Essentials in Immunology
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Module No. # 22
Evolution of Immunology
Lecture No. # 40
Evolution of the Immune system

Hello and welcome to this lecture on the evolution of immunology. Considering the evolution of immunology, needs to look at various aspects about molecules as well as cells of the immune system that have first appeared in the animal kingdom or so to say even the plant kingdom.

We all know that animals even for that matter, insects have some sort of primitive way by which they respond to external pathogens or infections. In fact, even plants can be infected by viruses and plants have their own way of responding to various kinds of fungal infections.

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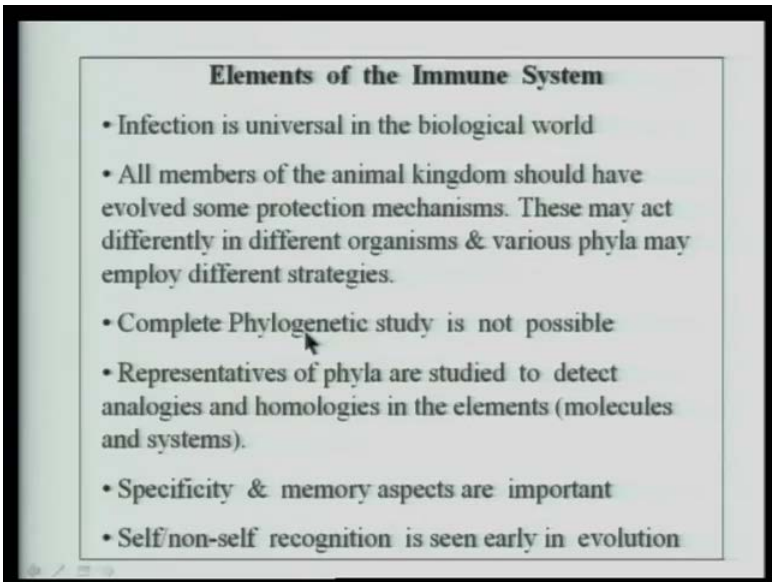

So, if you look at the animal kingdom, we realize that the infection is universal in the biological world and all members of the animal kingdom must have therefore evolved some sort of protection mechanisms in order to stay alive.

Now, these protection mechanisms may have different ways and they may work differently in different organisms and various phyla. And different strategies may be employed for inactivating the pathogen in question. It is obvious that during these evolutionary studies a complete phylogenic study of all the members to see what sort of mechanisms or what sort of molecules immune molecules is involved is practically impossible. Therefore, representatives of phyla are studied in order to detect analogies and homologies in the elements of the immune system that may be operating in that particular organism or animal.

Obviously, we all know that the immune system has got a variety of important elements and aspects to it and some of them are the specificity of the immune system or the specificity of the response to the incoming pathogen or incoming antigen. As well as the establishment of memory are very important aspects to say that the immune system is competitive, as we know it in the memmingen world.

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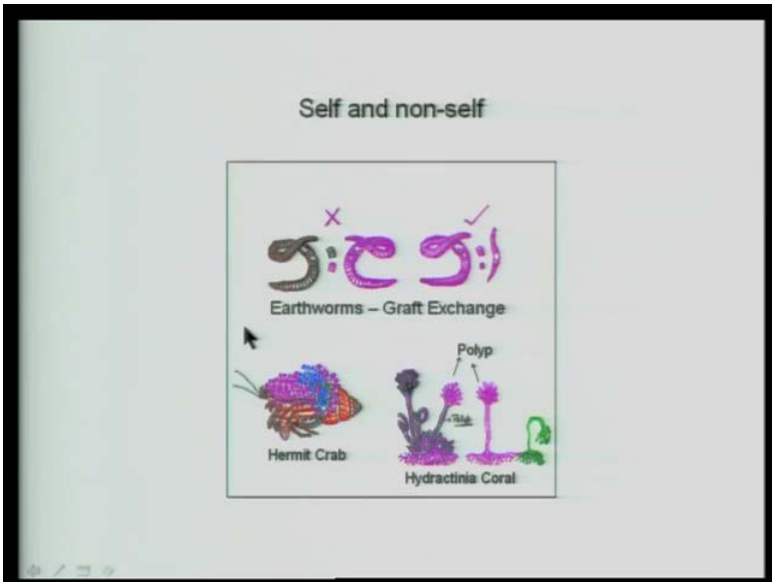

Needless to say the concept of self and non-self-recognition is also very important as in… and is in fact seen very early on in evolution. As mentioned in previous classes, the distinction between self and non-self is very evident in earthworms, where a graft exchange gives results, similar to the graft rejection reaction seen in higher mammals.

For example, if we take a piece of skin from one locality on the earthworm, one locality where earthworms are found and graft it on to earthworms obtained from a different

locality, there ensues a kind of a rejection reaction as opposed to the acceptance of that skin graft within earthworms obtained from the same locality.

Other examples that were described earlier was this hermit crab and corals, where what we realize in all these studies is that as organisms or cells became multicellular, there came a need to distinguish between self-cells belonging to one colony. And if cells from another colony is actually invading it, if this distinction was not there, then different cells or different organisms could come and invade different kinds of multicellular organisms and the identity of each one of these different kinds of multicellular colonies would be lost. And therefore, we see that this sort of self, non self-recognition is very evident even in polyps in corals.

These are reactions that abound in the animal kingdom, as you will see the distinction between self and non-self actually arose very early on in the animal kingdom. And this distinction between self and non-self actually is due to the evolution of molecules or protein molecules or receptors that are expressed on the surface of cells that are in question.

So, if you go on into some more aspects of the immune system that we have studied, we find that there are two parts to it, that is the innate or the non-specific immunity and they acquired or the specific immunity.

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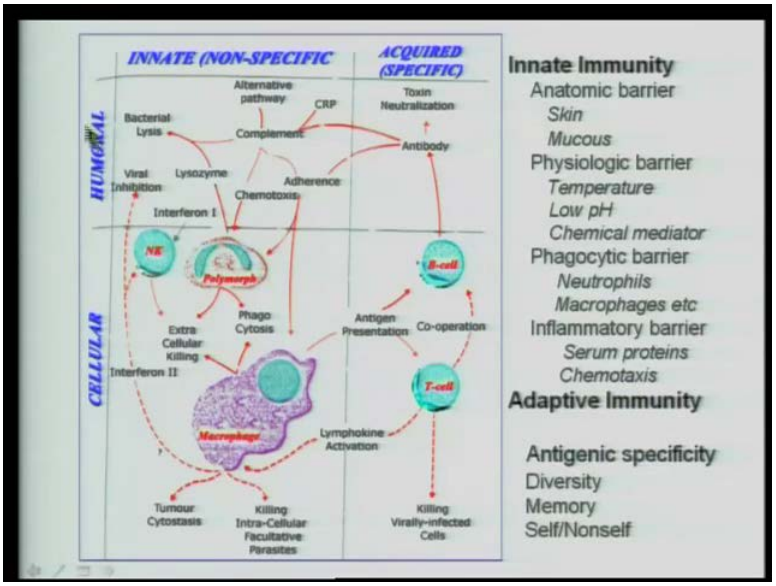

Innate being those that are obtained or got when you are born and therefore, involves a variety of strategies or mechanisms and cells involving macrophages, which was first discovered and thought to provide a protection against a thorn or those cells that surrounded the thorn and try to remove the thorn from the star fish larva.

So, you see there are a variety of molecules that are involved in this innate immune system, such as complement molecules, lysozyme and various kinds of cytokines and various kinds of molecules that cause chemotaxis of these macrophages. And considering the cellular immune system, you also have a humoral immune system and therefore, this humoral immune system consisting of various kinds of molecules, therefore when one looks at evolution, you have to look at a variety of aspects as to what sorts of molecules or cells began very early on in evolution.

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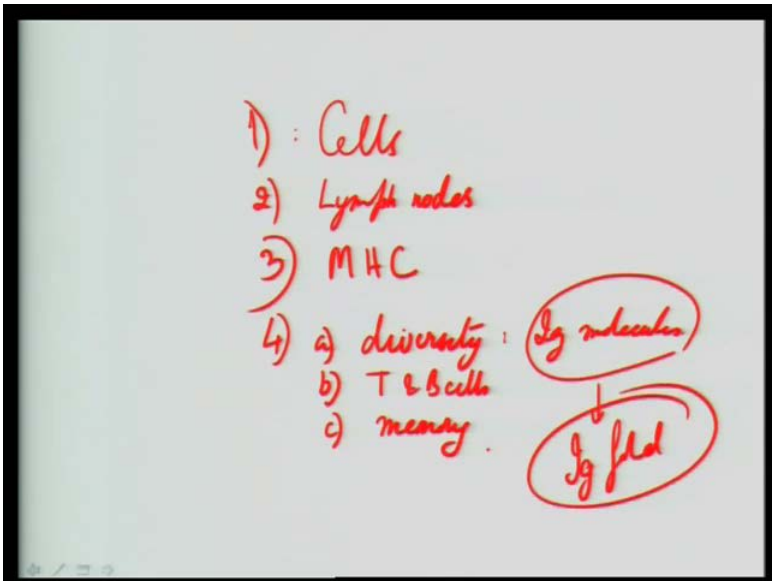

So, just to recapitulate a few things, what are those aspects that we need to consider when we study evolution. First of all you have to look at whether the immune system has got cells as we know it in the mammalian system, cells and organs of the immune system. So, what are these organs, are of course the lymph nodes and various kinds of other secondary and primary lymphoid organs and then of course you have the molecules that are involved in immune recognition, such as the MHC itself, a major histocompatibility complex, which is the basis for all immune reactions in mammalian

systems and also for that matter the rejection of skin grafts and the recognition between self and non-self.

