Essentials in Immunology

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Module B2

Lecture No. # 03

Cells & organs of the Immune system Part (Lecture) 2

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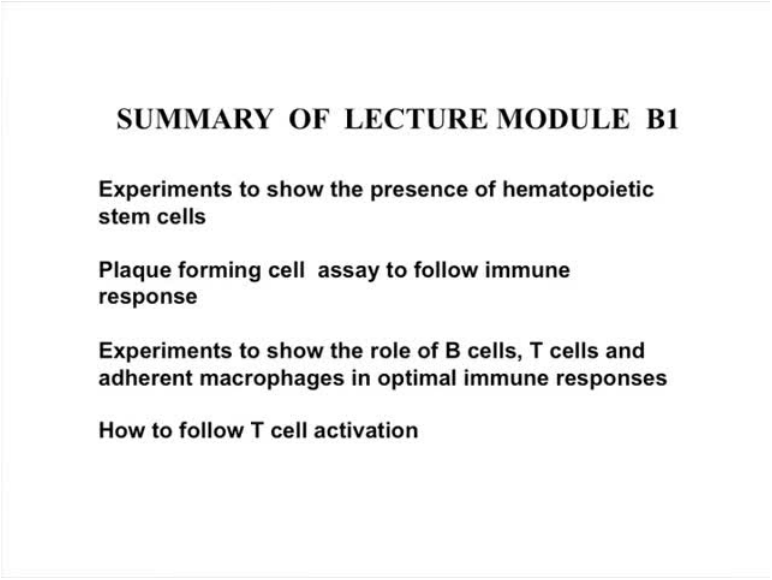

Hello and welcome. Let us first review some of the conclusions from the last lecture, which is listed in this slide. We went through some of the experiments, that showed the presence of hematopoietic stem cell, where we looked at how mice could be irradiated and populated with stem cells, that were isolated from the bone marrow. Then, we looked at, how immune responses were followed in the early experiments by using a plaque forming cell assays to quantitate the B cells that were producing the antibodies. And then, we went into some experiments to show the role of B cells, T cells and the adhering macrophages basically, to say, that although B cells were known to make antibodies, it was when they were put together with the T cells and more importantly, the adhering macrophage populations, only then, could they lead to optimal immune responses. And finally, we closed by describing ways by which you can follow T cell activation because this is necessary to understand the function of macrophages.

Now, during this T cell activation description, we saw how the activation of T cells leads to the proliferation of these cells. So, the hallmark of T cell activation being T cell proliferation, these activated T cells could then be followed by the incorporation of radioactive thymidine into their DNA. So, what we will do first is to try and see, what is the principle of this assay that involves T cell activation?

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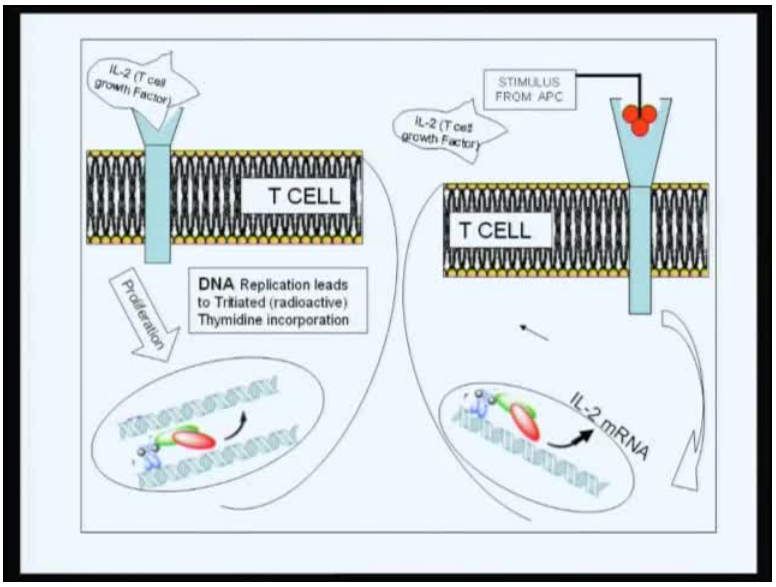

So, let us start with a blank slide and go on to the next one. If we have T cells in the mixture, these 2 T cells that are shown in this slide, let us see, how they can be activated? The T cell gets activated via the presence of a receptor, called as T cell receptor, which is shown here. We will not worry about how the T cell recognises the antigenic stimulus, suffice it to, stay, say at this present time, that the T cell can get activated in response to a stimulus, and so, we will put in something, like what you call as a stimulus, that has to come from another cell type, basically in antigen presenting cell or macrophage. Now, all this is being done to find out the function of macrophages because during those times, the experiments were evolving to see, how macrophage function, and basically, why they had to phagocytose and what they would do after phagocytosing bacteria or bacterial antigens?

So, let us see, what, what the stimulus from the APC would do? First of all, it has to bind to its receptor and this is done via the T cell receptor, and this T cell receptor, upon binding to its antigen, will transfer a signal into the cell with results in the activation of

the IL-2 gene. Therefore, IL-2 mRNA is transcribed from the IL-2 gene and this IL-2 mRNA results in the production of the IL-2, which is also a T cell growth factor. Being a T cell growth factor, it is secreted outside into the medium and this T cell factor, more of it is produced, and the T cell factor then goes and binds to its own receptor on a neighbouring T cell, or for that matter, into the same T cell, that produced the T cell factor.

So, this binding of the T cell factor to its receptor, which is also called as the IL-2 receptor, then transduces a signal, that results in the proliferation of the cell by synthesizing DNA and the synthesis of DNA can be found by the incorporation of radioactive thymidine. So, all one does is add tritiated thymidine to the activated T cell preparation, precipitate the DNA by using something like trichloroacetic acid, which precipitates all macromolecules. You filter out these precipitated molecules and count it in beta scintillation counter and therefore, more the radioactive incorporation, the more is the T cell activation.

So, now coming to this T cell stimulus, which is what our aim was to try and see what experiments could demonstrate? During those early experiments, the function of these adhering cells, which in the last lecture we concluded, that adhering cells when mixed with non-adhering cells, that contain the B and T cells, could lead to a better production of the antibodies and therefore, a better immune response.

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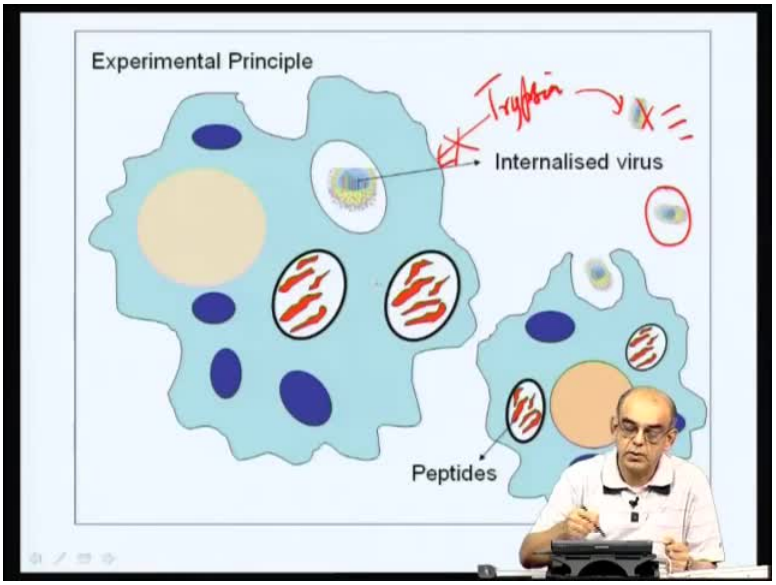

So, understand the macrophage function or the antigen presenting cell function, let us try to see, what the macrophage, done, does? It was already known, that macrophages could phagocytose, thanks to the experiments of Elie Metchnikoff and the later experimenters. So, what I have done here is to draw a macrophage here and this function of phagocytosis can also be mediated by cells, like neutrophils. So, what do these cells or antigen presenting cells do?

So, I have represented here a virus particle that would be found in a virus infected person, or for that matter, when you are trying to look at immune responses by using an antigen instead of the virus, there would be antigen that one would pulse into a cell preparation. So, these cell preparations would have this macrophage and if you want to follow the function of these macrophages, and if you take, took out cells derived from the spleen or other organs, which contain lymphocytes, one would have in addition to these macrophage or antigen presenting cell, it would also have the B cells as well as the T cells.

So, this virus particle is first taken up by the macrophage by a process of endocytosis or for bigger particles, there is phagocytosis; bacteria being phagocytosed, antigens being endocytosed. Now, the main important thing to bear in mind is that after phagocytosis, the antigen, whether it is a soluble antigen or a bacteria, or for that matter, viruses is taken up inside macrophage and therefore, not available outside here. In such a situation, if one was to put trypsin, if you used trypsin as a protease, it is a source of a protease and this trypsin has the ability to act on the antigen and clean it into smaller peptides, this trypsin is able to act on the virus particle or the antigen, only if it is available outside of the macrophage.

Now, if you take an internalized antigen or a virus preparation, the trypsin cannot enter the cell and therefore, cannot act on this virus particle. So, the experimenters took advantage of this kind of situation to try and see, if you put antigen along, along with an activated macrophage, how long would the trypsin be able to clean this virus particle? So, the basic experiment would be to see, you add antigen outside of the macrophage, allow these antigens to bind to the surface of the macrophage and then expose that to trypsin, and incubate the macrophage for various periods of time at 37 degrees. The phagocytosis needs 37 degrees and when phagocytosis or endocytosis occurs, the virus gets into the macrophage and then, so the trypsin would be unable to access that antigen.

So, therefore, as you incubate the macrophage at 37 degrees along with the antigen for longer and longer periods of time, more and more of these antigens are getting into the macrophage due to the process of endocytosis, and hence, unavailable for the action of trypsin.

Basically, what this antigen presenting cell does also, is to clip these antigens into smaller peptides, which are available inside the macrophage. And later on, what would happen to these clipped fragments is what determines the T cell activation?

