as a neutrophil chemo attractant and activating factor, and is involved in many, many inflammatory situations; so it is proinflammatory. It also attracts basophils and a subpopulation of lymphocytes. It is a potent angiogenic factor and induces adherence to endothelial cells.

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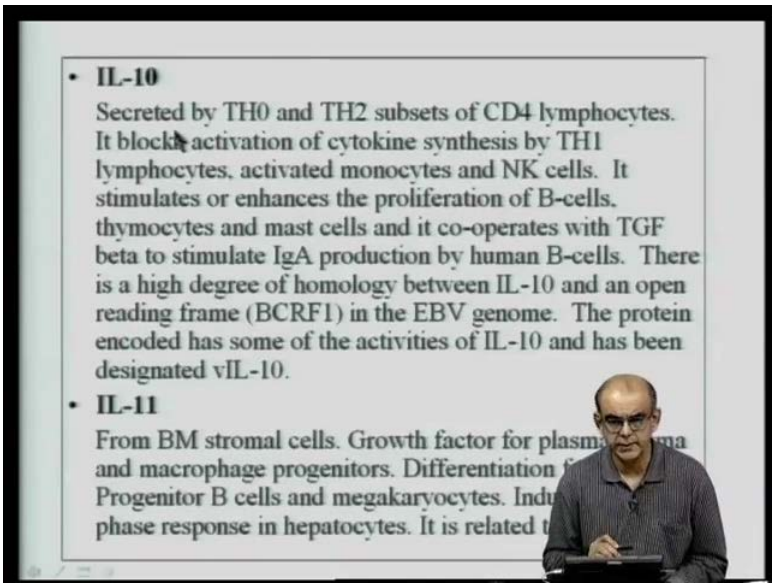

Then, going on further, another important cytokine being IL-10, it is secreted by Th 0 and Th 2 types of subsets of CD 4 lymphocytes. It blocks the activation of cytokine synthesis by Th 1 lymphocytes, activated monocytes, as well as natural killer cells, which stimulates or enhances the proliferation of B cells, thymocytes, and mast cells, and cooperates with TGF beta to stimulate IGA production by human B cells.

So, there are so many such actions have different kinds of lymphokines. Another important lymphokine being IL-12 which is involved in Th 1 responses. It is the important against intracellular pathogens, because it mediates, finally, the activation or production of cytotoxic T killer cells; it induces interferon gamma production by T cells and NK cells and enhances NK and ADCC activity; it stimulates the production of NK LAK cells are lymphokine activated killer cells. So, you can have killer cells that are activated by different kinds of lymphokines; basically, they, they, they have a killing action on their target cells. These LAK cells are the cells that were…, that are found to be very active against a variety of tumor cells, as they are arise this stimulates differentiation of Th 1 cells, and along with IL-2, it induces cytotoxic T cells differentiation.

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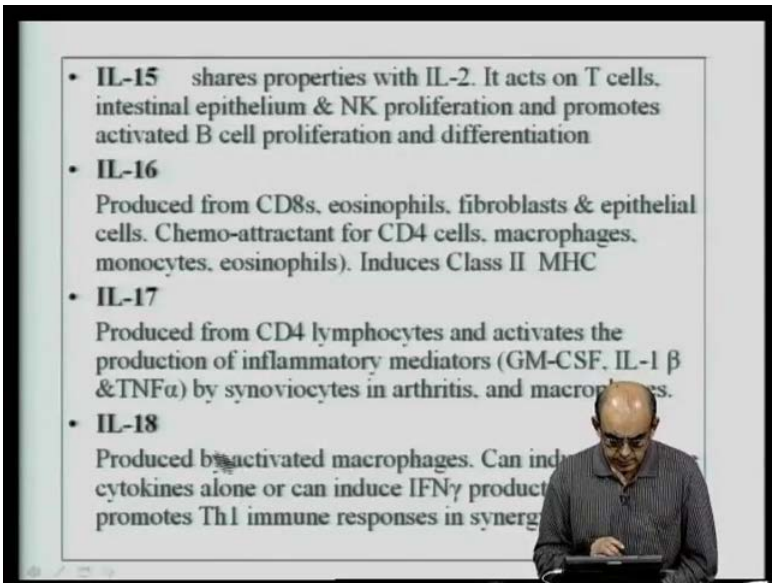