So, the question is whether MHC is evident early on in evolution. Then of course, what is adaptive immunity? Adaptive immunity is characterized by the presence of a variety of aspects such as diversity, which means to say or indicates that the multitude of pathogenic infections or multitude of antigens that the immune system gets exposed to in mammalian organisms always find a befitting response, where you get molecules that are equally diverse that can combined with these incoming antigens or pathogens, such as the immunoglobulin molecules. This diversity of course is brought about by a rearranging gene system, where you have different genes recombining to give you the variable segment of the immunoglobulin protein molecule.

Just to cut a few things short and to make things of this lecture interesting, it is very surprising or very interesting to find that such recombining antigens, where you have diversity is found also very early on in evolution. So, this diversification is not something very unique to us or higher mammals, it is present even in lower organisms such as you have drosophila, you have jawless vertebrates, agnatha. So, you see all these this sort diversification has actually evolved very early on in evolution.

Then of course, apart from the diversity, you have the participation of what are called as T and B cells and most importantly, the establishment of memory, which gives an infected person the ability to respond to primary infections in a more rapid manner. A familiar example being those who were recovered from small pox never ever come down with small pox and that is because of the establishment of what are called as memory cells.

So, with regard to all these aspects, various studies have been done to find out if the cells that we know of in the in the mammalian immune system is present early on in evolution and the presence or absence of different kinds of lymph nodes, the presence or absence of MHC or MHC like molecules and of course, the presence of immunoglobulin molecules.

One of the important things about immunoglobulin molecules is that it has a very characteristic domain or what you called as the immunoglobulin fold, which is a familiar

feature that you must have come across in a variety of subjects or topics that you have dealt with in the immune system.

A vast variety of molecules both within and outside of the immune system belong to what you call as the immunoglobulin super family of molecules. So, if you have the immunoglobulin folds in these early molecules, then they are actually related to the immunoglobulin molecule.

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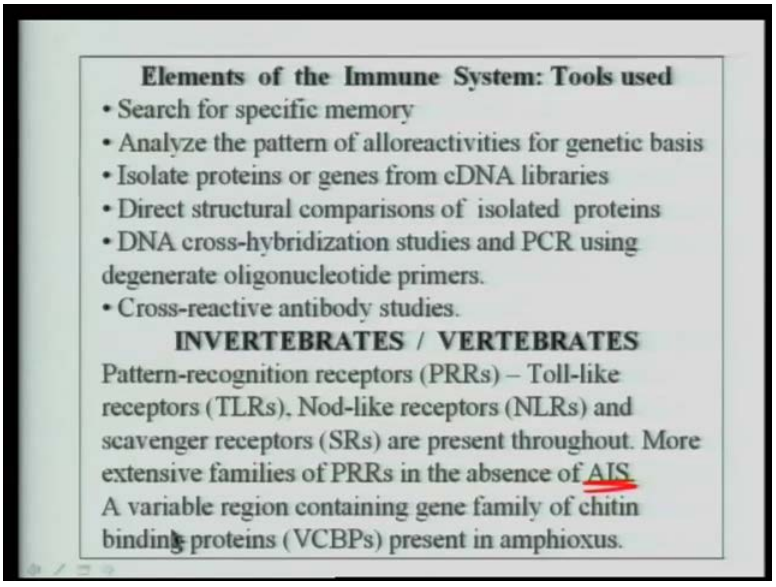

So, let us see how and what sort of studies were done in order to show some of these interesting aspects about evolution of the immune system. So, when you look at the immune system, one searches for specific memory, whether memory is present or absent and where actually memory is present in what sort of organisms, because that is the hallmark of a very efficient adaptive immune system.

Then you analyze the pattern of alloreactivities for genetic basis. Alloreactivities may occur without any relationship to genetic criteria, in other words, a major histocompatibility complex was discovered because there was as seen in those studies that there was genetic bases to these graft rejection reactions.

One sees that in primitive organisms like sponges and others, you do find graft rejection, but those graft rejection mechanisms need not be centered around molecules that have a genetic basis. Then of course, one has evidence of proteins that are participating in some

sort of immune system in the early organisms or early evolution, you look at direct structural comparisons of proteins that you isolate from these organisms.

Then you have of course cross hybridization studies using different kinds of DNA probes and using PCR degenerate oligonucleotide primers in order to find out whether there are any sequences that are similar in the DNA or genome of these organisms compared to what we have in higher mammals. And of course, one of the most important study is to take the antibodies that have been raised to various kinds of molecules in the higher mammalian immune system and see whether you can have cross reactivity to antigens that exist early on in evolution or animals in insects and so on and so forth.

So, covering both invertebrates and vertebrates one sees that the early on in evolution, the presence of an adaptive immune system or AIS, you will see this abbreviation coming up in many of the text books. These AIS is not present or not very efficient in the early organisms, this has been replaced by a kind of diversification or a more extensive innate immune system, which involves what are called as pathogen recognition receptors. So, this all of you have heard about and these pattern recognition receptors are those receptors that actually recognize the presence of a pattern or kind of a pattern in incoming pathogenic molecules or pathogens.

A familiar example is the toll like receptors, this toll molecule or the toll protein was first discovered in drosophila, while trying to study the embryogenesis in drosophila, later on of course, they found that this molecule has similar or homologous molecules, they called it toll like receptors, which are present in higher organisms. And there are various kinds of TLR's from 1 to 10, which play a very important role in innate immunity towards incoming molecules or different kinds of pathogenic protein molecules. Then of course, you have what are called as nod like receptors and scavenger receptors early on in evolution, these are present throughout or very well dispersed in the animal kingdom.

Now, in earlier also there is this variation or variable region that is present in the immunoglobulin molecule, has an equivalent, which is called as the VCBP's, which is the variable region containing gene family of chitin binding proteins. Now, since many of these cells are invaded by pathogens or different kinds of organisms, which are rich in chitin, they have evolved a kind of a protein immune protein system, where they inculcate the phenomenon of variability into these kind of proteins. There are several

examples and we will go through that in a little while. Now, this variable region containing gene family of chitin binding proteins is present in amphioxus.

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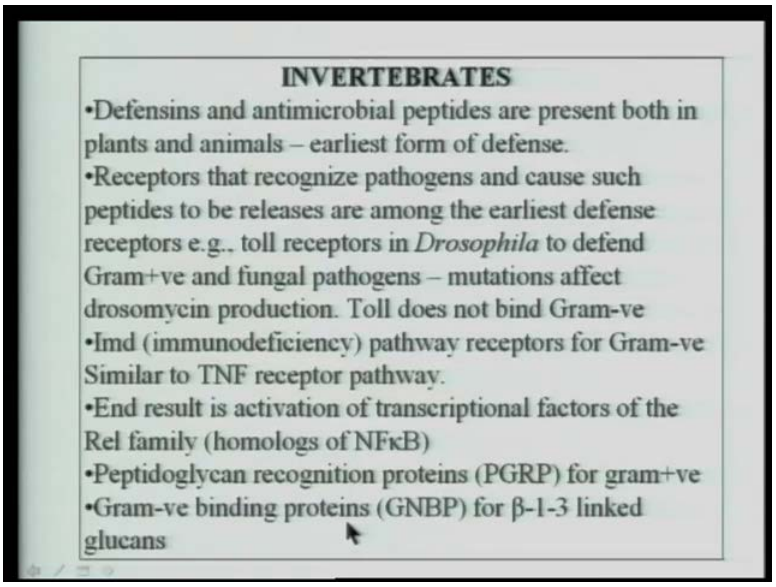

Now, as you go on in invertebrates, you find the presence of anti-microbial or anti-bacterial peptides that are synthesized in response to bacterial infections. For example, in amphibia and in insects, infection in insects is accompanied by an induction of the secretion of various kinds of anti-microbial peptides called as defensins, there are various other kinds of names to this, we will come to this we go on in the lecture. So, these are anti-microbial peptides, which can be inhibit the replication of growth of bacteria.

Now, these are present both in plants as well as in animals. So, these have been ascribed to be the earliest form of defense, now apart from proteins that have evolved early on, receptors that recognize pathogens, because there has to be a recognition of the pathogen, if the pathogen can induce an anti-pathogenic response, be it in very early animals, these receptors also cause such peptides to be released and these are also should have evolved very early on to defend that particular organism against infections.

So, you see the presence not only anti-bacterial or anti-pathogenic peptide proteins, you also have receptors that can recognize the incoming pathogen and these are of course present very early on, as I told you early, even in insects. For example, toll receptors in drosophila they are used to defend against a various a various kinds of infections such as gram positive and fungal pathogens. And they have found as expected that mutations in

these toll receptors actually had an effect on the production of anti-microbial peptide called as drosomycin into the hemolymph of these insects and therefore, came the connection that because anti-microbial peptides are being inhibited, when this toll receptor is being mutated, they said it has a function apart from embryogenesis and then it became very clear even in invertebrates that these toll receptors play a role in microbial defense.

Now, the toll actually does not bind to gram negative organisms in drosophila, but instead, it has another mechanism called as the IMD or IMD stands for immunodeficiency pathway. This pathway has receptors for gram negative bacteria and it is similar to the tumor necrosis factor receptor pathway in higher mammals.

So, you see there is a lot of comparison and it seems as if the sort of mechanism to evolve both the adaptive as well as the innate immune system has its roots very early on in evolution and it has been perfected diversified and more complex things were added as evolution took place.

The end result of all of these pathways of course is the activation of transcriptional factors, which activate various kinds of genes that in early or primitive like in insects is the induction of the secretion or induction of genes that will give rise to these antimicrobial peptides or proteins. Whereas in higher mammals, you have the induction of a variety of cytokines that will combat or activates phagocytose to phagocytose to different kinds of bacteria.

Now, in the insects, a very similar system that is homologous to the NF kappa B pathway is also is also present in drosophila and these belongs to the rel family as evidence by looking at the structure of these various kinds of molecules that are they are homologous. In addition to that you have other kinds of proteins called as peptidoglycan recognition proteins, PGRP in insects for the recognition of gram positive bacteria, because these bacteria have expressed the peptidoglycan, also gram negative binding proteins, gram negative bacteria binding proteins called as GNBP, they bind to beta-1-3 linked glucans.