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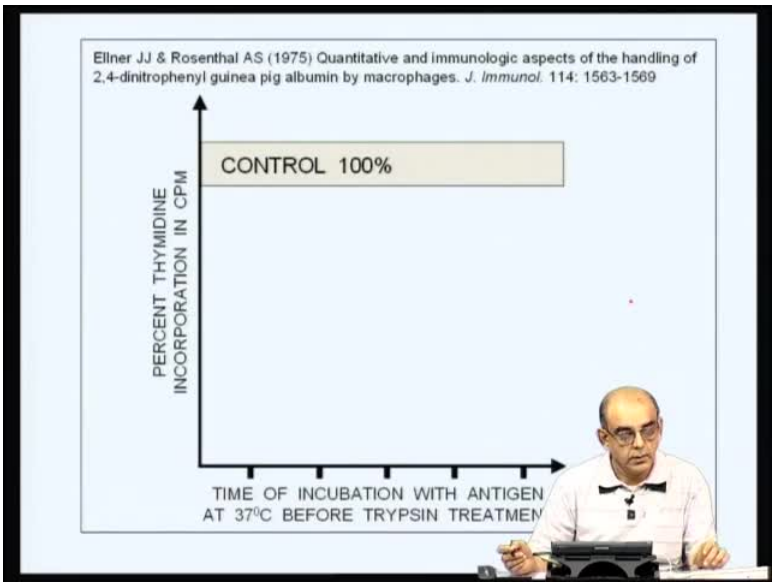

So, let us see, what happened in such an experiment that is done by Ellner and Rosenthal in the year 1975. What they basically did was to use macrophages that were isolated or taken from guinea pig. So, they had immunized guinea pigs with the antigens that we spoke of in the previous lectures, such as guinea pig albumin or KLH or more importantly, for this experiment, they used purified protein derivative, which is derived from micro-bacteria. So, what they did was to take cells from a guinea pig that was immunized with this antigen PPD, then isolated the various populations by taking these cells from the peritoneal cavity. So, these are called as peritoneal exudate cells (PEC) - peritoneal exudate cells.

So, what they did was to take the peritoneal exudate cells, which was their source of T lymphocytes and then, they have also macrophages, in that, put into that preparation,

which was the source for as antigen presenting cells, although at that time, there was no knowledge about the function of the antigen presenting cell or macrophage.

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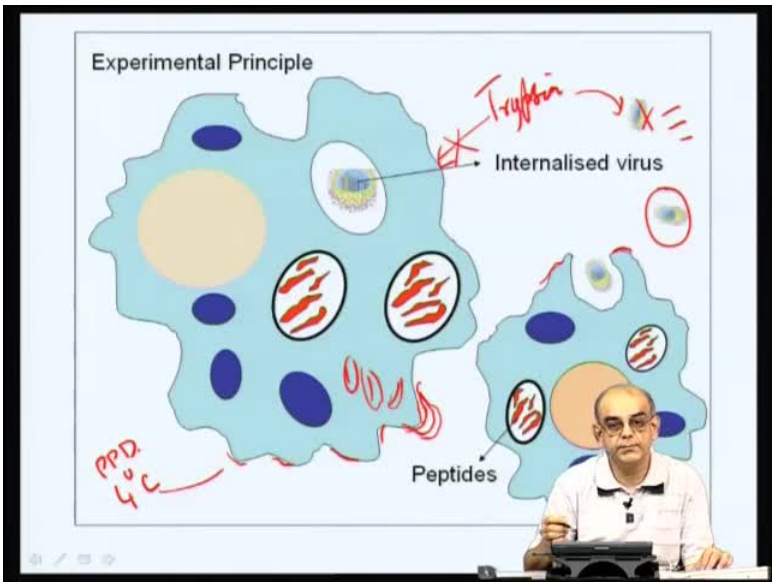

So, going back to the previous slide, what they simply did was to add PPD to this macrophage preparation, first at 4 degrees. So, when they added PPD at 4 degrees, all the PPD at 4 degrees can bind to the surface of this macrophage, we put it like this. After incubating for some time at 4 degrees, during which time phagocytosis cannot occur because it requires energy, they then exposed this incubated macrophage along with the PPD for various periods of time at 37 degrees. So, obviously, when at 37 degrees, this peptide would be phagocytosed and therefore, you would have a rite within the cell after some time. So, with increase in times, this PPD would be found inside the macrophage.

So, they exposed this whole mixture in the peritoneal exudate cells, which had the T cells, which were already activated because the guinea pig, were, was immunized to PPD and then, looked at the proliferation of these T cells. When they added these so called pulsed macrophages, pulsed because, you pulse these macrophages with PPD at 4 degrees for some time and then allow, wash the rest of the PPD off. So, there is no PPD, except for those that are sticking to the wall, to the membranes of the, of the cell. Then, when upon transfer to 37 degrees for various periods of time, this peptide would be internalized. So, the internalization of the peptide would make it inaccessible to trypsin.

Then, they would see, what would happen to the thymidine incorporation that is being followed by T cell activation, which was specific for this antigen PPD?

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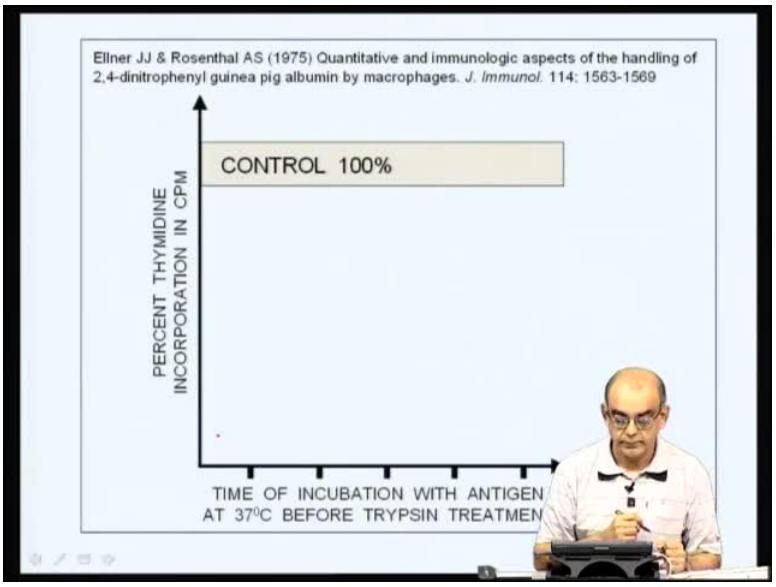

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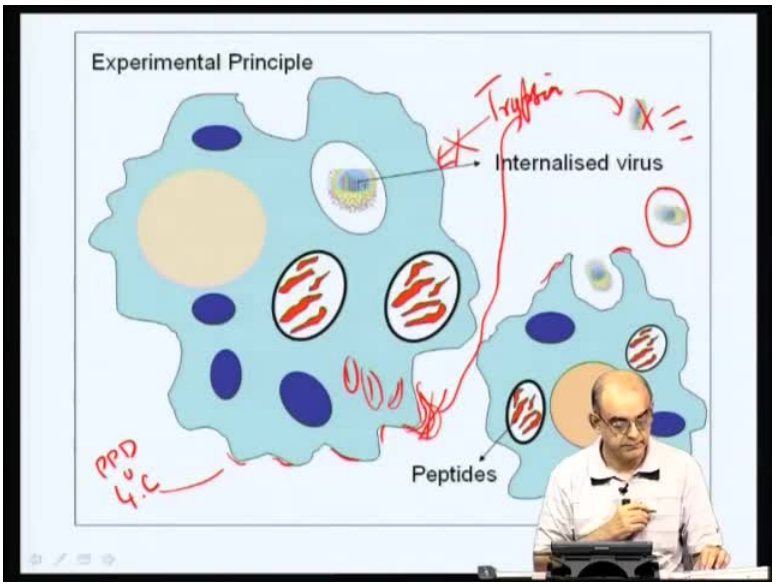

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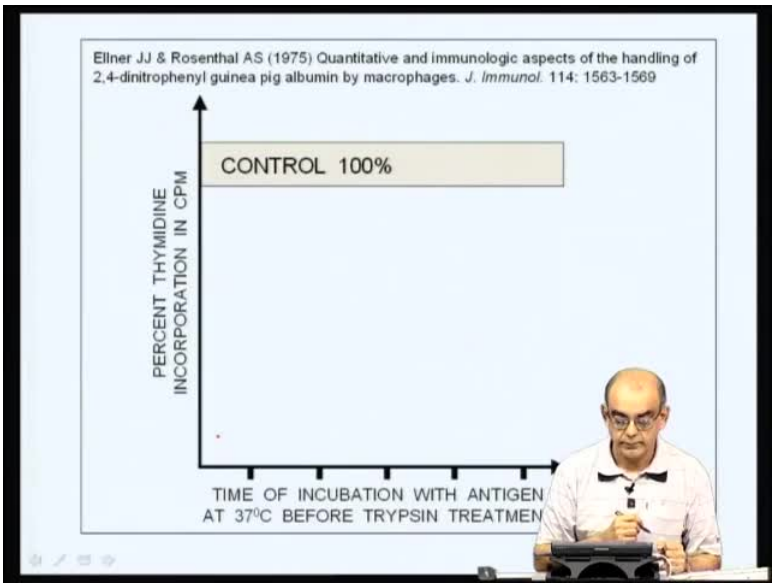

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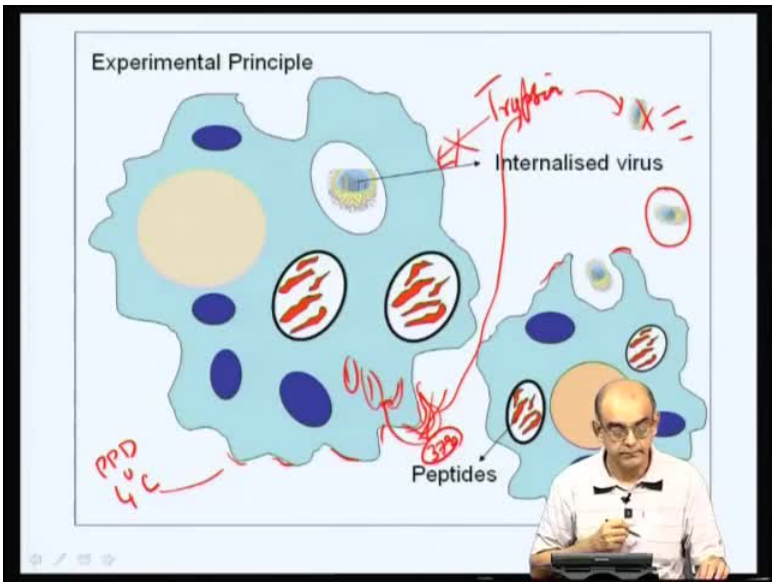

So, when they did that, they found, that at earlier time points, because the PPD was still outside, the trypsin could act on this and remove that PPD. So, there was no PPD available because of trypsin action, soon after 4 degree pulsing. So, at that time, what they found was, that is, at lower times of incubation, further incubation, 37 degrees, they had very little incorporation of the T cells, that had to see the antigen. But as time elapsed, we found, that, that in the last slide at 37 degrees, this would then go in and therefore, trypsin had no PPD to act on.