Going on further, you have IL-18 and IL-17, which are important– very important– cytokines. IL-17 produced from CD4 or T-helper cells activates the production of inflammatory mediators, such as GM-CSF, IL-1 beta, and TNF alpha, by synoviocytes in arthritis, which is, basically, an inflammatory situation. IL-18 is produced by activated macrophages can induce Th 2 type cytokines alone, or can induce gamma interferon production, and promotes Th 1 immune responses in synergy with IL-12.

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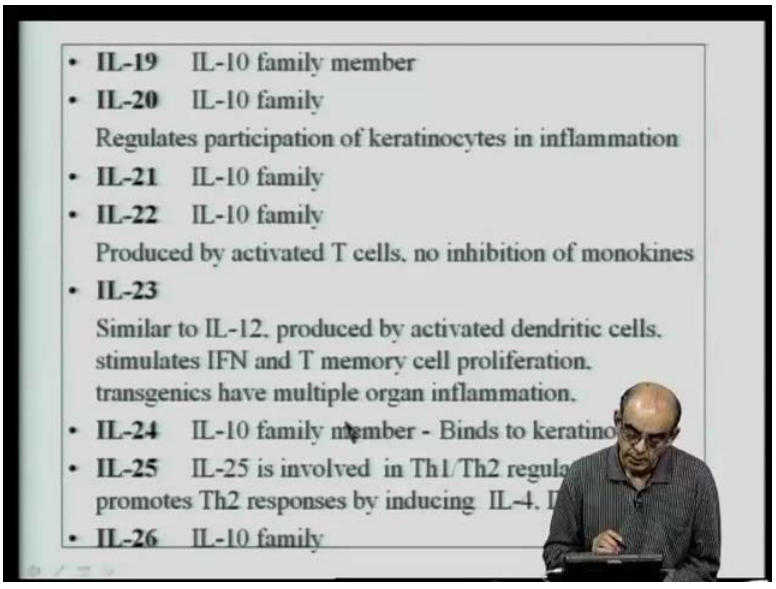

Now, IL-20– 20 is a, is a cytokine that regulates participation of keratinocytes in inflammation. Then going on further, of course, there are several of them which are IL-10 family members.

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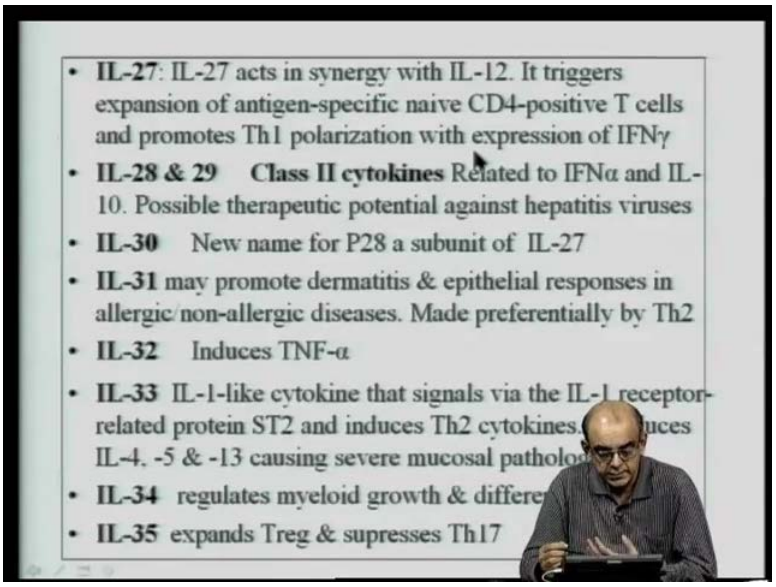