So, you see many of these pathogens that harbor or whose structure is made of these glucans, as well as this peptidoglycan, has kind of proteins that can bind to these bacteria. The whole idea being that when an infection occurs, these proteins can bind to these bacterial proteins and a kind of agglutinate them or precipitate them and then keep

them in one place and make a cyst around it and enclose them. So, that they do not go on causing infection within the insect or within the hemolymph.

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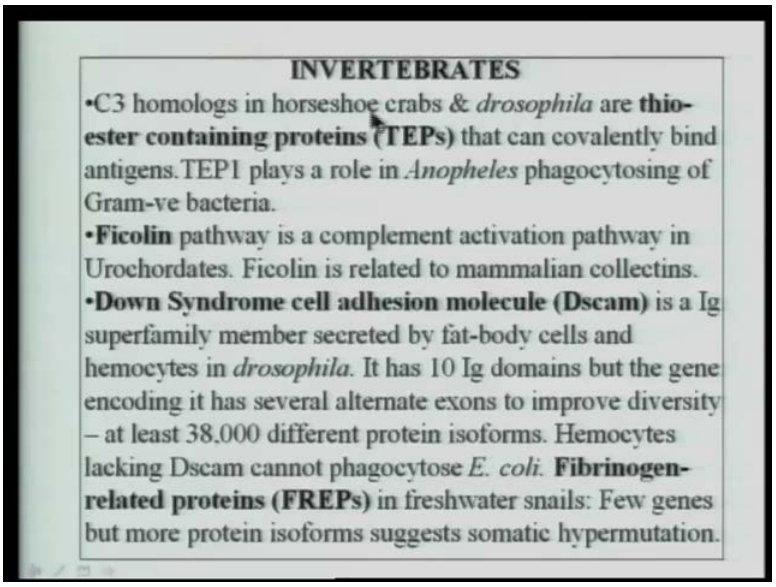

So, going further C3 or complement system, which is a very important pathway in higher mammals, is also present in different kinds of early organisms like drosophila as well as horseshoe crabs. Now, the reason why drosophila comes up again and again is that as I told you, it is very impossible for one to do studies. And all these different kinds of complicated experiments in all kinds of animals throughout the animal kingdom, so they are done in representative individuals and you find that the many of these results are actually true for other kinds of insects, as well like for example, lot of studies have been done even in mosquitoes.

So, you see C3 is a component of complement that is the third component that gets activated in the complement cascade. In the complement cascade, is involved in higher mammals to punch a hole into the infecting organism and C3 is the third component. So, these C3 homologs are present in these insects and the interesting or the most important point is that these are all containing thio-ester bonds, these thio-ester bonds have the ability to chemically or covalently conjugate to various kinds of proteins and therefore, these are called as TEP's, they can covalently bind to antigens. And they found that there are different kinds of these proteins and TEP1 is one such protein that plays a role in anopheles.

Now, in the anopheles hemolymph, there are cells that phagocytose gram negative bacteria, so they found that this TEP1, by binding covalently to the incoming pathogen plays a role in phagocytosis of gram negative bacteria. So, in other words, something similar to opsonisation that you find in higher mammals, where macrophages are activated to pathogens that are coated with these complement components.

Other molecule like ficolin is a new pathway, a different kind of complement activation pathway that is found in urochordates, it is related to mammalian collectin molecules. There is another important family of molecules as I told you, now you see these are the immunoglobulin superfamily member, which means that these are having the immunoglobulin fold and these are secreted by the fat body cells and hemocytes in drosophila. Hemocytes are cells that are present in the hemolymph of drosophila, so this is called as the down syndrome cell adhesion molecule or Dscam.

Now, the important thing about this particular molecule is that the protein itself has 10 immunoglobulin domains, so like what you have in the immunoglobulin IgG molecule, you have different domains. So, it has such domains 10 of them, but the gene encoding it has several alternative exons, like we have different kinds of genes in the variable, there are several variable region genes, both in the TCR, as well as immunoglobulin gene in the gene rearrangement pathway, here you have different alternate exons.

Now, these each one of these exons are chosen at random in order to improve the diversity of this molecule, in other words, from a single drosophila, you find that there are several such Dscam molecules that are having these immunoglobulin folds, but have having a different alternate exon and therefore, you have 38000 different kinds of protein isoforms, something similar to your variable gene rearrangement in immunoglobulin molecules.

The mechanism of course is still not clear and it has been found that hemocytes that lack this Dscam molecule and therefore, something similar to lacking immunoglobulins or having a deficiency in various kinds of immunoglobulin molecules like IgG deficiency IgA deficiency and so on, these molecules if they are lacking in drosophila, they cannot phagocytose E coli, so there is a kind of a relationship.

Similar such molecules such as FREPs or fibrinogen related protein molecules is found in fresh water snail, where this diversification does not happen because of alternative

exons, but it happens because of somatic hyper mutation that is found in higher mammals when IgG matures to form molecules or IgG molecules that have higher affinity.

So surprising, isn't it, that such different kinds of early on animals, have such kind of advance mechanisms that are similar, that is found in mammalian immune system, yet of course, many of these molecules are not as perfect and they do not do their job as perfectly is what happens in mammalian organisms.

Now, let us go into the elements of the immune system and then find out what are the important aspects that have happened during evolution in order to make the mammalian immune system or the mammalian immunoglobulin molecules so perfect.

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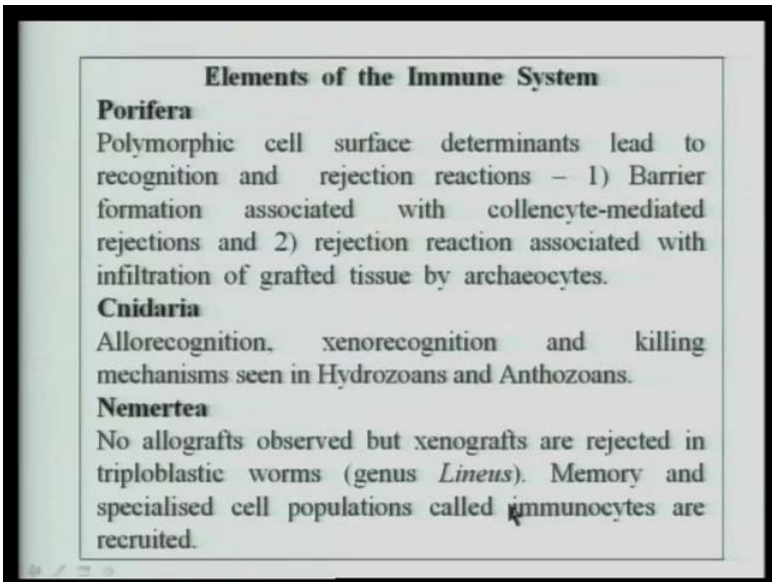

So, going into these elements, you find that in porifera, you find that polymorphic cell surface determinants actually lead to the recognition and rejection reactions as I told you earlier, that there are cell surface molecules and they are polymorphic. The polymorphism of course is not as extensive as what you find in MHC molecules in mammals.

Now, these are involved in barrier formation associated with the cell called as collencyte mediated reactions and porifera or sponges. Now, the rejection reaction associated is also associated with infiltration of grafted tissue by cytes cells called as archaeocytes,

something similar to what happens in graft rejection reactions, where T cells actually go and infiltrate or inflammatory cells go and infiltrate into the allograft and therefore, cutting the allograft circulation and rejecting that allograft, similar such function is actually mediated by these primitive cells called as archaeocytes.

Cnidaria, they have allorecognition, they also have xeno recognition, if you know what is the allo and what is xeno from the earlier immunological lectures. And there are killing mechanisms something similar to what happened with cytotoxic killer cells in higher mammals, they are seen in hydrozoans and anthozoans.

Also as you go on further, you find that of course this allorejection phenomenon as well as graft rejection or acceptance is not uniformly seen in all members of a phylum or class, some of them do not observe allografts, but there observes xenografts, the mechanism for which is not been yet worked out because of obvious difficulties in doing such studies with such difficult organisms.

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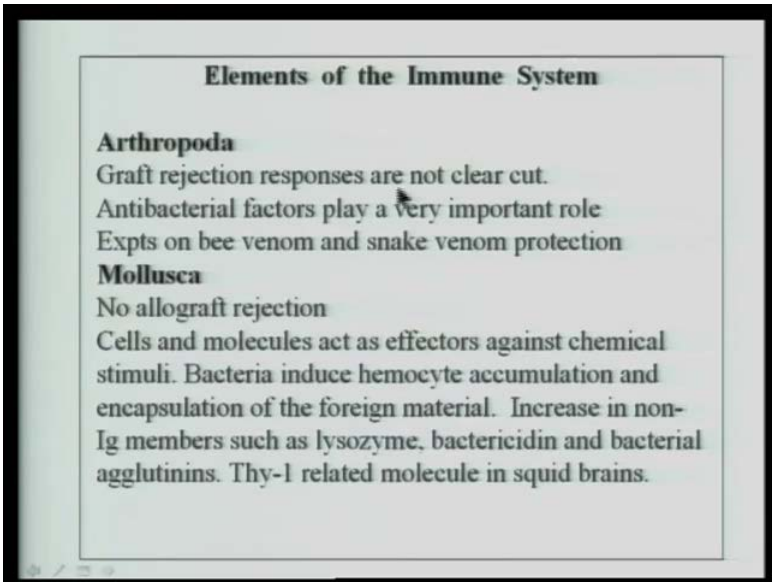

Memory and specialized cell populations called immunocytes are actually recruited within these organisms. In arthropoda, you find graft rejection rejections are not very clear cut that means graft rejections you cannot really pin down and say it actually happens in various kinds of arthropod members.