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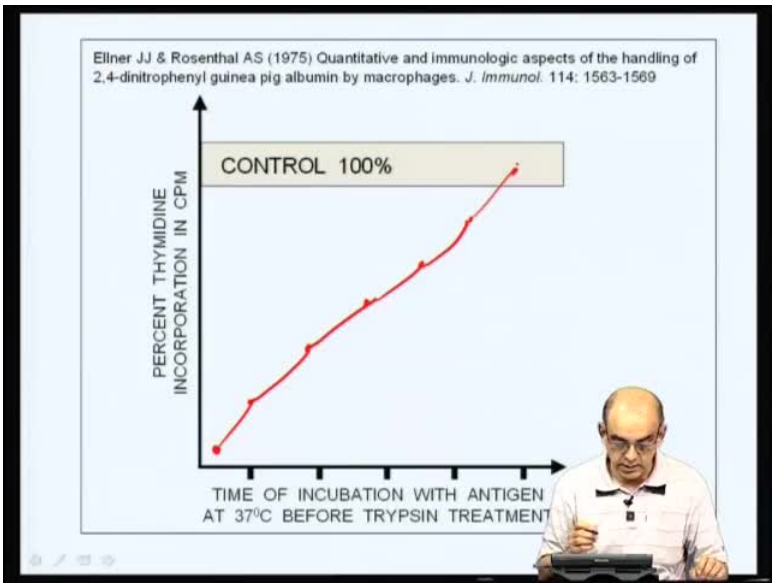

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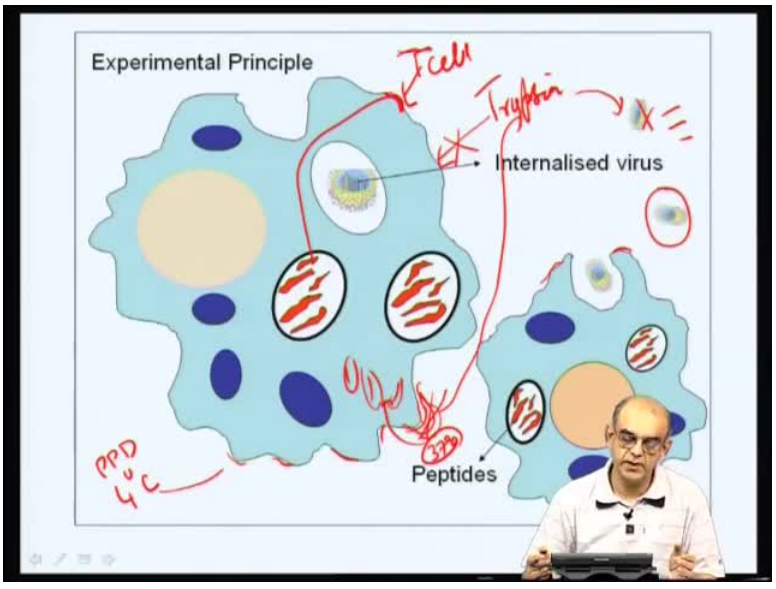

So, when they, when they did this incubation for longer period of time, they found, that before the addition of trypsin, so trypsin could act on that incubated macrophage, they found, that they got better and better incorporation of thymidine, in thymidine, into the cells showing, that, that the incorporation of thymidine increased because of DNA replication in T cell, which means, it is an indicator of T cell activation. So, therefore, what this meant was that you had this PPD outside at 4 degrees, therefore trypsin acted on it and there was nothing to be internalized. But you allow that PPD to go in by incubation at 37 degrees before the addition of trypsin outside the macrophage, you

found, that trypsin had, as time elapsed, there was much lesser PPD outside, that was available for trypsin action. And what it also meant was, that this processing of the PPD inside the macrophage, somehow led to the recognition, that T cells would come, T cells would recognize the macrophage, that was processing these antigens, which also meant, that since the T cell could only recognize antigen on the cell surface, it also meant, that these antigens had to come out some way on the cell surface. So, that is the property of T cell recognition and how the T cell recognizes is of course, we will come into the next slide.

So, what they learnt basically from this experiment was that the macrophage would internalize the antigen and the internalization then, is followed by a certain kind of a processing of this antigen, because larger virus particles had to be degraded to smaller sizes and this processed antigen would then be shuttled onto the cell surface, which would then be available for the T cell to see via its T cell receptor. So, what is the nature of this interaction between the T cell and the macrophage? So, this was the first indication, that came, that the macro for the requirement for adherent cells, vis a vis the macrophages, was because the macrophage had to take up the antigen and process the antigen in some way, which they did not know at that time except to say, macrophage contained lysosomes, which were acidic in ph. So, they used some blocking agent by increasing the ph of the lysosomes, by using ammonium chloride and lo and behold, the T cell activation was blocked.

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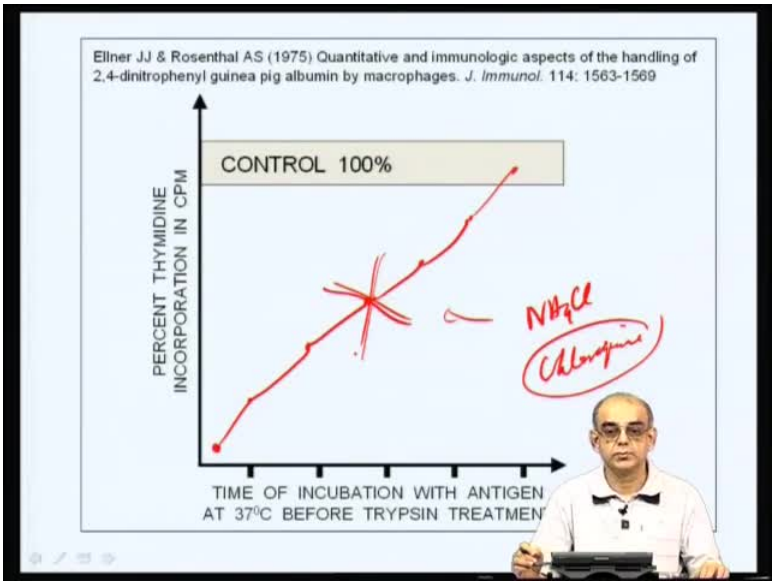

So, this process was blocked when you added to the macrophage, you added ammonium chloride or incubated the macrophage with ammonium chloride before the addition of the antigen PPD. There are other lysosomotropic compounds, like for example chloroquine, which all of you are known for its use in malaria. This chloroquine is also lysosomotropic because it goes to the lysosomes and alters the ph inside the lysosomes, the treatment of the macrophages with chloroquine before pulsing them with the antigen also, blocked this T cell activation. So, they knew from these experiments, that the acidic ph or the low ph of the lysosomes was required for the macrophage for its function.

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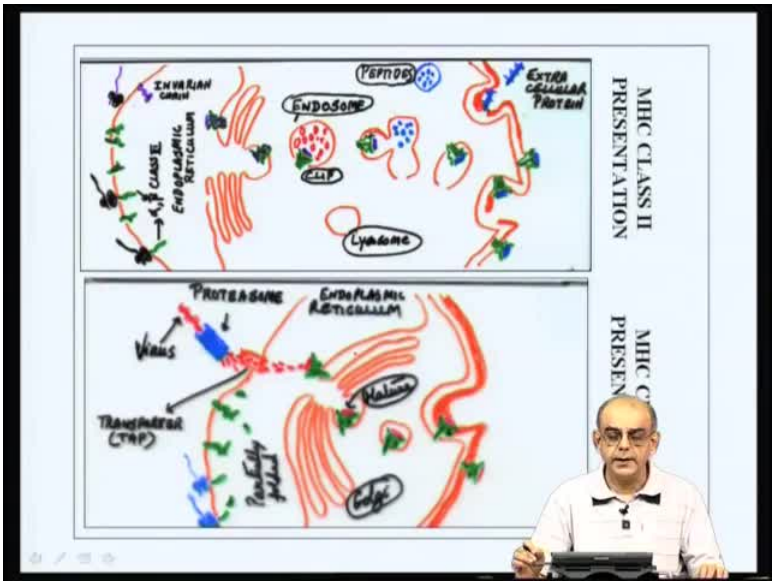

So, in retrospect of course, if you were to look at all these things, how macrophage function? We now know, there are 2 types of presentation - one presentation, which takes up the soluble antigen, that is available to the, available to the macrophage. So, you have extracellular proteins, soluble protein, or for that matter bacteria, which is taken up by the macrophage and the macrophage, these antigens that are taken up in the macrophage, are clipped up in the endosomes. And then, you have these compartments playing a very important role in how these molecules or peptides are loaded onto structures, called as MHC molecules or major histo-compatibility molecules and then, brought out on the cell surface.

As opposed to soluble antigens, you had to, you have to think about immune responses to viruses, that are not available outside. For example, retro virus, characteristic example

being AIDS virus. You have the virus going inside and replicating inside, so how does an immune cell deal with a pathogen that has entered the cell? So, there is another pathway, antigen presentation, which is we call as class 1 presentation because the antigen arrives as endogenously, it is not taken up from outside, which is exogenous antigen presentation. The endogenous antigen presentation also involves clipping of the antigen into smaller peptides and presentation along with a different type of class MHC molecule, called as the class 1. All these aspects of presentation will be taken up during the antigen presentation lectures.

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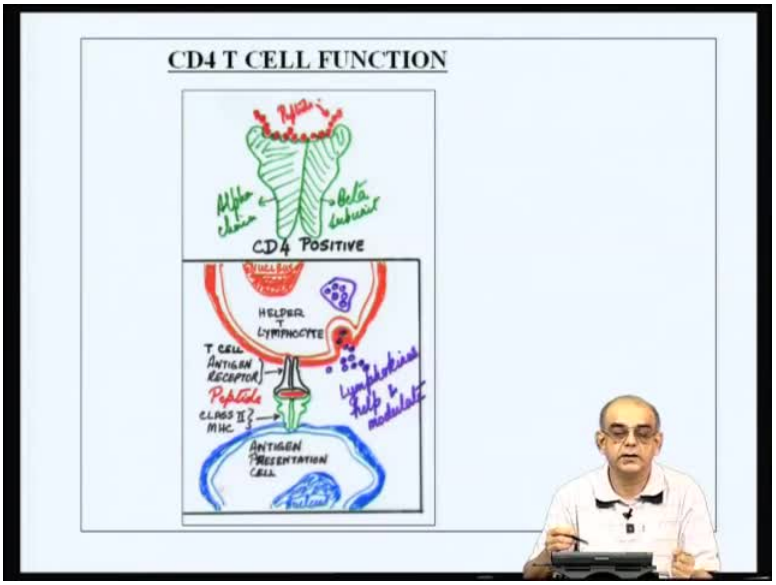

Now, again to summarize, T cell function or C D 4 T cell function is nothing but T helper cell proliferation. So, what happens there is that the T cell receptor has got 2 subunits, which is, the T cell receptor has got, I am sorry to say, T cell receptor actually has several subunits, but the main functioning receptor has got 2 subunits, which interacts with the MHC molecule, which is now present in this peptide shown to you in red. So, the green is the MHC class-2 molecule, which holds up this peptide inside of it like a bouquet and this bouquet is what is recognized by the antigen recognition portion of the T cell receptor. Now, in response to this recognition, the T cell gets activated and spews out lymphokines and these lymphokines are the ones that play a major role in giving B cell help. And various other functions of cytokines will be discussed in the cytokine class.