And if you look at other kinds of cytokines, there are basically up to 35 of them. You can go through them in at your own time, and all these cytokines, as I mentioned, work in a kind of a network. All these pathways of networks are quite exhaustive, and can be found in our in in different kinds of textbook, as well as in different kinds of catalogues of different companies– biological companies– which sell the cytokines or different kinds of antibodies. So, you will see that Promega company may have a website that that may have their own articles on different kinds of lymphokines, and these networks pathways are given, and I would encourage you to look at these different kinds of pathways or charts that are available at different websites, since going through these networks and to describe them is, kind of, limiting in this in this one hour of lecture.

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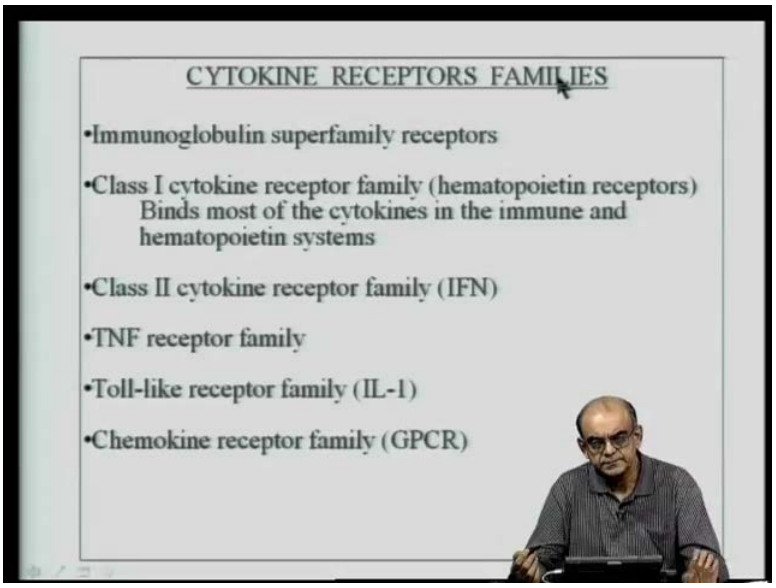

Then, going on from cytokines to their specific receptors, so if you look at these receptors, there are different. Again, they can be categorize as different families. As I told you, each cytokines has its own receptors. So, if you look at all these different kinds of receptors to the different cytokine families, you have what are called as the immunoglobulin superfamily of receptors; you have class 1 cytokine receptor family, which is also known as hematopoietin receptors. Basically, this is the largest group which binds most of the cytokines in the immune and hematopoietin systems.

Apart from class 1, you have the class 1 cytokine receptor family, which is, basically, the interferon receptors; then you have the TNF receptor family; then you have the toll-like receptor family, and then you have the chemokine receptor family. So, you have these broad receptor families that have been classified.

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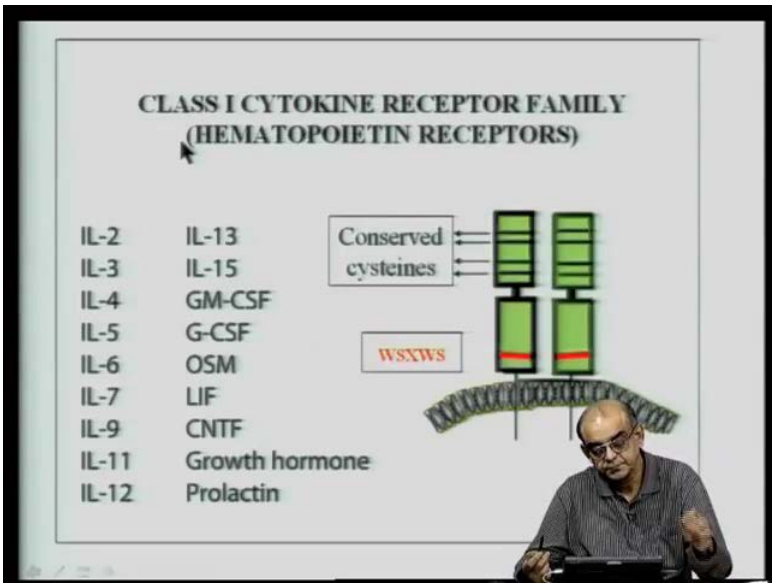

