Essentials in Immunology

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Module No. # 10

Lecture No. # 19

The major histocompatibility complex – Part 1

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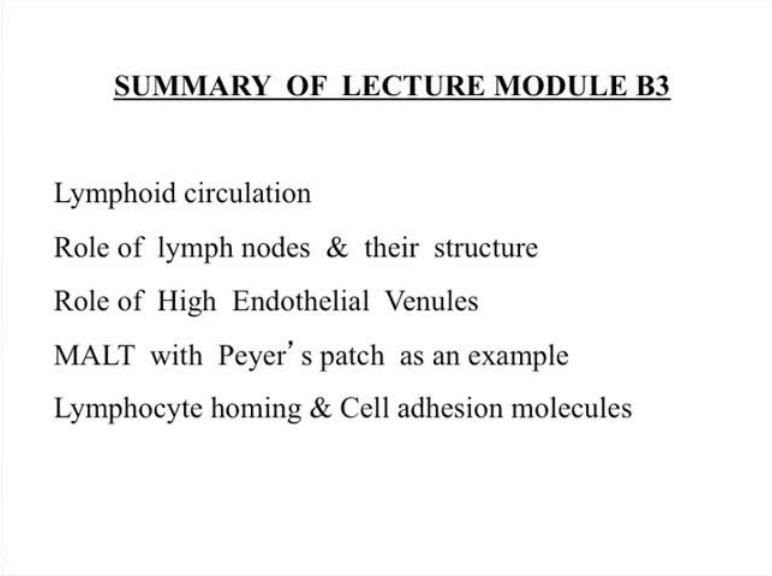

Hello and welcome to this lecture in this series, essentials in immunology. First, let us summarize the aspects that were covered in the previous lecture which is given in the slide. We covered some aspects of lymphoid circulation. The importance of how the thoracic duct in the circulation associated with the lymph plays an important role in how the different lymphocytes can congregate in the lymph nodes as well as the role of lymph nodes and their structure in mediating an immune response.

We also considered the role of the high endothelial venules, which play an important role in the migration and trafficking of lymphocytes from different areas of the body, especially from the blood into the lymph node as well as some of the other lymph nodes that were associated with the secretory surfaces such as the mucosal associated lymphoid tissue of the lymphoid system, where they pay the organization of the Peyer's patch was

studied as an example and we also considered the molecular interactions in the protein-protein interactions that play an important role in lymphocyte homing.

So, after having considered all these different kinds of cells, the B cells, the T cells as well as the macrophages, the forerunner of the antigen presenting cells that play important role in orchestrating immune responses to different pathogens, we now need to consider how these B cells and T cells, for that matter, different types of lymphocytes are identified. As I told you, historically, it was looked at from the point of view of morphology - so, different types of lymphocytes being different in size and their appearance being different, when stained, after taking or bleeding a patient or for that matter, an animal.

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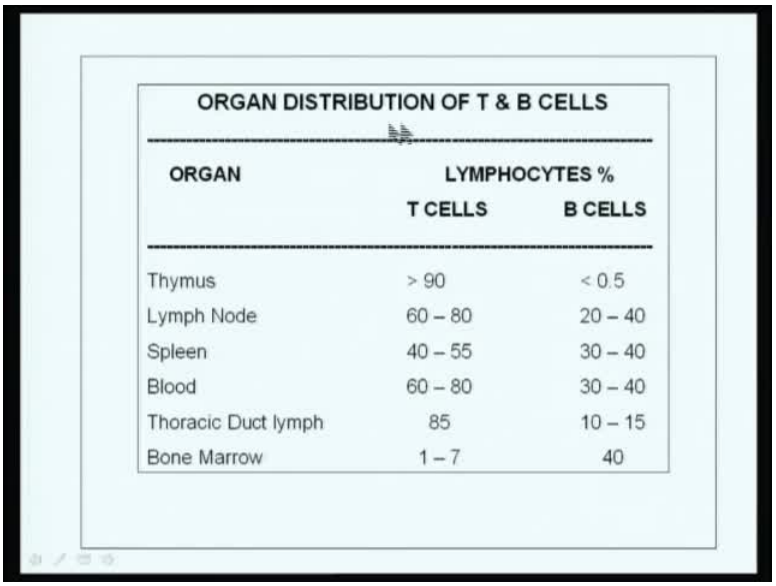

So, when we look at these different types of lymphocytes, the contribution of the percentage of lymphocytes that are present in these different organs is shown in this slide, where you look at the organ distribution of T and B cells. So, if you were to look at the thymus, the thymus being the seat of T cell differentiation or the location of the primary lymphoid organ, which teaches the immune system to distinguish cell from non-cell and not react against the cell, 90 percent of the cells that go into the thymus or migrate from the bone marrow as a precursor into the thymus, most of them actually die and most of them that form the population of the thymus are 90 percent T cells. Of

course, there are a lot of stromal cells and other type of epithelial cells that actually belong to the thymus, which take part in educating these incoming and outgoing T cells.