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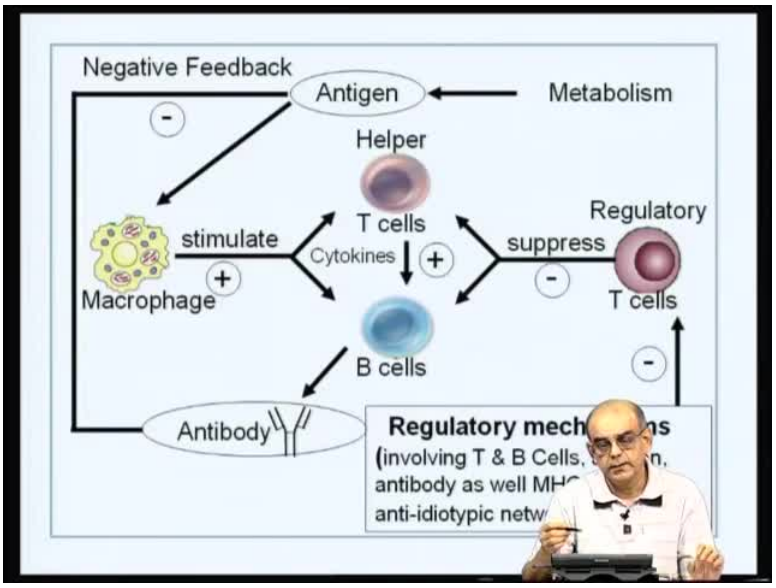

So, basically then, you have all these cells functioning in a, in a co-operative way and basically, just to summarize for you, you have of course the antigen, that is arising from the pathogen and by various other processes in the body. This antigen stimulates the macrophage or is taken up by the macrophage, processed inside the macrophage into smaller peptides, which then, upon interaction with a T cell, stimulates the T cell to secrete cytokines; all other major cytokines being interloping, which helps in the proliferation of not only T cells, but B cells as well. So, other cytokines help the B cells to make the different classes of antibody. So, therefore, antigen taken up by the macrophage, which stimulates the T cell, help T cell to give cytokines or give T cell help upon T cell macrophage or antigen presenting cell interaction, which then interacts with the B cell, and the T cells interact with the B cells in order to produce antibodies and therefore, their function.

As opposed to stimulation, the immune system also has various regulatory pathways. The antigen by itself, and the antibody also, in a variety of complex processes, can feedback regulate this stimulation of the immune system in the event, that it gets stimulated very much and goes beyond what is required for an optimal immune response. So, these regulatory mechanisms include, what are called as, generation or differentiation of regulatory T cells, which will be dealt with in future classes. So, this is the summary of, of how the antigen is recognized and results in final T cell activation via the macrophage.

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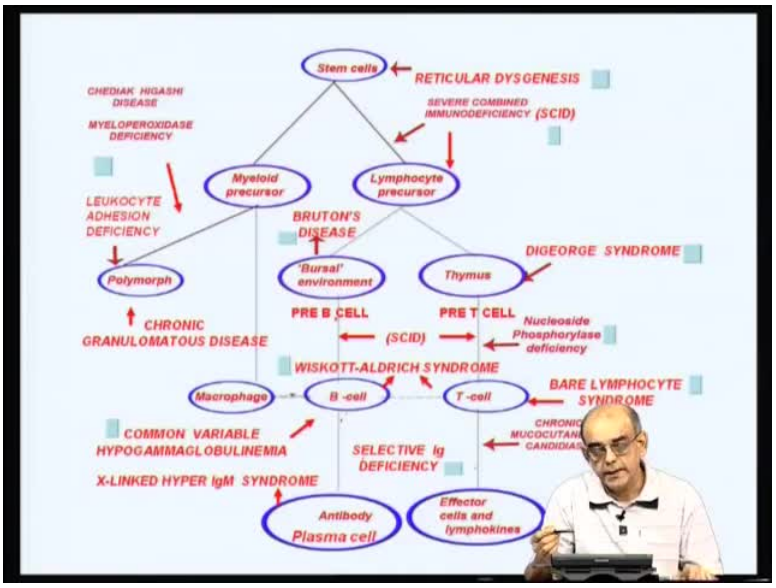

So, let us go on, then further, after having learnt about the size of the immune system, although there are so many complex things, that are performed in a very optimal way, the immune system has its own abnormalities or immuno-deficiencies or disorders. So, going into some of these disorders of the immune system or immune deficiencies, I have given to, I have given here some of the pathway by which these stem cells differentiate into the lymphoid and myeloid precursors, which we went to, in the, in the previous class and at what steps these various immuno-deficient diseases affect this differentiative pathway.

So, we all know now, that stem cells, via their precursors, can finally differentiate into T lymphocytes or plasma cells or from B cells. So, let us look at what are the diseases that affect stem cells.

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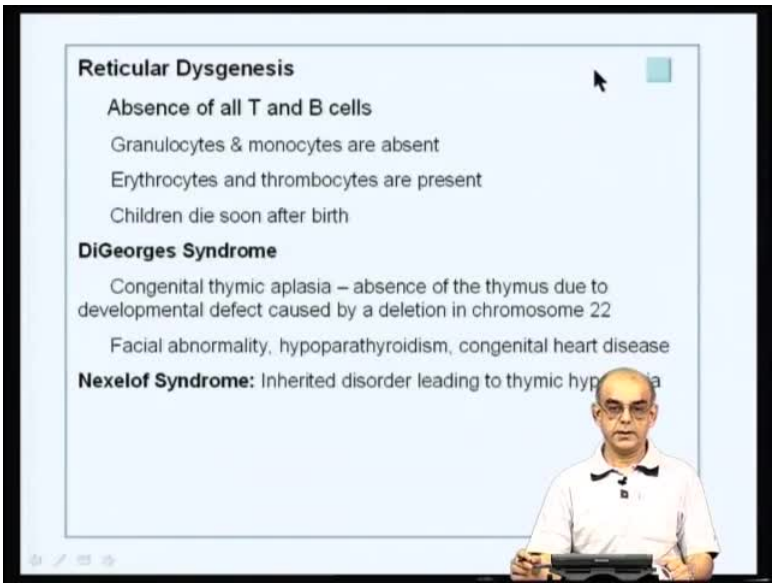

So, we have diseases that affect stem cells, called as reticular dysgenesis. Now, what is reticular dysgenesis? So, if you were to look at what reticular dysgenesis does is, it is characterized by an absence of all T and B cells; granulocytes and monocytes are absent, but erythrocytes and thrombocytes are present and children die soon after birth because of the absence of all T and B cells.

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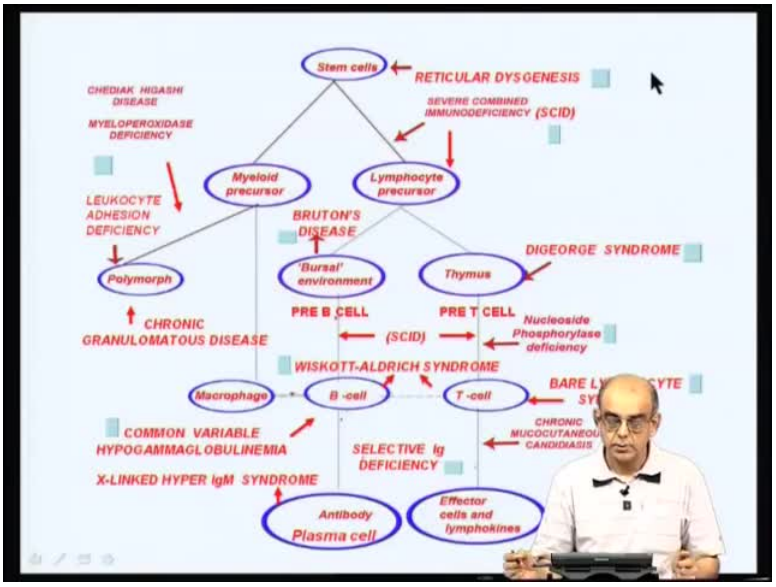

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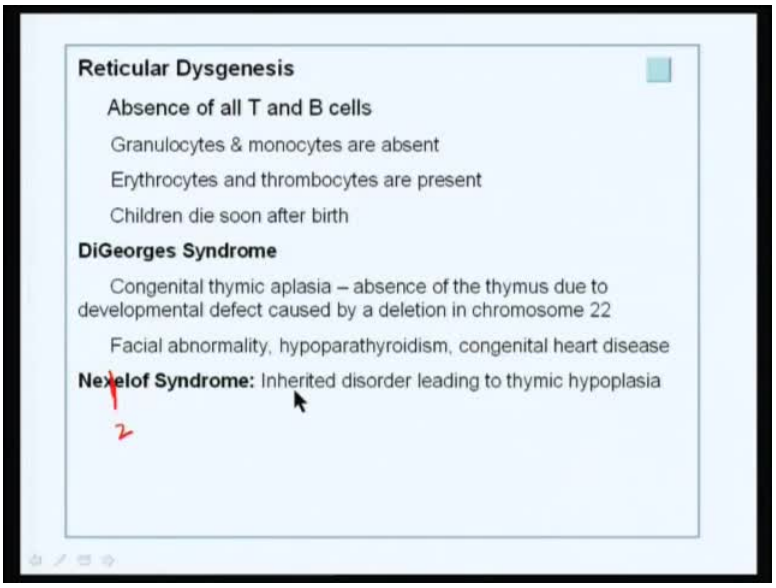

Now, going back to this particular diagram, we also have other diseases, which affect the primary lymphoid organs, like the thymus and the bursa or for that matter, the synthesis or differentiation of pre B cells and pre T cells. So, if you look at the thymus, there is this deficiency called as the DiGeorges syndrome. This DiGeorges syndrome is a congenital thymic aplasia; aplasia means the thymic is not fully functional, so we have the absence of the thymus. It is actually due to developmental defect, which is caused by a deletion in chromosome number 22. All these patients have abnormal facial abnormalities; they are hypoparathyroidism and congenital heart disease. Some of the photographs of these patients can be seen in popular text books, like Kuby immunology.

Then you have, what you call as, the Nezelof syndrome. I am, this, this has to be, this has to be z, nezelof syndrome, there is a typing error over here. So, Nezelof syndrome is characterised by also, hypoplasia, thymic hypoplasia. And while the DiGeorges syndrome is a developmental defect, this is an inherited disorder.