There are of course antibacterial factors, which is a major important role or important mechanism by which they combat organisms and there is a lot of experiments that have been done with bee venom and snake venom, they inject snake venom and bee venom into cockroaches or other kinds of arthropods in order to see what sort of mechanism is put into action.

In Mollusca, there is no allograft rejection that has been reported and cells and molecules act as effectors against chemical stimuli, so if you give chemical haptens, inject chemicals haptens into Mollusca, you do find some sort of a reaction, where certain kinds of cells called as hemocytes, they actually play some role and they get induced and they play a role encapsulation of bacteria or these foreign material.

As found in early on in evolution, by the way if you remember that even in bacteriophage, infection of bacteria you find, the phenomenon of restricting DNA, which is actually can be considers as an immune mechanism that has evolved early on in bacteria in order to cut up the bacteriophage that are infecting into the bacteria.

Similarly, like you have the presence of lysozyme, lysozyme is required to lies bacteria, that is what it is called as lysozyme. So, you find that non-immunoglobulin superfamily members such as this lysozyme, bactericidin and bacterial agglutinins, these play a very important role in mollascs. And important to say thy 1, which is present in the thymus or on the brain cells is actually found in squid brains.

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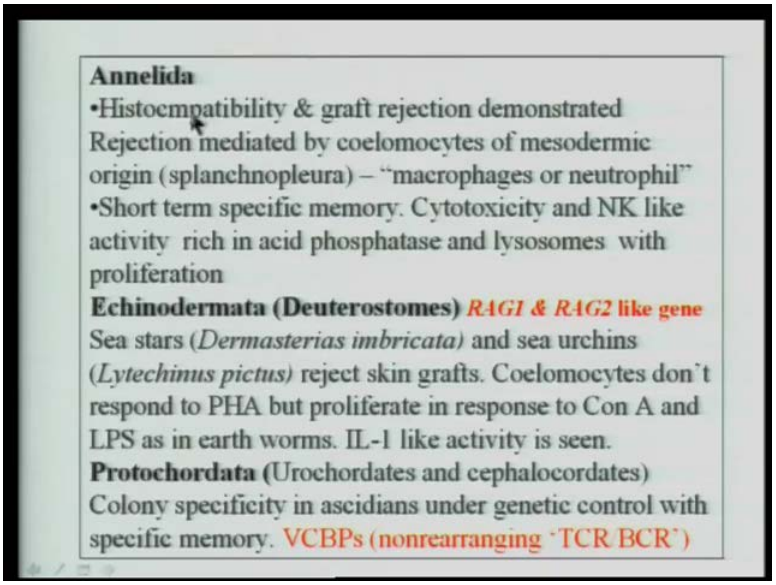

Then going on to Annelida, you find that there is some sort of graft rejection has been demonstrated here and this is actually mediated by coelomocytes and they have some sort of a short terms specific memory. In other words, if you take the same skin graft and again graft it to the same earthworm, there is a somewhat accelerated rejection in terms of time, so there are certain kinds of cells that are rich in acid phosphatase and lysozymes.

Now, when you come to echino dermata, where you have like sea stars and sea urchins, now you find the early presence of what are called as RAG1 and RAG2, which I will go to a little more in detail, in the coming slides. So, these are the first organisms where RAG1 and RA2, like genes are present within the genome of sea stars and sea urchins. Just like mammalian cells, proliferate in response to con a, these cells coelomocytes that are obtained from sea urchin also proliferate when add con A to them, which means that con A has corresponding receptors on the coelomocytes.

LPS, which is a very important ligand for toll receptors also, activates coelomocytes in earthworms. And for the first time, you find that IL like activity, IL-1 is a cytokine, is an interleukin that has been found or plays a very important role in higher mammals and that is found very early on in sea urchins or IL-1 like molecules.

Protochordates, urochoradates and cephalochordates, these are, they have colony specificity, like you must have heard about the ascidians, these colony specificity or rejection reactions, now this point in evolution seems to come across as being under genetic control. So, perhaps the first evidence where MHC like molecules may be playing a role and of course, you have these chitin binding proteins, which are the early forerunners for the non-rearranging T cell receptor or the B cell receptors.

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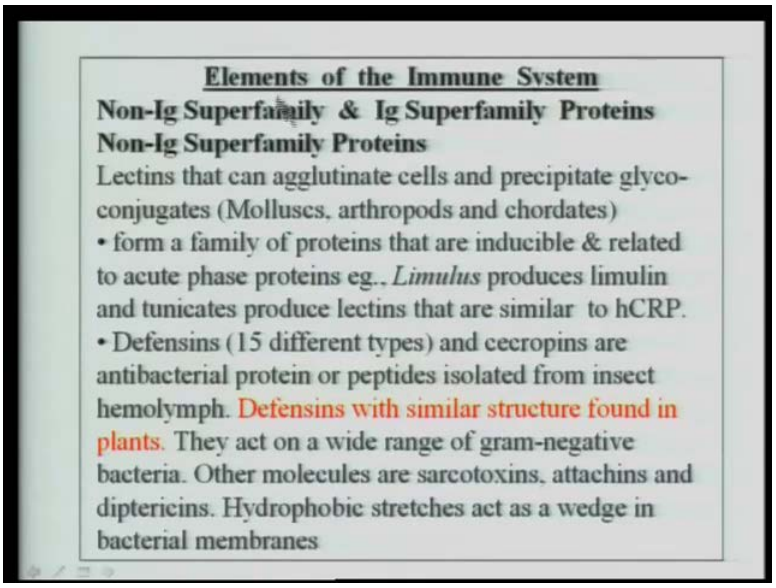

Now, going on further, looking at the elements of this immune system of both non Ig as well as Ig superfamily of proteins, you find that in mollascs arthropods as well as in chordates, lectin or lectin family of molecules, they play a very important role in agglutinating cells and precipitating glyco conjugates, especially in insects.

They form a family of proteins that are inducible by bacterial infection or injection of bacteria into the hemolymph of, let say for example drosophila and they are related to the mammalian acute phase response or acute phase proteins. For example, in limulus, they produce a protein called as limulin and tunicates, produce lectins that are similar to human C reactive protein. And of course, defensins, the different types of defensins which are antimicrobial in nature and other kinds of antimicrobial proteins are peptides are cecropin and then you these are all proteins or peptides isolated from the hemolymph.

Defensins with similar structure, when they have looked at structure after crystalizing these molecules, they found that is similar even in plants of course with some variations. They are not exactly similar, these act in a variety of gram negative bacteria. Remember there are different types of defensins, therefore different types of bacteria could be combated with. Other molecules of antibacterial nature or sarcotoxins, attachins and diptericins, these all seem to have hydrophobic stretches that act as a wedge into the bacterial membrane and that is how the inactivate the bacteria.

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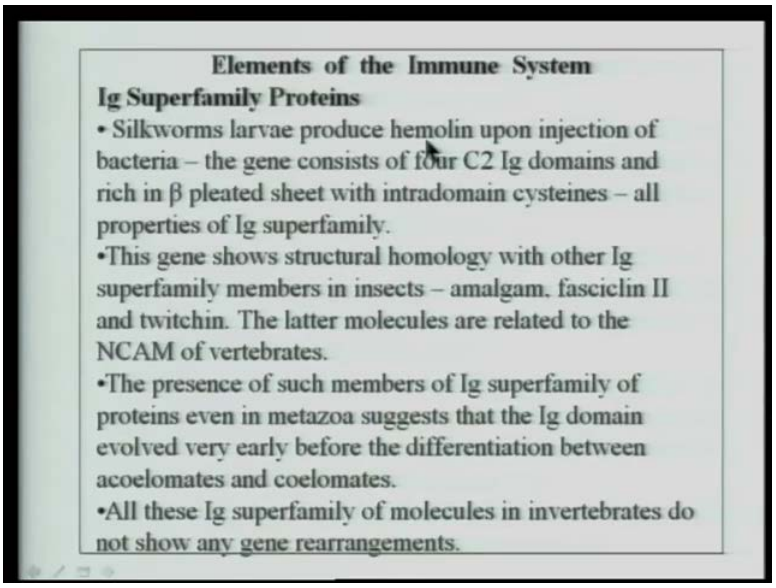

So, then coming on to immunoglobulin superfamily of molecules, you find that silkworm larvae, the familiar silkworm that that we know of making silk, these larvae they produce a molecule called as hemolin, this upon injection of bacteria and of course, infection of silk worms is a very important thing for the silk industry and they have worked on these silkworm larvae and they found that this hemolin is actually secreted upon infection of with bacteria and this gene actually consists of four C2 immunoglobulin like domains. So, like you have the C2 is a domain that is present in the IgG molecule, similar such four of these domains are present in these hemolin, therefore making it a member of the immunoglobulin superfamily.

It is rich in beta pleated sheets and they have intradomain cysteines, which are characteristic of the immunoglobulin fold. So, this gene shows structural homology with other immunoglobulin superfamily of members in insects, amalgam such as amalgam, fasciclin 2 and twitchin. The latter molecules are related to the neural cell adhesion molecules of vertebrates, you see what is the different kinds of connections that you find in these protein molecules and the analysis that has gone on to prove, but that perhaps that many of the cell adhesion molecules that play a role in trafficking of lymph of lymphocytes to lymph nodes actually arose very early on in evolution.

The presence of such members even in metazoa suggests that this immunoglobulin domain actually evolved very early before the differentiation between acoelomates and

coelomates. Coelomates and acoelomates related to the coelomic cavity that has arose during evolution, but the important point to take home about all these things, although they are all belonging to the immunoglobulin superfamily of molecules, these molecules that are present in these early animals or invertebrates, they do not show gene rearrangements. So, it is not something similar to what happens with antibody molecules.