So, looking at the class 1 cytokine receptor family of receptors, you find the hematopoietin receptor as, basically, made up of domains or portions of the receptor that have certain kinds motifs. They have conserved cysteine motifs in these domains, and you have another motif called as the tryptophan serine unknown amino acid tryptophan serine motif.

So, in their primary amino acid sequence, these are the motifs that are available in the cytokine receptor. Basically, they are dimmers, and the ligands that bind to them are listed over here, basically, being IL-2, IL-3, IL-4, 5, 6, 7, 9, 11, 12, and so on.

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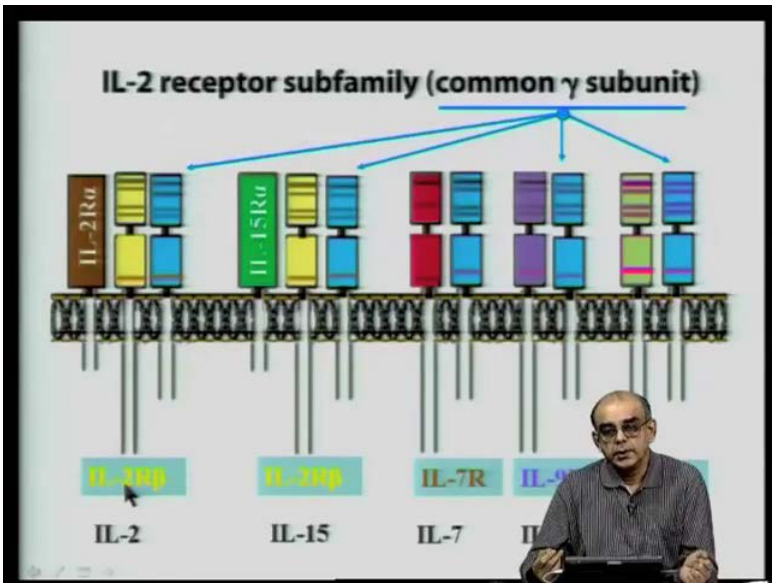

Going on to the subfamily of this class 1 or hematopoietin family of receptors, you have several subfamilies based upon their structure, and the way that their subunits are, are makeup this receptor. So, you have what is called as the IL-2 receptor subfamily, which share a common gamma subunit. Basically, you have for IL-2 and IL-15, they have three subunits, and these three subunits are made up of the alpha, the beta, as well as the gamma subunit. So, you see, you have the IL-2 receptor– have the IL-2 receptor alpha– which has the short intracytoplasmic tail, not enabling it to mediate trans membrane signaling after it binds to its lymphokine IL-2.

You have the other two subunits having longer intracellular segments, which enable them to mediate signaling events below the membrane; that is what results in the IL-2 action on the genes that it has to activate. We will come to that in a little later on, then, in the next cytokine class.

So, basically, if you look at this color, you see that all these different cytokines share this particular subunit, and they have, of course, the XWS motif, and they have conserved cysteine motifs. So, in addition to this common gamma subunit that is shared between IL-2, IL-15, IL-7, IL-9, and IL-4, the other subunits have their specific lymphokine binding subunit. For example, IL-5, IL-15, shares the IL-2 receptor beta, but it the alpha subunit binds to the IL-15 lymphokine, and the IL-7 receptor subunit alpha binds the IL-

7 and so on. So, these have been classified together based upon the sharing of this gamma– common gamma subunit– and called as the IL-2 receptor subfamily.