In the lymph node, you find that 60 to 80 percent of the cells that occupy a lymph node are T cells and about 20 to 40 percent are B cells. In the spleen, which is a more organized secondary lymphoid organ, you find about 40 to 55 percent making up T cells and 30 to 40 percent B cells in nature, and in the blood, it is about 60 to 80 percent T cells and 30 to 40 percent B cells.

Now, in the thoracic duct lymph, 85 percent being T cells, which are migrating from the different tissue spaces and going back into the systemic circulation and about 10 to 15 percent of the cells that are formed in the thoracic lymph duct are B cells in nature. In the bone marrow of course, the seat of stem cell production as well as B cell production, you have only 1 to 7 percent - this is in the humans, now 40 percent B cells, whereas in the bursa, the birds you have majority of them being B cells.

So, we have spoken about these different percentages of T cells and B cells. Now, how are these T cells actually identified as T cells and identified as B cells apart from the technique that is used to stain them and look at them microscopically. For this, you need to learn a little bit about the structure of the antibody and how the antibodies that are produced from B cells can be used to look at certain kinds of proteins and how these can be applied in different kinds of techniques.

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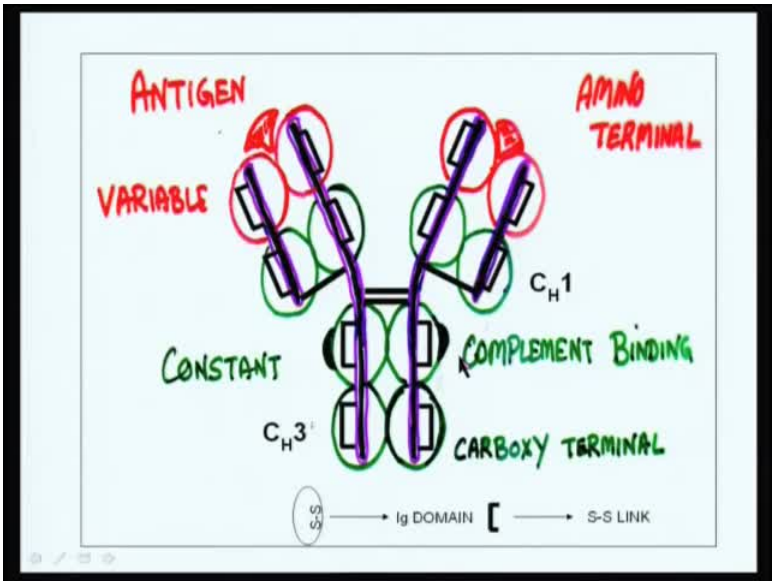

So, looking at the antibody, a crude antibody structure that is given in this transparency, it actually, as I had mentioned earlier it is got two chains. One of them is longer than the other one and therefore, it is called as the heavy chain, which is given here and you have a light chain, which is shorter in nature, and these heavy chains these two heavy chains and light chains are linked by disulphide bounds, which is given by this link over here, which is the black structure that I have drawn to bridge these two heavy chains over here; that is the two disulphide bounds as well as a single disulphide bound that bridges the heavy chain and the light chain, which is present on both these chains on either side.

Now, these two chains - two heavy chains and two light chains, makeup your antibody structure and you have this red portion is called as the variable domain or the variable portion of the antibody, which is responsible for binding the antigen. This is the region which confers specificity of the antibody molecule to the antigen that it has been raised against and these green circles represent the constant regions of the antibody molecule and it has got in the IgG molecule, IgG being a class of antibody molecules, you have three domains called as the constant heavy domain 1, the constant heavy domain 2 and the constant heavy domain 3. So, these are constant in primary amino acid structure, while in the variable portion, the primary amino acid structure is variable in nature because it has to recognize different types of antigens and different types of epitopes.

So, this constant region of the antibody molecule is also a portion of the antibody molecule called as Fc, which imparts the functional properties to this antibody molecule. For example, some of the things that are going to be relevant later on is the complement binding portion of the antibody molecule and is also called as the Fc portion as I just now said.