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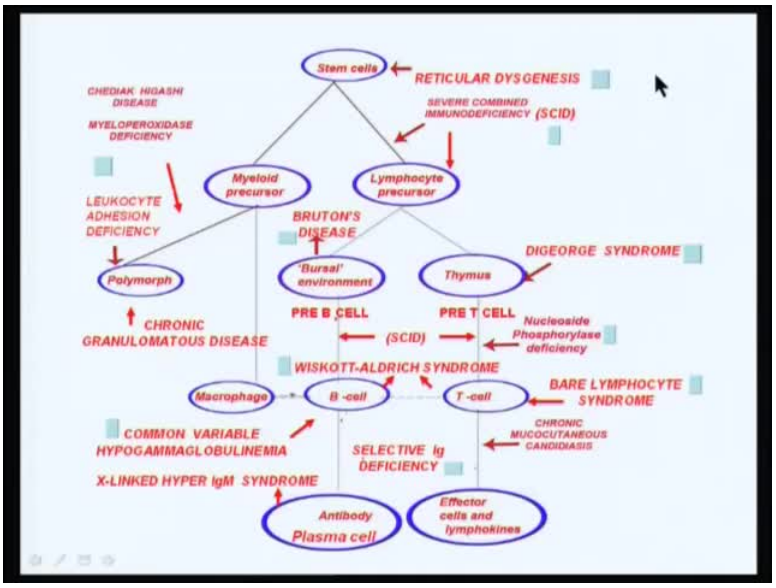

So, going back to this tree, let us see, what happens in the immuno-deficiency, that affects the bursal environment or for that matter, the pre B cell.

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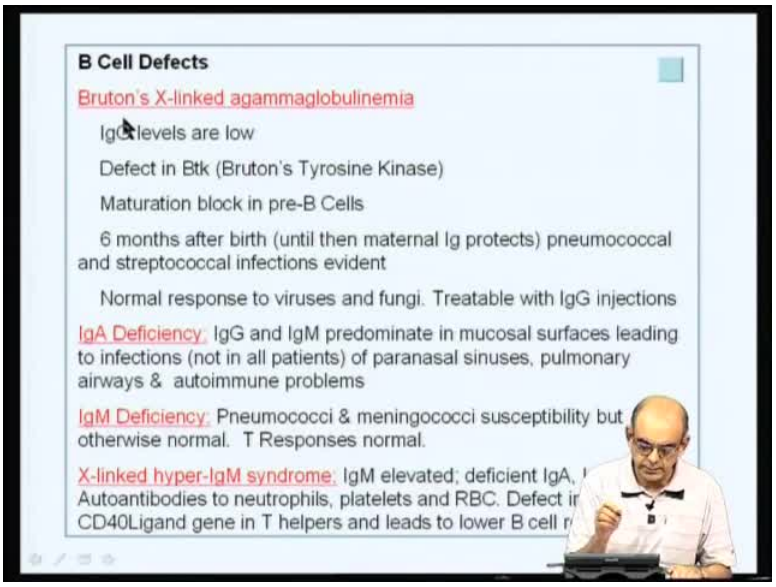

So, if you were to look at this, you have what is called as Bruton's X-linked agammaglobulinemia. So, here, the IgG levels are low and the defect stems from basically, in Btk or Bruton's Tyrosine Kinase. There is a maturation block in pre-B cells and patients 6 months after birth, they come down with pneumococcal and streptococcal infections, until then, the maternal IgG protects the new born. They have a normal

response to viruses and fungi, and to a certain extent, they are treatable with IgG infections.

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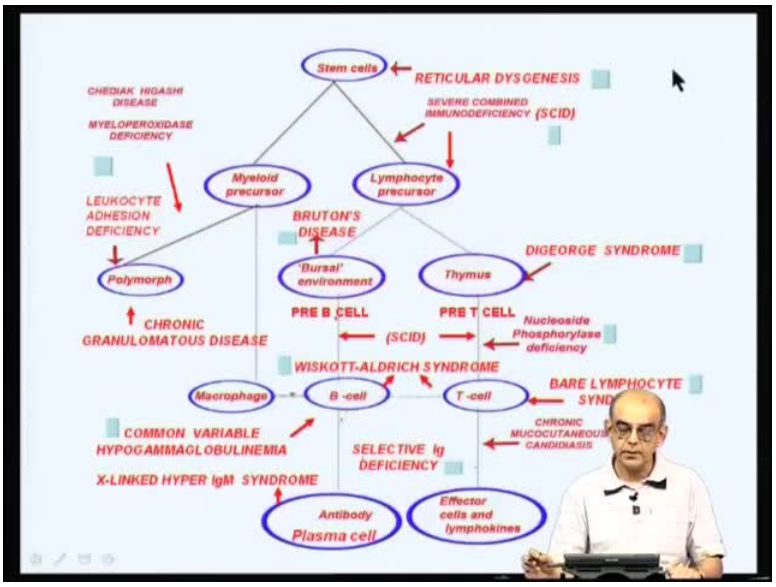

So, having done, looked at the immuno-deficiencies, that affect the stem cell thymus and the bursa environment, you have some of the very common immuno-deficiencies, such as severe immuno-deficiency syndrome or severe combined immuno-deficiency disease. So, in SCIDs, this is a family or a group of, group of immuno-deficiencies.

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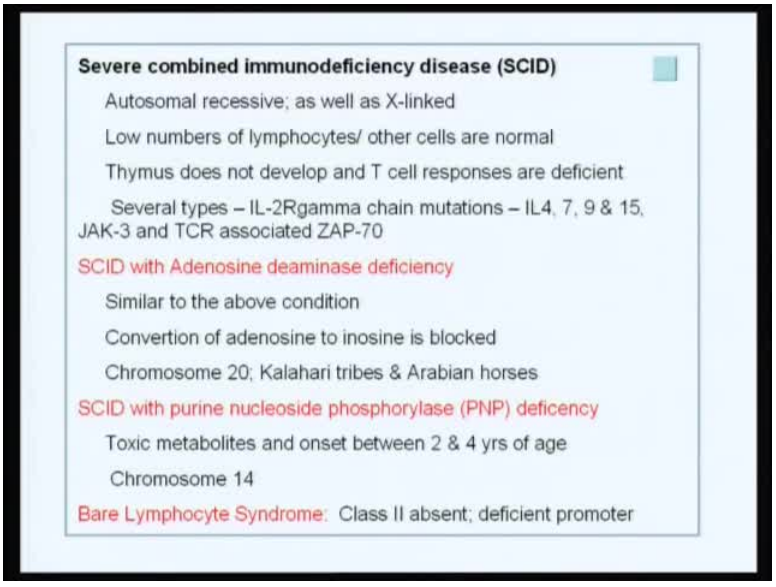

If you look at what are the different kinds of immuno-deficiencies in severe combined immuno-deficiency disease or SCIDs, they are usually autosomal recessive, some of them are X-linked, they are characterized by low numbers of lymphocytes, but other cells are normal, the thymus does not develop very well and T cell responses are deficient. There are several types of this, of these SCID diseases and one of this has to do with the absence or mutations in the IL-2 receptor gamma chain. The IL-2 receptor has 3 chains and the gamma chain is shared between other lymphokines, such as IL-4, IL-7, IL-9 and IL-15. So, therefore, this mutation in IL-2 receptor causes a deficiency in the function of IL-4, IL-7, IL-9 and 15. Therefore, leading to several abnormalities in immune cell function, which we will look at, when we see, how cytokines, all these various cytokines, such as IL-4 and IL-7 function. In addition to mutation in IL-2 receptor gamma chain, there is a SCID or diseases, where JAK-3 or Janus Kinase-3 is affected. In addition to that, the TCR associated protein called as ZAP-70 is also deficient in function, leading to a deficiency T cell function.

So, as you can see, there are several types of SCID diseases. Then, in addition to that, you have other things, called as SCID with adenosine deaminase deficiency or ADA deficiency. So, they, they also share, that the condition where the numbers of lymphocytes are abnormal, here mainly the conversion of adenosine to inosine is blocked. As a result, several toxic chemicals accumulate within the cell and therefore, lead to the non-development of the cells, which need the adenosine deaminase and that is found, the gene is found in chromosome 20. And many of these deficiencies are found in Kalahari tribes and in Arabian horses for some reason.

In addition to adenosine deaminase deficiency, you have SCID with purine nucleoside phosphorylase deficiency or PNP deficiency. There also, toxic metabolites accumulate and the onset occurs between 2 and 4 years of age, and this particular gene is found in chromosome 14.

So, another type of SCID or deficient lymphocyte function comes from, what you call as, the bare lymphocyte syndrome, which is due to the absence of class 2 or MHC molecule, the type-2 MHC molecule on the expressed, on the surface of various kinds of T cells and this is because of deficient promoter, that, that helps in this and the transcription of the gene.

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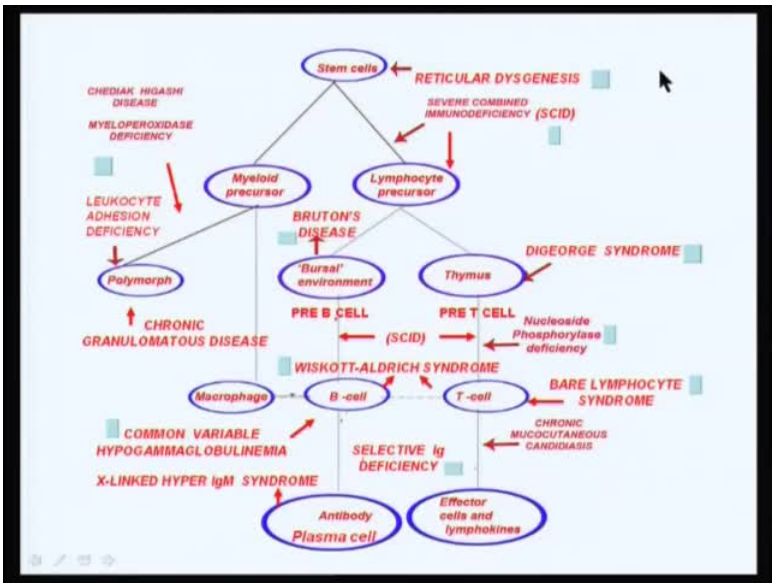

So, going back to the tree, you find, that we have covered the PNP deficiency, the Bare lymphocyte syndrome, which affects the T cells and of course, all these, the Bruton's disease. And then, let us look at other kinds of diseases, that affect the B cells, which is called as the Wiskott-Aldrich syndrome, and let us see, what, what sort of deficiencies affect the B cells or the B cell pathway.