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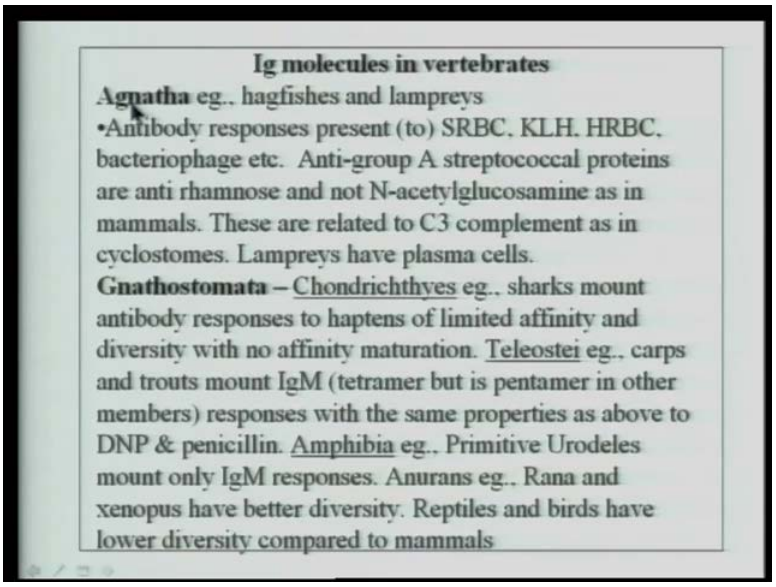

Then of course going on to the agnatha, which are characterized by hagfishes and lampreys, so these there you have antibody responses, they can respond to sheep red blood cells, keyhole limpet hemocyanin or injection of red blood cells. And of course, bacteriophages, and you find that these kind of antibody proteins or antibody like proteins, they are all against the streptococcal anti-group A, so their anti-carbohydrate, anti rhamnose, anti N-acetyl, glucosamine kind of reactive antibody.

Now, all of these are related to C3 complement and it has been found that lampreys have actually plasma cells. Plasma cells similar to plasma cells or the differentiated B lymphocyte that starts to make or secrete large quantities of immunoglobulin molecules. In gnathastomata, for example, chondrichthyes, for examples being sharks, sharks have a very good immune system, they mount antibody responses even to hapten, but they have a limited affinity and the diversification is not there, as well as affinity maturation that happens in higher mammals is kind of limited. In bony fishes, you have carps and trouts, they mount an IgM response, this is a tetramer in carps, but is a pentamer in many other

kinds of teleostei. And of course, they respond to various kinds of drugs like, haptens like, you know molecule like penicillin, dinitrophenyl hapten. So, when you come to amphibian, primitive urodeles mount only IgM responses, they do not have other kinds of immunoglobulin molecules, but anurans like rana, you know xenopus, frogs have a better diversity. Of course, coming on to reptiles and birds, they have a more advance system, immune system, but their diversity is lower compared to the mammalian immune system.

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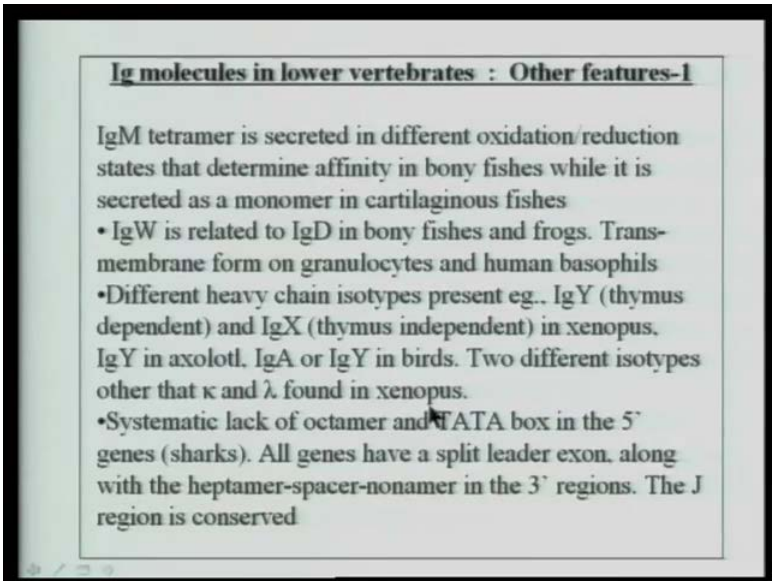

Now, going on further, some of the important features you find that there is a molecule called as IgW, which is related to IgD. These are IgD in mammals in mammalian B cell, these are present in bony fishes and frogs. In fact, this IgW is found on granulocytes and because of these studies that they found that IgW is related to IgD, they found that IgD is actually a membrane molecule in human by basophils. So, you see how these studies were helped or became possible because they found that IgW was found in granulocytes in frogs and they were similar to IgD molecules.

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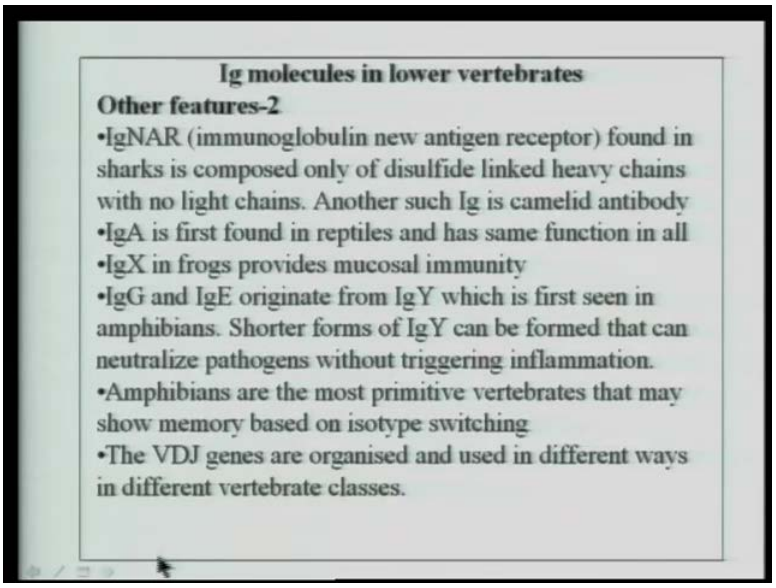

Similarly, you have different kinds of, like for example, those molecules that do not have a light chain, two heavy chain containing molecule called as IgY, which all of you know about. So, these are present in xenopus and these are supposed to be thymus dependent molecules and IgX which is the forerunner for IgA suppose supposed to be a thymus independent kind of antigen. And of course, in all of these, for example, in sharks, there is a systematic lack of octomer motifs which will come to a little later on during the gene rearrangement that occurs in immunoglobulin molecule, that their region is conserved and not having different kinds of gene segments during the immunoglobulin gene rearrangement.

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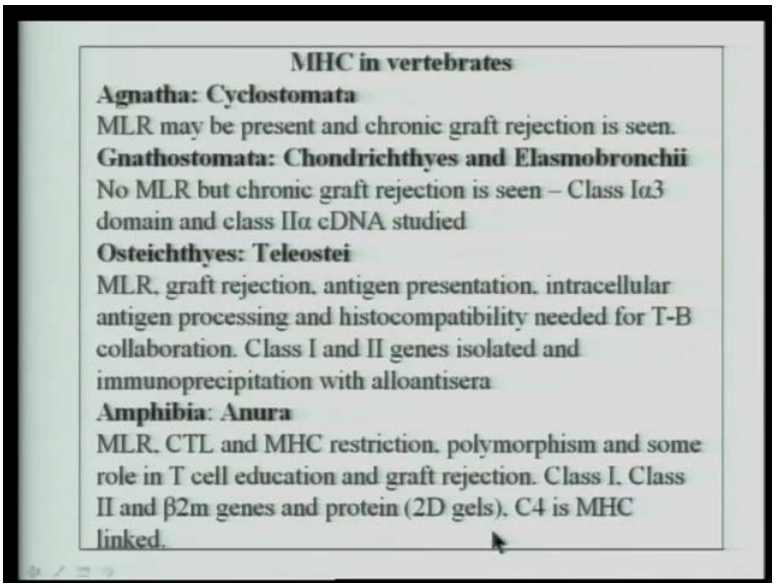

So, you see several such features are there, which I will not go into for the lack of time, but you can browse through in these slides. Now, going on further, MHC, now MHC MLR is present in many of these, they start to show their presence in agnatha and the MLR mixed lymphocyte reactions, their a chronic graft rejection is seen in chondrichthyes and elasmobronchii. Now, teleostei, you have MLR, graft rejection, antigen presentation, intracellular antigen processing. Class 1 and class 2 genes have been isolated by immunoprecipitation and of course, you have beta 2 gene, beta 2 microglobulin genes have been found by 20-dimensional gel electrophoresis, C4 or complement 4 component was MHC linked in amphibian.