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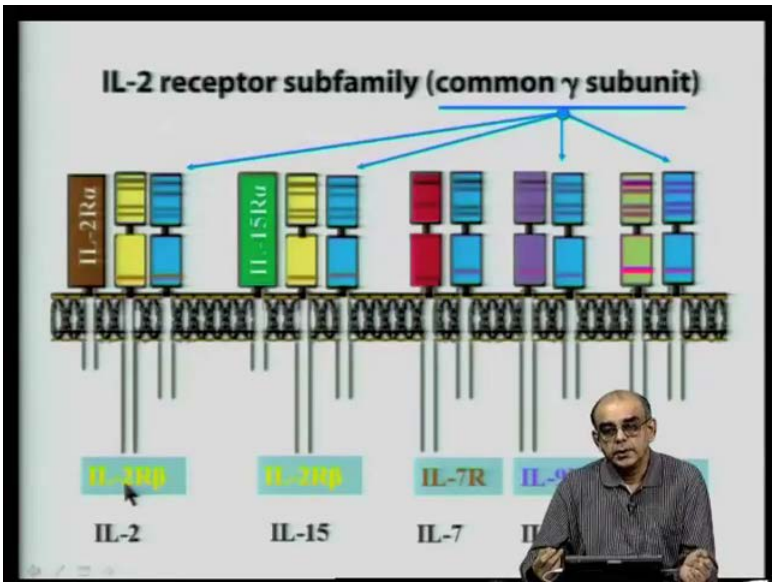

Then, you have the IL-6 receptor subfamily, which shares a common glycoprotein subunit of 130 kDa. So, you have these, over here, that are designated IL-6. Of course, you have two of these GP 130 units, and then, if you have the other lymphokine binding subunit, again, characteristically having a short intracytoplasmic segment, basically, saying that the GP130 mediates, or able to mediate, the trans membrane signaling, subsequent to the binding of its lymphokine. So, you see, IL-11 share the same GP 130, but has a different IL-11binding subunit; so on for the ciliary neurotrophic factors– C N T F– or the oncostatin M factor, or the leukemia inhibitory factor.

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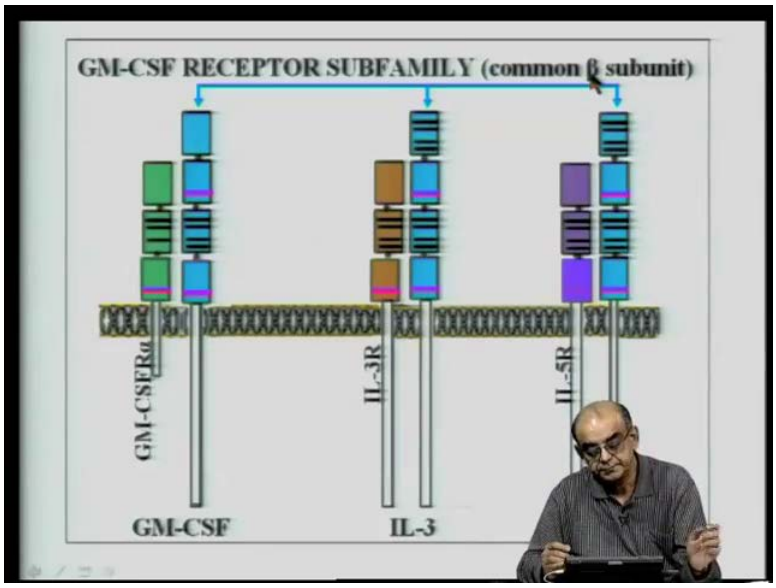

Then, you have the GM-CSF receptor subfamily, which shares a common beta subunit, as shown over here, in this color. So, you have the the receptor specific subunit, and then you have a longer intracytoplasmic segment which enables the trans membrane signaling, which is the common beta subunit between GM-CSF, IL-3, and IL-5, IL-5.