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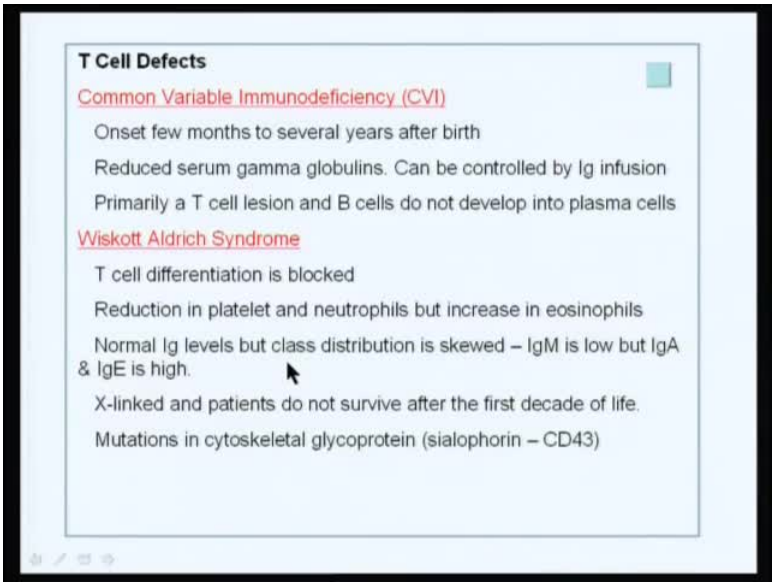

So, here you find that the Wiskott-Aldrich syndrome is actually, the blockage in T cell differentiation. It results in reduction in platelet and neutrophils, but there is an increase

in eosinophils and normal IgG levels predominate, but the class distribution of IgGs is skewed; you will come to know more about the various classes and subclasses of immunoglobulins, suffice it to say, that IgM is low, but IgA and IgE levels are higher. This is a X-linked disorder and patients do not survive after the first decade of life. The mutations that affect the syndrome or results in the syndrome, is basically in a cytoskeletal glycoprotein, which is called as sialophorin-CD42. Now, CD42 stands for cluster of differentiation.

Now, the other T cell defect is also called as the common variable immuno-deficiency, which is also called as CVI. It has an onset few months to several years after birth and it is characterised by reduced serum gamma globulins. Because of reduced T cell help, it can be controlled by immunoglobulin IgG diffusion, it is primarily a T cell lesion and B cells do not develop into plasma cells because of T cell lesions.

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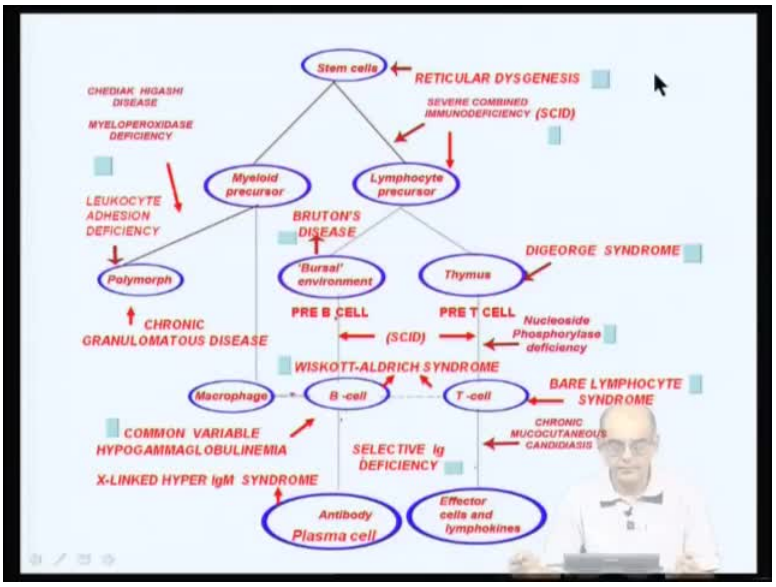

So, going back to the tree, we have covered the Wiskott-Aldrich syndrome, as well as, the other diseases. Let us come to the other diseases, which affect the B cells, like the X-linked hyper IgM syndrome or the common variable hypogammaglobulinemia. Now, here, I think I have done a mistake in the, let us go back and see what happens to B cell X-linked hyper IgM syndrome, which was not expanded upon by a link. The hyper IgM syndrome results in more of IgM in some of these diseases; this is also X-linked disorder.

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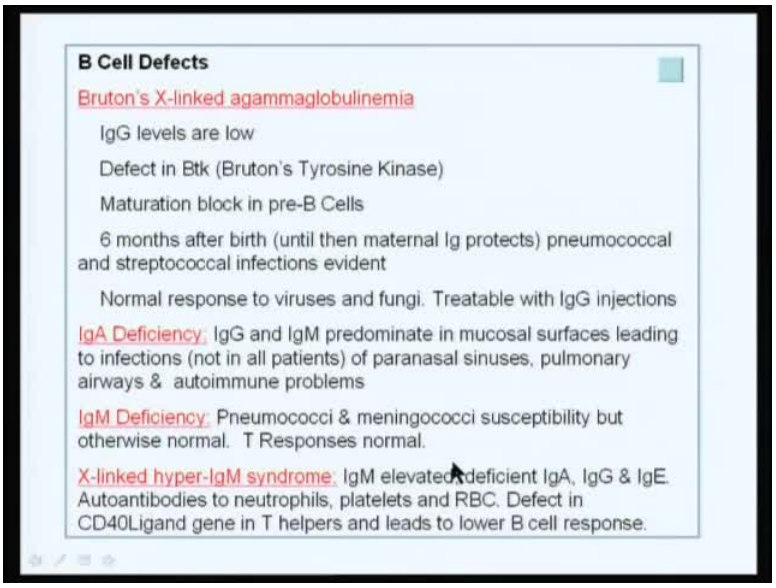

So, let us go into some of things, yes there you are, this is the B cell defects, which is the Brutons' X-linked hypogammaglobulinemia, which I described to you just a little earlier. You have syndromes where you have selective IgA deficiency or selective IgM deficiency, where you have IgG and IgM predominate in mucosal surfaces because IgA, as I told you, predominates in when the malt tissue or mucosa-associated lymphoid tissues, this actually predomination of IgG and IgM in some patients, leads to infection of paranasal sinuses, the pulmonary airways and leads to say, autoimmune problems. These problems are more predominant in all patients of this syndrome.

Now, going onto IgM deficiencies, these patients are very much susceptible to pneumococci and meningococci infections, but otherwise they are normal and the T cells are normal in this particular deficiency.

Now, as I told you, the X-linked hyper-IgM syndrome, where the IgM is elevated, but the other sub classes IgA IgG and IgE are deficient, actually leads to autoantibodies to neutrophils, platelets, as well as erythrocytes. The defect is actually, due to the CD40Ligand gene in T helper cells and leads to lower B cell response.

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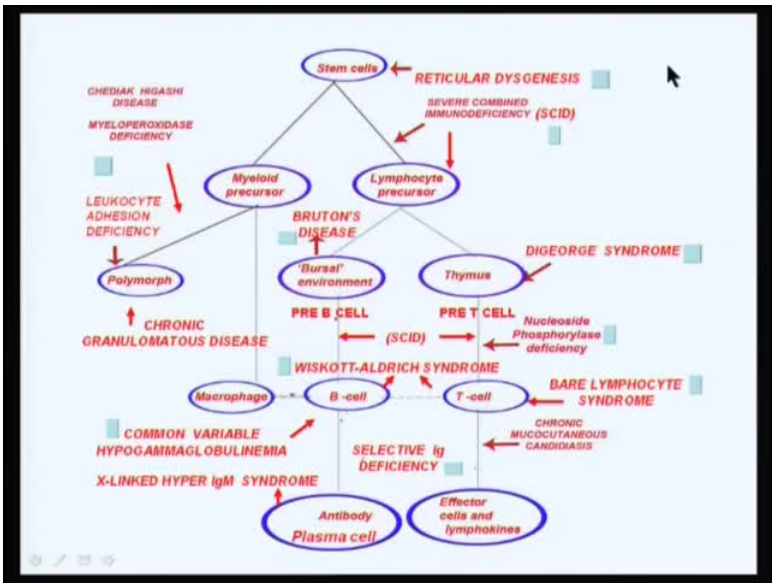

So, going back to the tree, now having covered the lymphocyte lineage, we will try, we will see, what are the diseases that are associated with the myeloid pathway. So, the myeloid pathway is affected by disease, such as the Chediak Higashi disease, leukocyte adhesion deficiency, which affects the neutrophils or the polymorphs and also the chronic granulomatous disease.

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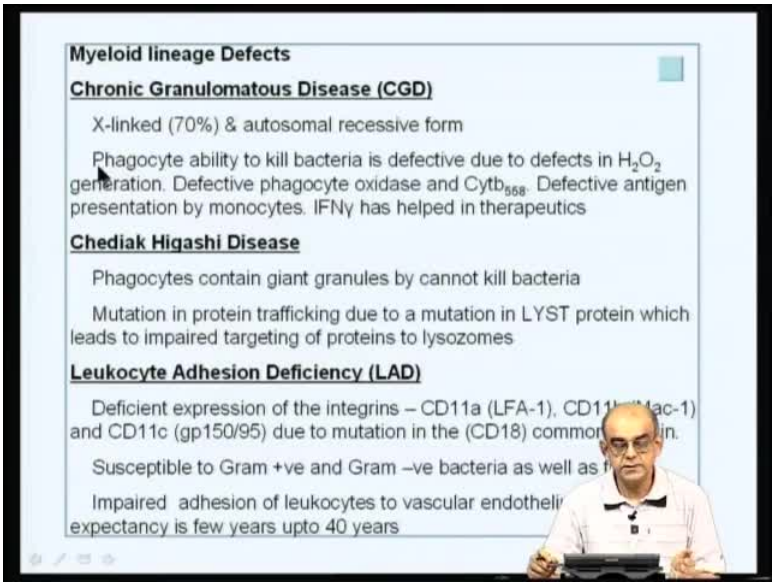

So, going to this, these deficiency diseases, the myeloid lineage defects, such as the chronic granulomatous disease or the CGD, which is, which is an excellent disorder in

70 percent of the patients, while some patients also have the autosomal recessive form. The phagocyte ability to kill bacteria in these patients is very much defective to, due to the defects in hydrogen peroxide generation, because one of the mechanisms by which phagocyte kills or lyse bacteria is by the production of hydrogen peroxide, as well as, super-oxide radicals. So, they are defective in hydrogen peroxide generation due to the presence of a defective phagocyte oxidase enzyme, as well as, cytochrome B558, which are involved in the production of these various radicals. This of course, as I told you in the earlier classes, is part of the innate immunity.

Now, this also leads to a coincident defective antigen presentation by the monocytes from these patients. Now, in such patients, the therapeutics involving the infusion of gamma interferon has helped in the activation of these phagocytes. So, perhaps, some of these patients have benefited from on-going studies, wherein you can actually use gamma interferon for therapy.

In addition to CGD, you have, what is called as the Chediak Higashi disease, which affects the myeloid lineage, where the phagocytes contain giant granules. You know, granules also contain various chemical enzymes, proteins that are helpful in lysing bacteria. These granules are bigger in size, they are giant in size, but they cannot kill bacteria. So, the ability to kill bacteria in phagocytes from these patients is very much compromised. This is actually, due to a mutation in protein trafficking, due to a mutation in what a protein called as LYST. Now, the function of this protein is actually to target various kinds of proteins to lysosomes for mediating degradation of proteins and you know degradation of proteins is involved in antigen presentation.