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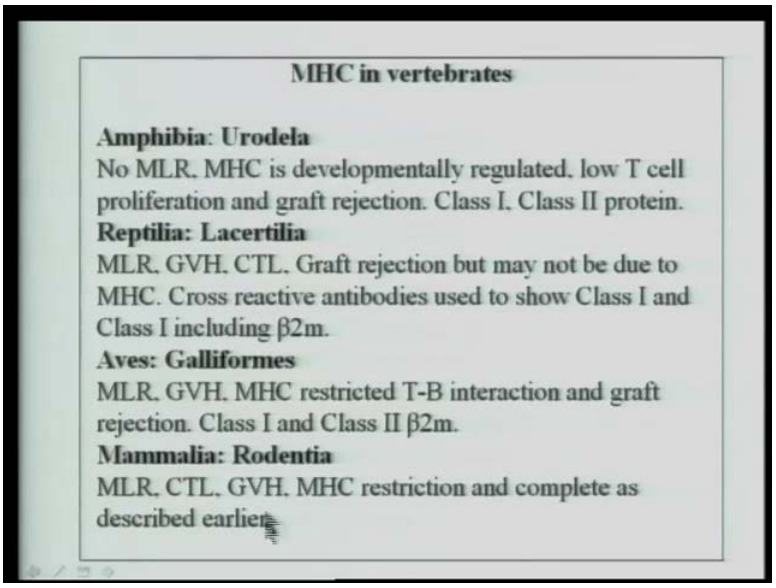

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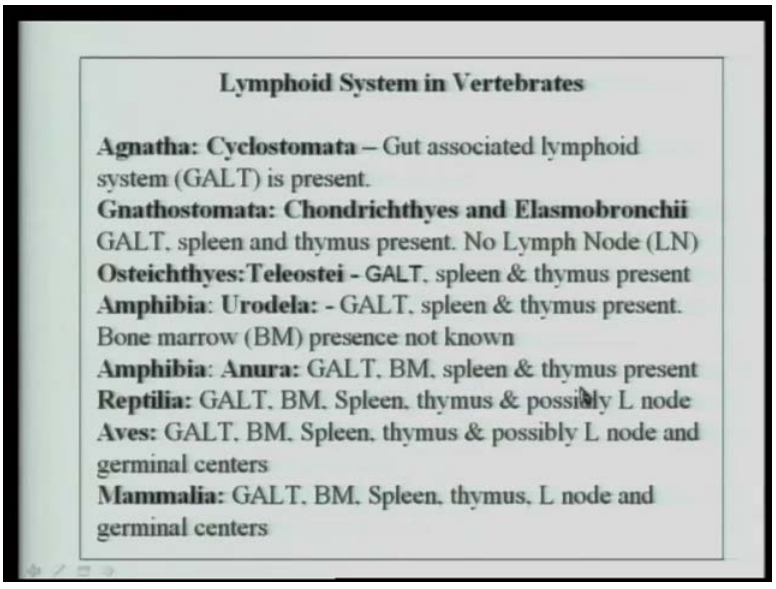

So, like this a several such studies have been going on into various kinds of, different kinds of organisms. And when you looked at the lymphoid system, you find that the galt is present in cyclostomata and these are all specialized the gut associated lymphoid system. So, you see how these lymph nodes or specialized types of lymphocyte containing organs are coming into place in evolution.

So, you see galt bone marrow spleen and thymus is present in amphibia, but the bone marrow is not known in earlier amphibia like urodeles. And then of course, as you go on

to reptilian, aves and birds, you have all these different kinds of lymph nodes that are present.

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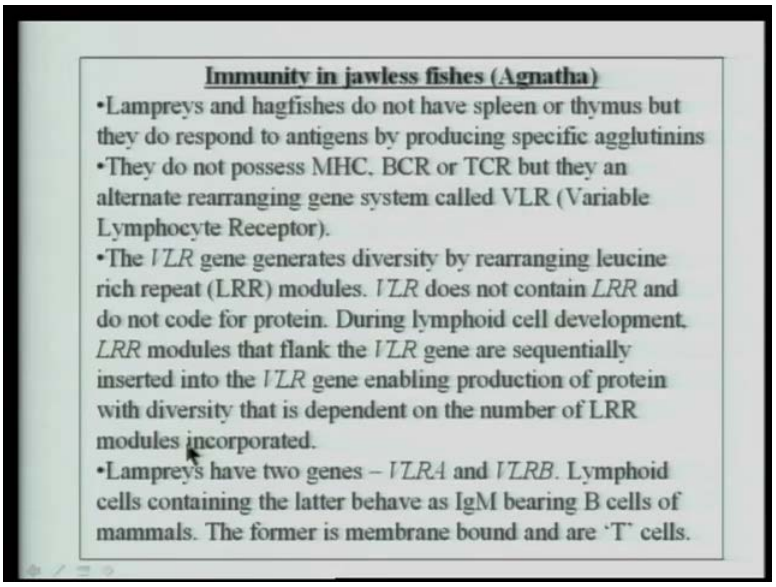

Now, what is the very important thing that we need to take home during this evolutionary lecture. This actually was discovered by the discovery of a phenomenon or a kind of a gene system that is present, which was thought not to be present in earlier organisms because the T cell receptor and the B cell receptor are molecules that rearrange. So, it was thought that this rearranging system was not present in primitive animals, but they found such a rearranging system in lampreys and hagfishes, they do not have the spleen or thymus, but they do respond to various different kinds of antigens by producing specific agglutinins.

They also do not possess MHC, they do not have the B cell receptor or the T cell receptor as we know it in higher mammals, but they have an alternate rearranging gene system. Very important and very interesting point, this is called as the variable lymphocyte receptor.

So, what is this VLR gene? This VLR gene is the one which creates diversity, these VLR genes have what are called as rearranging leucine rich repeat modules. This LRR molecule are found even in higher mammalian immune system molecules, so these LRR molecules are actually taken and jucksed opposed onto the VLR gene.

The VLR gene is actually the protein is not made and not functional, but when there is an infection in these lampreys and hogfishes, there is some rearranging mechanism, where it takes the leucine rich repeats and puts it together with this VLR gene. Once this juksed up position occurs, the gene become active and secretes out this protein and this protein is very important for combating incoming different kinds of organisms.

So, you see you have a rearranging system and many of these members of this rearranging gene like VLRA and VLRB play a very important role in what you call as the B cell immunoglobulin receptors, something similar to that or the T cell or so to say they are pretative B cells or pretative T cells.

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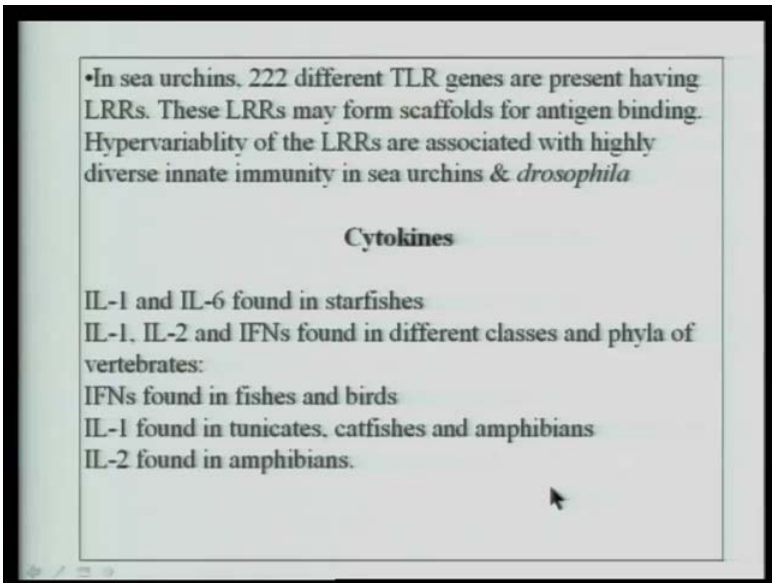

Then of course, going on into sea urchins, you have several such LRR taking part in immune recognition. And IL1, IL6 and interferon's are cytokines that have been found in early vertebrates, so they have been found earlier on, for example IL1 has been found in tunicates, catfishes and amphibians, IL2 is also found in amphibians.

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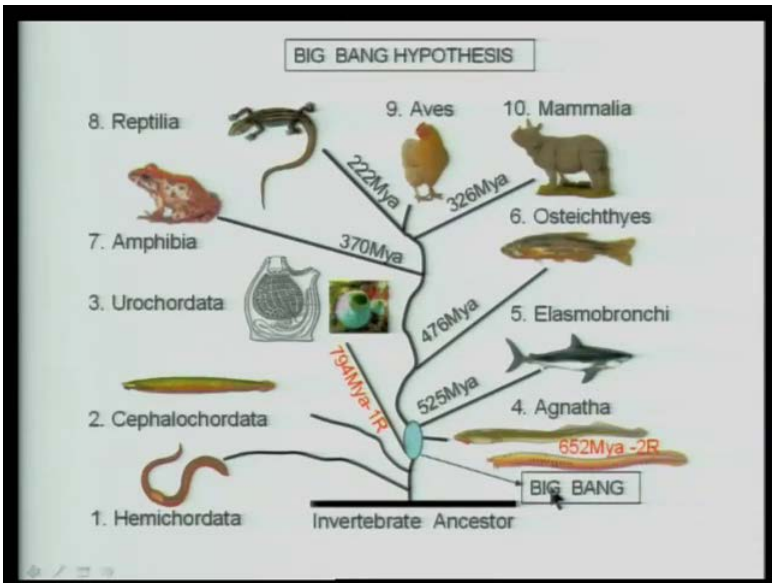

So, you see IL1 and IL6 actually first appeared like molecules, first appeared in starfishes. So, you see how early the such advance molecules show their presence, so in order to understand this particular concept about what happens in diversification, we need to understand what is called as the big bang hypothesis.

What is this big bang hypothesis something that we see in astronomy? So, this big bang hypothesis was hypothesized after looking at the evolutionary scale. Keeping in mind to look for the events that happen in the adaptive immune system, what are the important point that happened in the adaptive immune system.

Now, what happens during immunoglobulin synthesis? When immunoglobulins are made, you have immunoglobulin gene rearrangement. So, this gene rearrangement takes the help of various different kinds of variable genes takes it out from the chromosome or from the DNA and takes it next to what is called as VJ segment. And the VJ segment is then jucks opposed onto the constant region gene, which happens in the light chain. In the heavy chain of course, you have the diversity segments playing a role. So, there is a mechanism or proteins or enzymes are involved in doing this whole process.

So, the proteins or the enzyme that are involved in this process are called as RAG1 and RAG2, recombinase activating genes. So, without this recombinase activating genes, there would be no immunoglobulin or no T cell receptor. For example, in humans, you

find a particular syndrome called as a omenn's syndrome. This omenn syndrome is characterized by the lack of rearrangement immunoglobulin or T cell receptor molecules.

So, you see how important the RAG1 and RAG2 genes are for the process of doing the recombination. This recombination by RAG1 and RAG2 genes is actually mediated because of the presence of certain recognition sequences called as RSS or signal sequences recombinase signal sequences.