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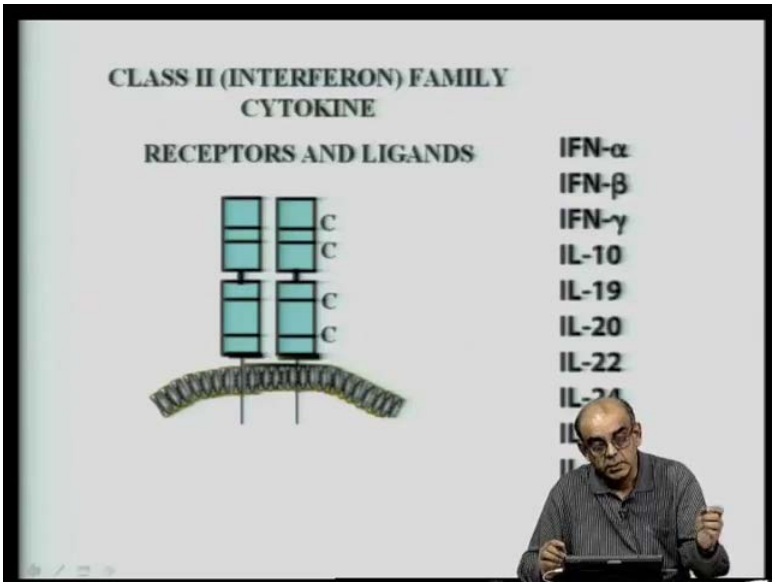

So, here, of course, going on to the class 2 family, this is not a subfamily– this is separate family which is called as the interferon subfamily, which has the different kinds of

interferons. Only 3 of these are listed over here– alpha, beta, and gamma, but there are several different other kinds of interferons that have been discovered.

In addition to this, of course, you have all these other kinds of lymphokines. IL-10, which is a immunosuppressive cytokine, basically, being a dimer and consisting of cysteine motifs; then you have the TNF receptor family, as mentioned earlier in the classification, having this single subunit that, basically, has three motifs called as the C 1, C 2, and C 3. Basically, they have TNF binding– they are TNF bindings. So, TNF alpha, beta, and then you have CD 27 ligand, the nerve growth factor, and the Fas antigen.

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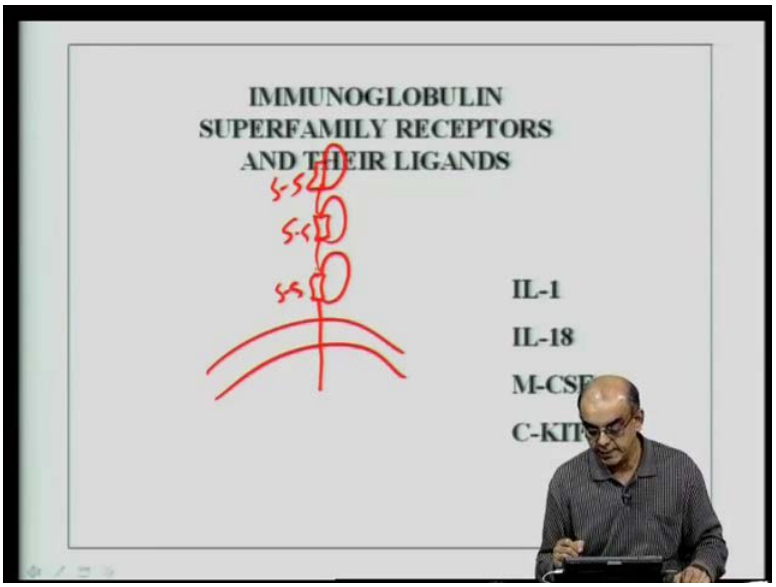

So, then, of course, you come to what is called as this– the immunoglobulin superfamily. What is the immunoglobulin superfamily? It is, basically, the disulphide bonded domains that is available in, in the immunoglobulin domains that are found, and many of these domains are shared between different kinds of molecules, both in the immune system and outside the immune system.

So, if you look at these molecules, you find, of course, as, as, as you saw earlier, you have the membrane, and then you have these immunoglobulin domains forming this subunit. So, these are the disulphide bonds, which is characteristic of these immunoglobulin domains, and these are the immunoglobulin superfamily of receptors, and the ligands being IL-1, IL-18, M-CSF, and C-KIT.