Now, going onto leukocyte adhesion deficiency or lad, they have deficient expression of integrins, are addition molecules, which we will come to next lecture or perhaps in the same lecture. Now, these integrins are expressed on the surface of lymphocytes, as well as other kinds of cells, which help in the mediation of cell surface adhesion. You might remember some of the, one slide that I showed you in the first class, where I showed you, how the cells migrate out from the capillaries through the vascular endothelial cell junctions because they have, they are undergoing chemotaxis in response to a chemotactic factor, that is released from the cell wall of bacterially infected, bacterially infected sites.

So, these integrals, they play a very important role in these adhesive, adhesive roles or adhesive reactions that are taking place between cells, between the cells themselves, as well as between the cells and the capillaries, vascular endothelial cells in the capillaries. So, these, some of these integral molecules or proteins are called as CD11a or LFA-1, which was, it was previously known as lymphocyte function associated 1 molecule because this is a LFA-1, because there are other LFAs, such as LFA 2, 3 and so on. So, the CD11b is another molecule, that is affected in this particular deficiency, it is also called as Mac-1 antigen and the CD11c is also called gp 150 or gp 95.

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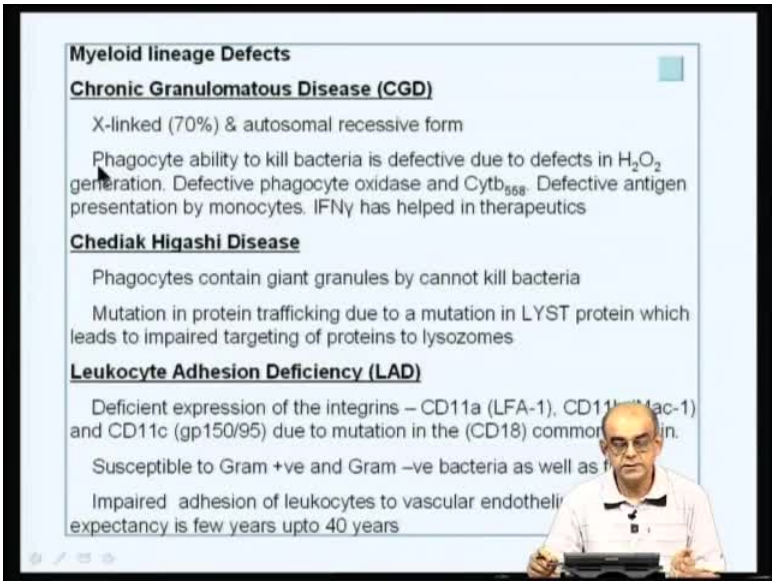

Now, this, all these expressions are actually, due to the deficiency in the expression of these integrins or due to the mutation in the CD18 or the common beta chain that they share between themselves. In all these lymphocyte interactions and in immune responses, you will come across a variety of molecules especially cytokines, but that share various kinds of protein subunits. So, they are called as alpha, beta, gamma. So, you will, certain receptors sharing the gamma chain, as you saw in the case of IL-2 receptor and in certain other cases like these integrals, share a different kind of protein subunits. So, different kinds of integrals share common subunits, so if the common subunit is absent, the expression on the cell surface of these integrals are blocked. And therefore, the functions, that are associated with these integrin molecules where adhesion reactions are also blocked, so therefore, it, this imparted adhesion of leukocytes, neutrophils, so on and so forth, to the vascular endothelial cells is blocked and leaves actually, to the

susceptibility to gram positive and gram negative bacteria, as well as fungi. So, this deficiency actually leads to a life expectancy of a lowered nature, like just a few years or up to 40 years.

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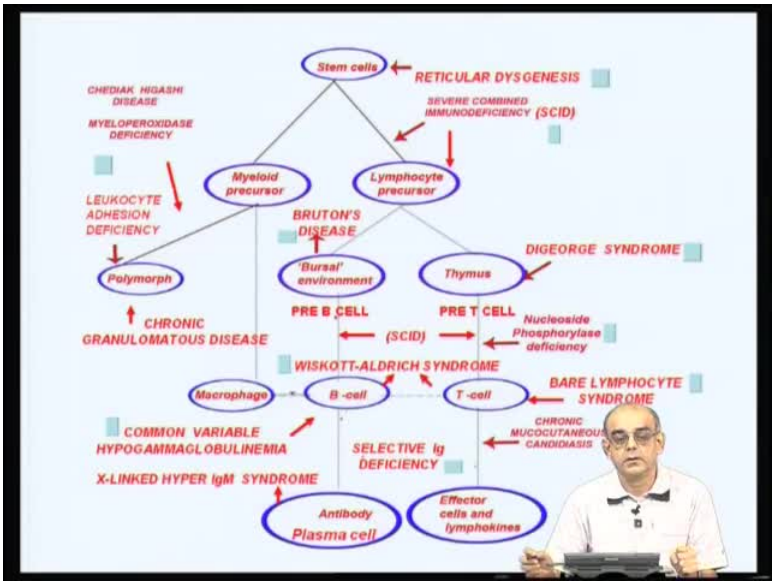

So, you have now seen all the different kinds of immuno-deficiencies that are associated with this lymphoid lineage differentiation pathway. We have covered some of the main immuno-deficiencies, but there are other kinds of deficiencies, that you can see in popular textbooks, some of them include Ataxia telangiectasia, which is due to the deficient kinases, that are associated with this disease and there are several other diseases, which we will see in various other popular textbooks, but suffice to say, that these are the main immuno-deficient diseases, that can be covered to see, in what steps, that they, that they affect in the pathway, which is shown by these arrows.

So, so far, we have now covered up to now, how the Hematopoietic stem cell differentiated into these various kinds of T lymphocyte to B lymphocytes, as well as the monocytes or the differentiated macrophages and the associated immuno-deficiencies. Then, we have covered some experiments, that demonstrated at that time, how a Hematopoietic stem cell could give rise to all the different kinds of cells in the immune system using the, or referring to the Tim and Markeloff experiment, using irradiated recipient mice. Then, we went into, how experiment showed the presence of the B cells, the importance of the bursa and the bone marrow, as well as, the importance of the

thymus derived T cells, and the experiments, that were done to show, how the T cells were shown not to produce or not being involved in antibody production, but the B cell was more important antibody production. And the more important contribution by the presence of the function of macrophages and the consequent antigen presentation and the experiment, that was done with pulsed, PPD pulsed macrophages derived from guinea pigs, and how the T cell activation occurs?

So, having seen all these things about the cells of the immune system, now we see how these cells are actually organised within the body? Now, various kinds of, parallel kinds of comparison can be made between the immune system and the immune or the defensive forces of a particular nation. You know, just like you say, immune function which, which recognizes self from non-self and also the defence forces have to recognize the difference between the national citizens and the aggressor. Similarly, the immune cells also differentiates or is able to make out the presence of the aggressor or like danger, distinguish danger from non-danger, as well as, what are called as pathogen recognition molecules, which we will come to in the lecture on innate immunity. So, how are these cells organised?

Now, a comparison can be made to a nation, which is at war. When a nation is at war, there have to be mechanisms by which they transport troops from where they are normally present to the active war zone, which need not necessarily be at a zone that is normally occupying the forces at peace time. So, all these troops are organized by organizing various kinds of railway procedures, where the trains are dedicated to transport troops to the border.

Similarly, in the immune system, the antigen or the bacterial infection can come in any place in the body and the lymphocytes that are circulating in the blood have to come out from the blood vessel, in the place of aggression or in the place of the lesion. So, how are these done? And what sort of, what sort of mechanisms are employed by the immune system in order to orchestrate these kind of immune responses and for that, we go into our next series of lectures, which includes the organs of the immune system.

So, as I told you in the earlier class, there are different organs of the immune system, classified as primary lymphoid organs, which includes the thymus and the bone marrow and you have, what are called as, secondary lymphoid organs because they play a

secondary function, and are not basically involved in the differentiation into T lymphocytes from precursors or the differentiation of B cells from B cell precursors. Now, these secondary lymphoid organs are the ones that play a very important role in detecting the antigen. So, the mature lymphocytes, all grow into these secondary lymphoid organs and it is at those locations, that the antigen is actually detected; so, what are these secondary lymphoid organs?

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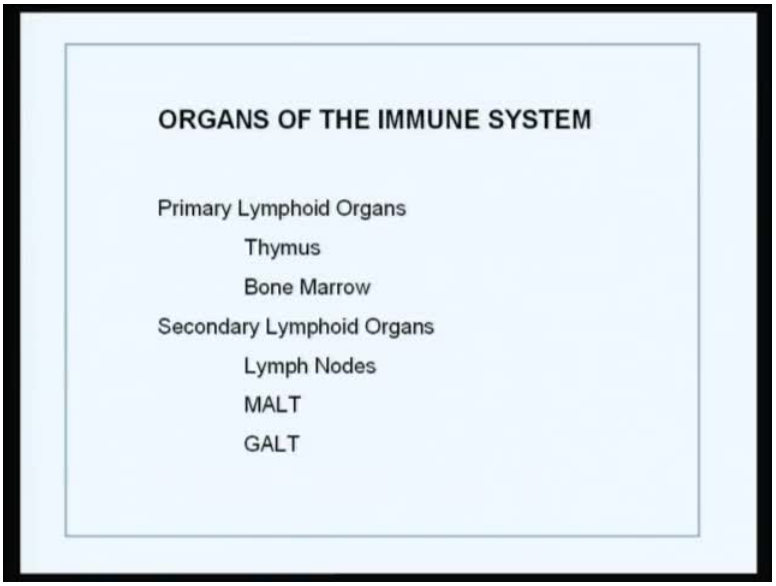

So, you will see, that these secondary lymphoid organs are described in your books as lymph nodes or the mucosal associated lymphoid tissue, which I alluded to in the previous class, which is main, mainly connected with the mucosal surfaces, as well as, certain lymph nodes, that are associated with the gut, this is called as the gut associated lymphoid tissue. Remember, even the gut is, the gut is, actually the gut is one of the very important locations, which is constantly bombarded with microorganisms or pathogens or all kinds of bacteria and viruses because the food that we eat, all, always contains some sort of bacteria or the other, whether it is pathogenic or non-pathogenic depends upon, how they get across the intestine. So, the gut is constantly dealing or bombarded with these kinds of bacteria, that is derived from the food that we eat and yet the body is not being infected all the time. So, the gut associated lymphoid tissue has a very important and a very unique way of blocking this, these kinds of pathogens. So, looking at all these secondary lymphoid organs, we need to, we need to learn, how these secondary lymphoid organs actually function.