So, when you look at these they found that they are the hypothesized, that as these different kinds of organisms evolved and here you find in the red, I have put these, this is Mya stands for million years ago. So, as you evolve and this 2R stands for whole genome replication, so during this process at this stage, there was some sort of invasion by a transposon, we will come to that in a little later on. This invasion of a transposon followed by gene duplication or whole genome duplication is what is supposed to make up this phenomenon or hypothesis called as the big bang, which is now of course has got extraordinary proof and they believe that such a big bang hypothesis did occur.

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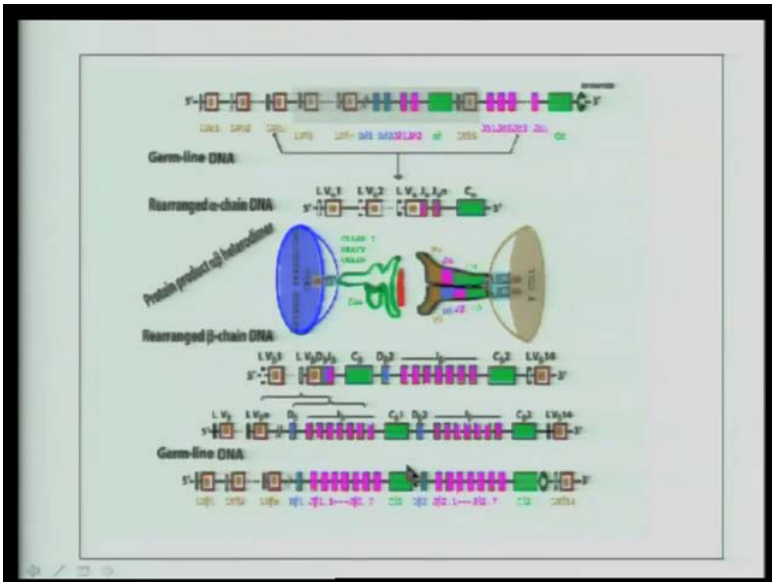

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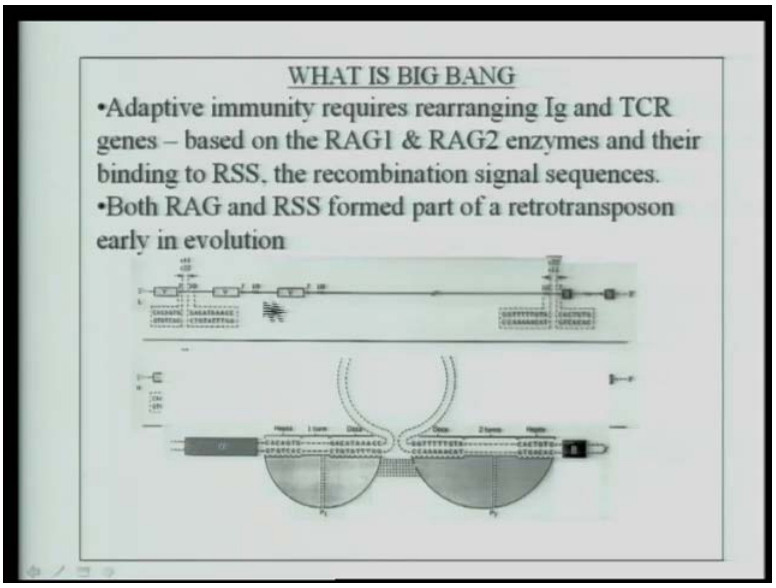

So, what are these transposons. Now, you will see that this is what I just told you earlier and that is, that you have to have gene rearrangement, they are jucks opposed over here in order to make the final T cell receptor. So, this is the rearranged alpha chain gene, these are the leader peptide and on either side of these segments, you find the presence of what are called as this RSS or recombination signal sequences. So, you have either a haptomer or an octomer, so this is also called as a 12, 23 base pair root in higher mammals.

So, what happens is that these enzymes sit on these recognition sequences, so they come and they come together after sitting on those recognition sequences, this intervening sequence loops out and is cut out and then the genes are jucks opposed. So, you have the jucks opposition of the variable region with the joining segment and that is what is shown over here. This is the rag gene that is sitting on the aptamer and decamer sequences and then the other rag gene that is sitting over, here they come together loop out this intervening sequence and bring the variable region together with the J region segment.

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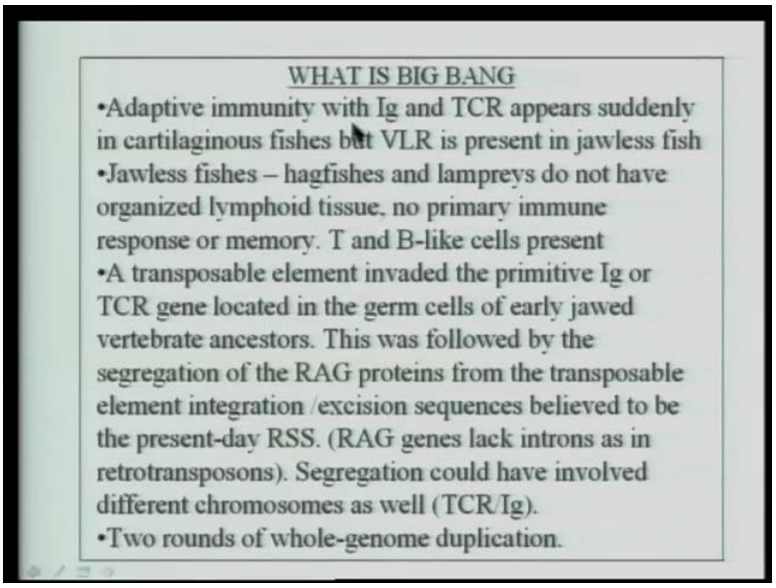

So, therefore, you see that the RAG1 and RAG2 enzymes are very essential to make this whole thing loop out and bring them together and connect them together. So, these are the enzymes that are very important and of course, these sequences are also very important. Now, what they have found is that the adaptive immunity with immunoglobulin and TCR this appears suddenly in cartilaginous fishes.

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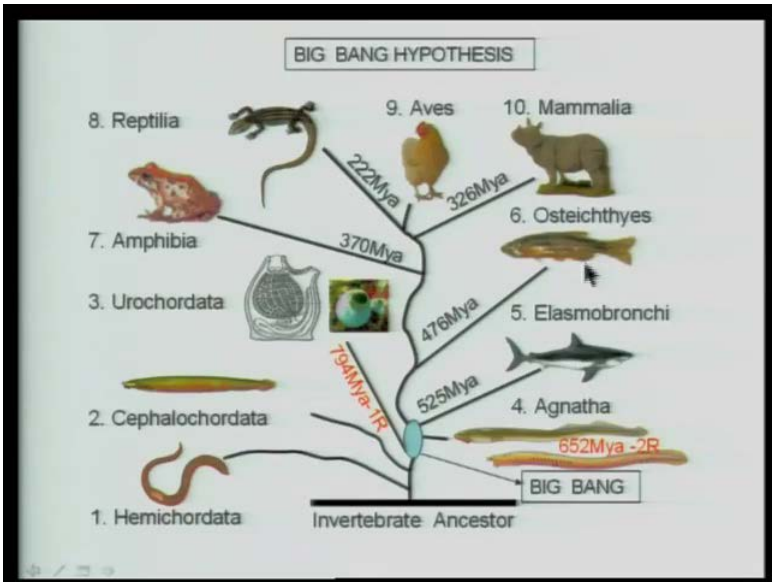

What is this cartilaginous fishes? Cartilaginous fishes is, these are the cartilaginous fishes, over here these are osteichthyes and these are elasmo elasmobronchi, the bony

fishes and cartilaginous fishes. So, you see, if you look at this arrow that is where this big bang supposed to have taken place.

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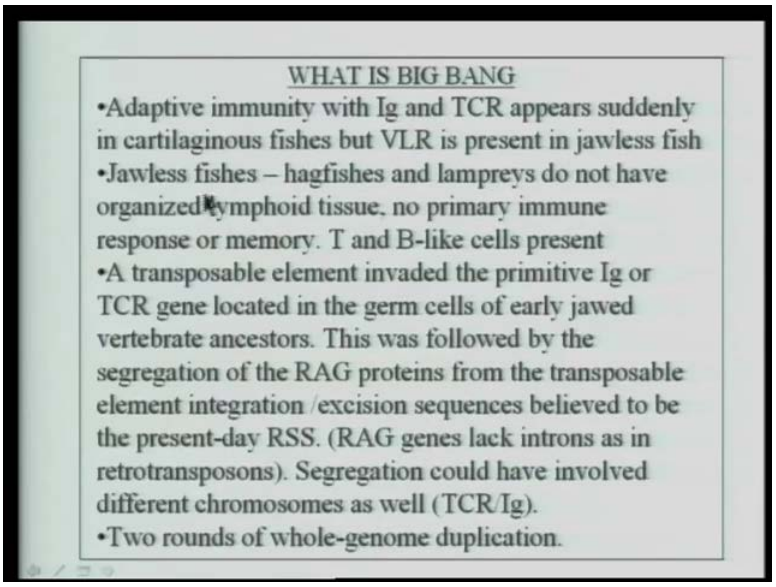

So, in these jawless fishes, hagfishes, they do not have organized lymphoid tissue nor do they have a primary immune response or memory. So, they have T or B like cells and they proposed that the transposable element invaded the primitive immunoglobulin or TCR gene located in the germ cells of early jawed vertebrate ancestors. So, soon after the jawless, soon after hagfishes and lampreys, there is supposed to have been an invasion of the germ cell by this transposon.