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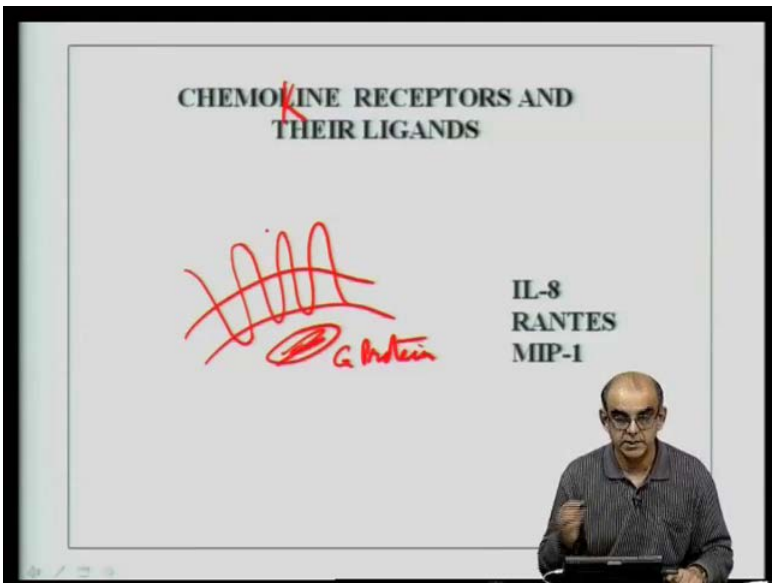

Going on further, you have the chemokine. I am sorry about this This has to be the chemokine receptors, and their ligands– you have the ligands being IL-8, RANTES, and MIP-1. So, in this case, these are different from the rest of the receptors in being…, in having trans membrane domains. So, they have 7-trans membrane domain. So, you have the receptor. So, you have something like this, and these receptors or the chemokine receptors, are coupled under the membrane to G proteins. So, these are G protein-coupled receptors, basically, for the purpose of downstream signaling, or the trans membrane signaling events subsequent to the binding of the chemokine to its chemokine ligand.

So, basically, then you see that looking at cytokines, they were first discovered in a, in a fashion where they looked at supernatants of allo, alloreactive lymphocytes. Subsequently, these, these, these different factors, or the genes for these different factors were found and the receptors were characterized, and all these different kinds of lymphokines structure was worked out, and based on their structure and function, they were classified into different families and superfamilies.

So, all these different kinds of lymphokines bring about their action by binding to the receptors, and are regulated in a very unique way. So, the way they are regulated,

actually, has a lot of relationship with different kinds of receptor families, that we just now receptor… that we just now recognized, and that is having a common subunit.

Basically, if you have a common subunit shared between different receptors, you can envisage a situation where a dearth or a limitation of the subunit can actually cause a limitation in the kind of lymphokine action. Although there are different kinds of lymphokine binding subunits that are available, even if more number of subunits are available for a particular lymphokine, if the common shared subunit is, is limiting in number, then you would find that there would be a limitation in the outcome of that lymphokine, although the lymphokine is produced in large amounts.

For example, a very interesting situation arises. When you have T cells proliferating, T cells secrete IL-2. When the… When the, when the activation is strong, you have more of this growth factor, or more of IL-2 being synthesized in the medium, and therefore, one would think that the more the amount of IL-2, it should go on resulting in a continuous increase in the number of, of, of lymphocytes that are responding to this lymphokine IL-2.

But what actually happens, in vivo, or for that matter, in vitro, also, is that after a period of time, these lymphocytes failed to respond to IL-2, even though large amounts of IL-2 are available in the surrounding medium, a nd this is a case of receptor down regulation, or altering the affinity of a particular of that particular receptor. So, it does not respond to excess of lymphokine. In fact, this is how lymphokine responses are regulated, and we will come to that in the next class. So, this class has dealt with the various kinds of lymphokines, their structure…