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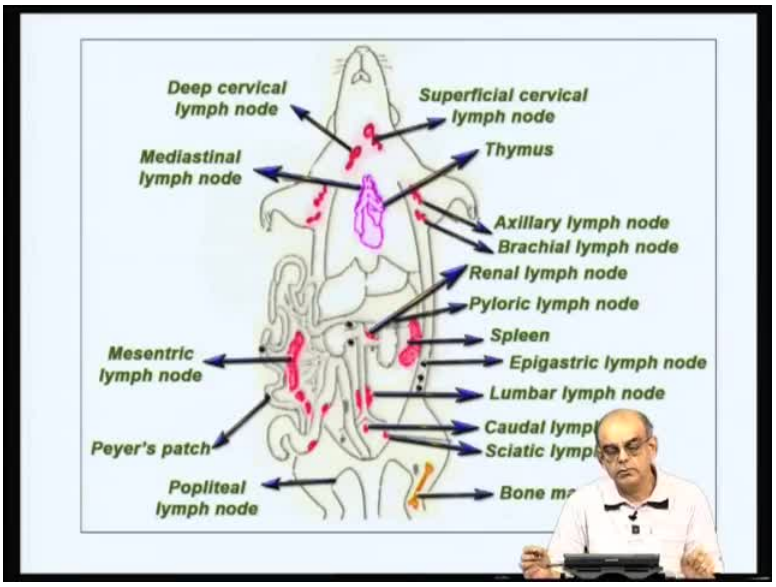

Now, when you look at how the antigen infection or the pathogenic infection occurs, we need to look at, what sort of these different kinds of lymph nodes that are present? If you were to open a mouse, you will see that there are so many, so many of these lymphoids, lymph nodes are secondary lymphoid organs. So, as you will see here, these are all called by different names, suffice it to show you, that a particular mammalian body contains an array of different aggregation of different lymphoid tissues or lymph nodes, that are found in different places in the body, like for example, these mesenteric lymph nodes are just found attached to the peritoneal of the intestine and then, you have of course, the Peyer's patches within the intestine itself in order to encounter the incoming bacteria from the food that we eat. And then, you have all these different kinds of lymph nodes, that are found under the arm and under the neck and so on and so forth, which is also present even in the humans.

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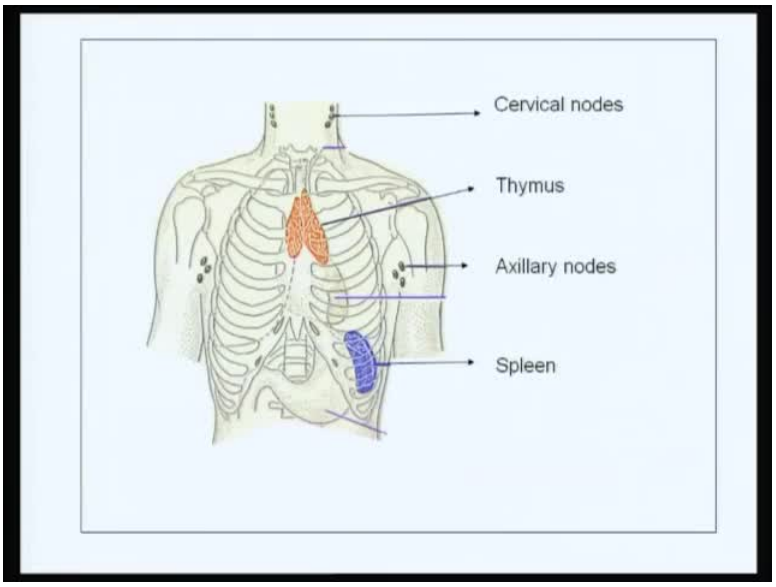

So, just to, just to see, that under the armpit you have axillary nodes and then you have the thymus just below the sternum, then you have the cervical nodes, the thymus being a primary organ and all these different kinds of nodes being secondary lymphoid organs and one of the major secondary lymphoid organ, is the spleen. So, in order to understand the function of these lymph nodes, which are very much distributed all over our bodies, we need to understand the structure of the lymph node.

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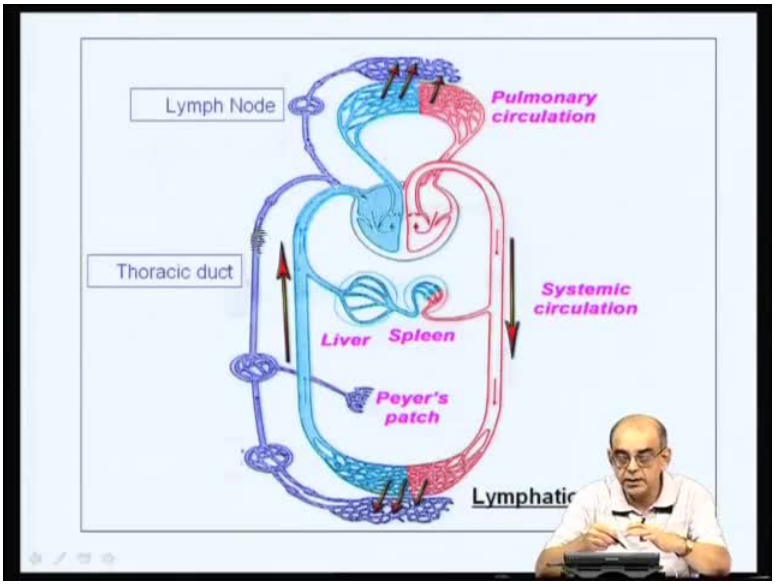

Although we would not like to go into the anatomical structure of these secondary lymphoid organs, which is, which we will find very well described in various kinds of textbooks, the effort, that we will put in these classes is to understand, basically what these secondary lymphoid organs do, in respect or with respect to how the antigen is perceived?

Now, to understand the lymph node, the role of the lymph node in immune responses, we need to recognize, that in addition to the systemic circulation or the circulation of your blood within the body through the heart, basically to say, you send in oxygenated blood from these ventricles and then, get this oxygenated blood, losing its oxygen or giving up its oxygen to various tissue cells and then, getting the deoxygenated or the oxygen-depleted blood back into the heart.

So, what happens in these situations is that in addition to this systemic circulation, you may also have to recognize the presence of, what is called as, the lymphatic circulation because the lymphatic circulation is a circulation that involves the fluid that drains the various tissues spaces. For example, if you were to have a kind of redness in your skin and a small sort of a patch, that develops an irritation that develops over there, there is oedema of fluid. Now, where is all this fluid coming from? And how does it come from and how does it and where does it go? So, during such situations or even under normal situations, the fluid that drains the various kinds of tissue masses or tissue cells is actually being drained into certain kind of paths and this path, when they all accumulate and make a sufficient kind of a fluid, is called as the flows in a duct, called as the thoracic duct; so, this is the lymphatic circulation. So, the thoracic duct contains all these drained tissue fluids these tissue fluids, if there is an infection, will carry that infectious organism or the toxin that comes from it and this is actually circulated by these various kind of lymph nodes. Now, these lymph nodes also have some property of contraction, so these contractions actually, let the fluid go from one lymph node to another and finally, it empties out into the heart via these veins.

So, then of course, the heart takes care of how the blood is dealt, oxygen depleted blood is pumped into the lung and then, you have the exchange of oxygen into the haemoglobin, and then it comes back into the heart, where the aorta then is taking the oxygen and then completes oxygenated blood and completes this circulation. So, all the tissue, all the tissue spaces have this fluid, which collect this antigen and drains them into

this thoracic duct. So, that is how, these antigens come into the circulation in addition to being in the systemic circulation.

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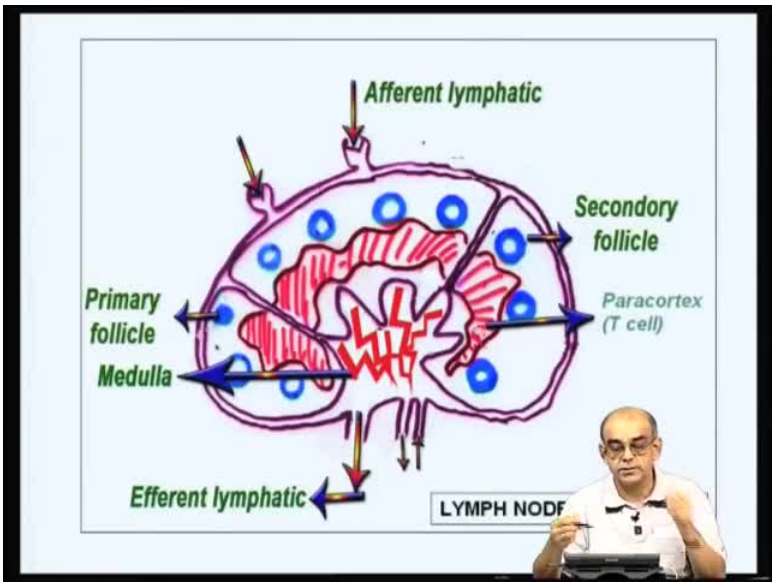

Now, if you were to look at the structure of a lymph node, you will see that it contains a kind of a capsular structure, just like way many other secondary lymphoid organs, so it has a capsule on the outside and inside we call it as the medulla. So, the main thing, that we need to recognize before we end this class, since the time is becoming short, this capsule has got entrance points like you see over here, either vessels that are coming from the thoracic duct or from various other kinds of spaces, these are called as the afferent lymphatic ducts. The afferent lymphatic duct takes the lymphatic fluid and let it come and drain into this lymph node. So, the actual lymphatic duct or the lymphatic fluid, that contains the antigen during infection of some tissue space, contains the antigen and comes within and into the lymph node in this fashion.

Now, within the, within the lymph node, you have various kinds of structures and we are not going to the actual details of anatomical structure, just with respect to the immunological aspects. You have B cell areas, where B cells have congregated and then of course, you have the T cell areas, or what you call as, the Paracortex, which contains T cells and your antigen presenting cells represent in this entire lymph node. So, all the 3 cell types are available in this organized kind of tissue, where you can have them interacting with each other and giving rise to the optimal amounts of lymphokine in

order to encounter and react to the antigen, that is being drained in from the afferent lymphatics.

So, there are 2 kinds of structures over here, what is called as the primary follicle and a secondary follicle. A primary follicle contains a follicle, which is ready to encounter the antigen and contains B cells that are mature, whereas a secondary, a secondary follicle contains B cells, that have got activated by virtue of interacting with T cell and seeing the antigen and contains, what is called as the germinal centre along with the dividing lymphocytes, that surround it.

So, we will leave it at this point and tell you more about the lymph, the lymph node structure in the next class. So, we will, we will end this lecture and summarize the things that we have done or learnt today, was to look at, how the antigen presenting cells present antigen and this presentation of antigens are detected by T cell activation and how all these various types of cells congregate in certain structures, called as the lymph nodes, and we will get into the role of secondary lymphoid organs in the next class.

Thank you very much.