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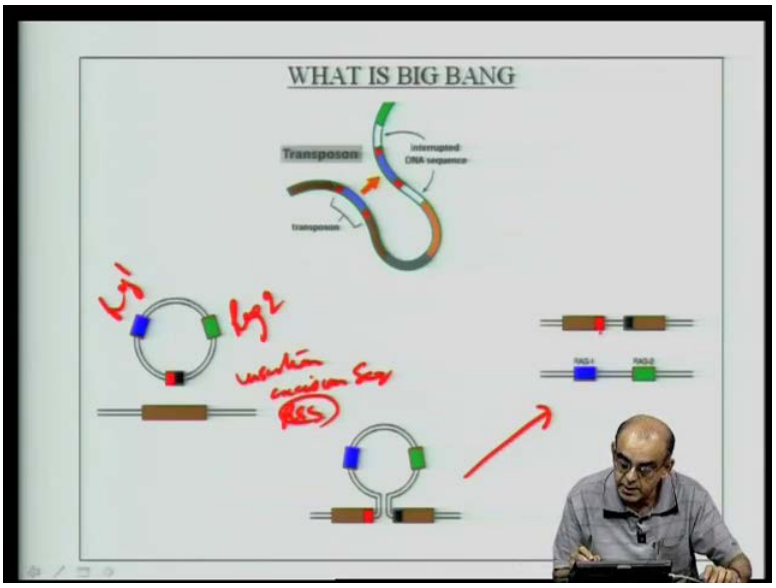

What is this was followed by the segregation of the proteins that are involved in this transposon. So, what is this transposon? Transposons are movable genetic elements, these are known as jumping genes in maize and which have which have obtained Nobel prize for Barbra Mcclintock. So, you have these transposons, this transposon contains these transposase enzymes, which have the ability to cut out the sequence and to cut out the sequence are these different kinds of sequences, which are the recognition sequences.

So, here it is called, in the early, in these transposon, they are called as insertion excision sequences. So, you see how they jump from this part to another gene, where they insert, they cut the DNA of the gene and insert the insertion excision sequences. So, they go on jumping from gene to gene, so these are your transposons.

So, if you look at transposons over here, the early big bang hypothesis, you have the insertion excision sequences and you have what you call as the rag gene, RAG1 and putative, this is the RAG1 and this is the RAG2 gene part of the transposon. And these are your incision, insertion, excision sequence or RSS for short and this is of course so called germ cell genome.

Now, this transposon, because it has the ability to go and insert into the genome, it has inserted over here, as you will see over here, you have the insertion sequences put into the clip, the genomic DNA or the genome and insert themselves into that particular place. So, you see the RAG1 and RAG2 genes, and after this what happened or supposed

to have what happened is that this particular chromosome or DNA that has the RAG1 and RAG2 genes and the RSS actually segregated during recombination in the germ cell. So, when they separated, the RAG1 and RAG2 genes got separated into a different chromosome and the insertion sequences were left and these insertion sequences was the place where you have the immunoglobulin genes coming in together.

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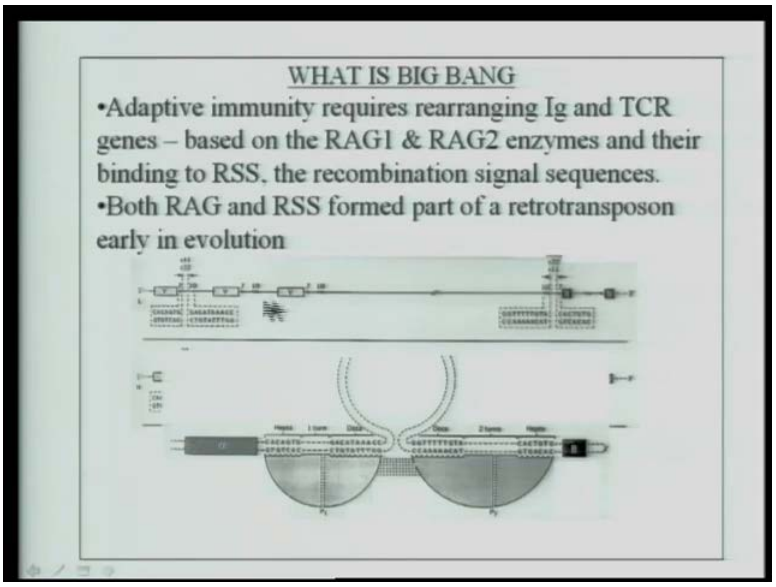

So, in other words, you find this is RAG1 and RAG2 has to recognize these two sequences, which are incision excision sequences or the so called todays RAG1 and RAG2 or RSS recombination signal sequences and these are your RAG1 and RAG2 enzymes. So, if you go back to this, you will see the RAG1 and RAG2 enzymes actually sit on these RSS's in order to bring about recombination.

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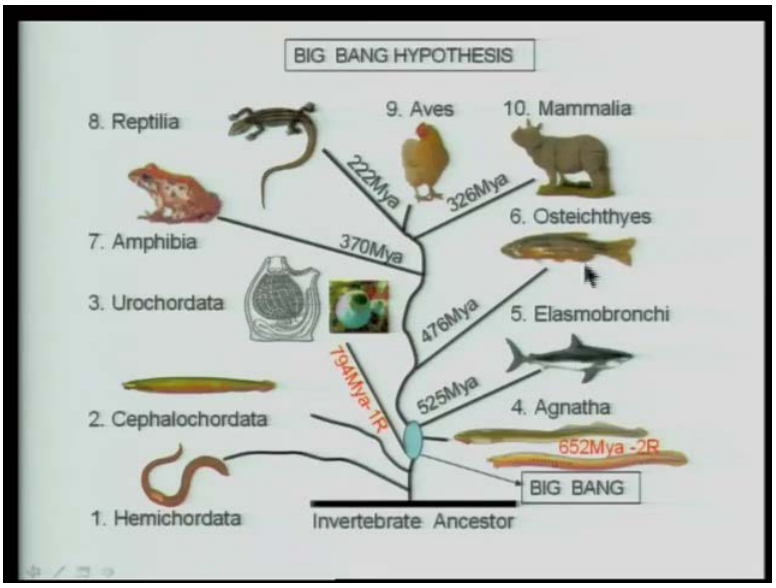

So, you see this is the big bang hypothesis, which is supposed to have occurred very early on in evolution just before the evolution of jawed vertebrates. So, this is a lot of proof has accumulated in to show that actually this sort of insertion or transposon mutagenesis happened and it was probably a very lucky event, which is happened only in the germ cell of those jawed vertebrates.

So, this is the big bang hypothesis and this actually concludes our lecture on evolution. And I have told you about the segregation of the RAG1 and RAG2 from the RSS, after this segregation happened, the entire genome actually duplicated once or twice and proof has been obtained for this duplication over evolutionary studies that people have studied. And during this duplication, you have all these different kind, they are separated into different chromosomes and then of course, now, you know, it as RAG1 and RAG2 and you have the hapten, the 12-23 base pair root. The RAG1 and RAG2 cannot act if they do not have these RSS's and both RAG1 and RAG2 just like transposons, they do not have introns. So, these are some of the features that tell people that actually RAG1 and RAG2 actually came from a transposon.

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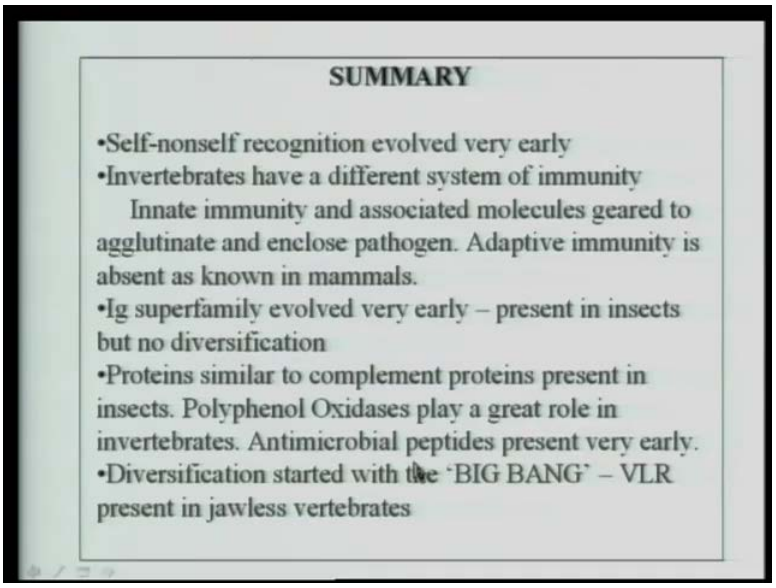

So, in order to summarize this lecture, you find that this self non self-recognition happened very early on in evolution. This basically was to prevent the colonization of multicellular colony forming cells to be colonized by other types of cells and therefore, the individuality would be lost and perhaps very important species would be lost during evolution. And this perhaps evolved because of the evolution of cell surface molecules that could react towards each other or recognize each other.

Invertebrates have a different system of immunity and they their innate immunity and associated molecules are geared to agglutinate and enclose pathogen or encapsulate pathogens. And their adaptive immunity is absent as known in mammals or there are several harms of this adaptive immunity, which are having common element in mammals, but needless to say that the mammalian adaptive immunity is very much advance.

The Ig superfamily evolved very early and some of these molecules are present in insects, but this is characterized by lack of diversification except for certain known molecules like what we saw the chitin binding, variable chitin binding proteins and the variable lymphocyte receptor present in agnatha and jawed vertebrates later on.

Protein similar to complement proteins are present in insects and one of the most important molecules do not covered earlier in this lecture was the presence of polyphenol

oxidases in insects, these play a very important role in invertebrates to combat pathogens and to agglutinate them.

The antimicrobial peptides of course evolved very early as a component of defense in earlier animals and diversification. What started it all was the so called big bang, which is represented or evidenced by the presence of what is called as variable lymphocyte receptor, that is present in jawless vertebrates, which are otherwise not having components of the adaptive immunity; thank you very much.