So, looking at these different regions of the antibody molecule and some of the terms that we used in the previous class to designate certain families of adhesion molecules, for example, we term some molecules, superfamily consisting of immunoglobulin domain. Now, the immunoglobulin domain is one such domain over here, which is found in many molecules in the immune system. Basically, it is this domain that is linked by disulphide bound which is written in the following manner.

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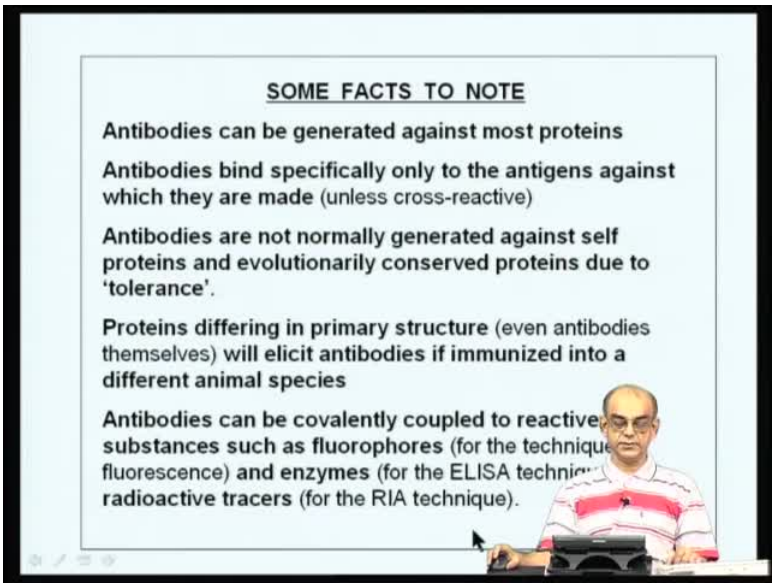

So, going on how can these antibodies actually be used in terms of identifying B cells or T cells? So, some of the facts that we need to note are that antibodies can be generated against most proteins. Now, these antibodies that are generated against any specific protein, bind specifically only to that protein antigen and if it is a small molecule, if it has been raised against a small molecule, it will be specific to that small molecule, which is also called as a hapton.

So, antibodies bind specifically only to the antigens against which they are made or generated, unless of course, if these different protein molecules share certain regions or share certain amino acid regions and therefore, have certain domains that are cross reactive and the antibody will show some cross reactive binding on such proteins that share some of their regions.

Now, these antibodies are not normally generated against self proteins and also evolutionary conserved proteins that have the same amino acid sequence across the animal kingdom or for that matter, across mammals. This is because of the phenomenon called as tolerance. Tolerance deals with a subject, which concerns the lack of recognition or the lack of reactivity to certain antigens that are self or perceived as self.

Proteins that are differing in primary structure, for that matter, even antibodies themselves because they have a variable domain to them and therefore, these antibodies

that are raised against different protein molecules are variable in nature in the variable domain.

So, even antibodies that differ, those regions that differ in the primary amino acid sequence, will also elicit antibodies, provided they are used to immunize into different animal species; again, because of the phenomenon of tolerance. So, for example, you can raise antibodies to let us say, pigeon cytochrome c or cytochrome c that has been isolated from pigeon, only when it is injected into a different animal species, for example, a rabbit or a rat.

So, this particular aspect has been utilized in the applications that had been using these antibodies in different techniques. For example, one uses different antibodies that are raised to the antibodies themselves. So, it is called as a double antibody or an antibody to an antibody and how does one raise this. If you take rat immunoglobulin molecules, if you inject this purified rat immunoglobulin IgG molecules into a different animal such as the mouse or for let us say a pigeon or a sheep, then you will get the sheep will make antibodies to the mouse immunoglobulin IgG molecule. So, in short, they will say sheep anti mouse IgG or sheep anti mouse IgM and so on and so forth.

Now, one of the important things that have been useful for using these antibodies in different techniques is their ability to be covalently coupled to reactive substances. For example, fluorophores; fluorophores that emit fluorescence when stimulated with UV light. So, this has been used in the technique of immunofluorescence test, which are routinely used in immunodiagnostics and several other techniques.

These antibodies can also be covalently coupled to enzymes that can be used to produce colour. For example, the ELISA technique or the enzyme linked immuno sorbent assay technique, which uses this coupling of enzymes so that a colour is produced, when this particular antibody is used. They can also be covalently coupled to radioactive chemicals so that you can use it as radioactive tracers in the technique of radio immuno acid, which can be covered later on in different in later lectures.

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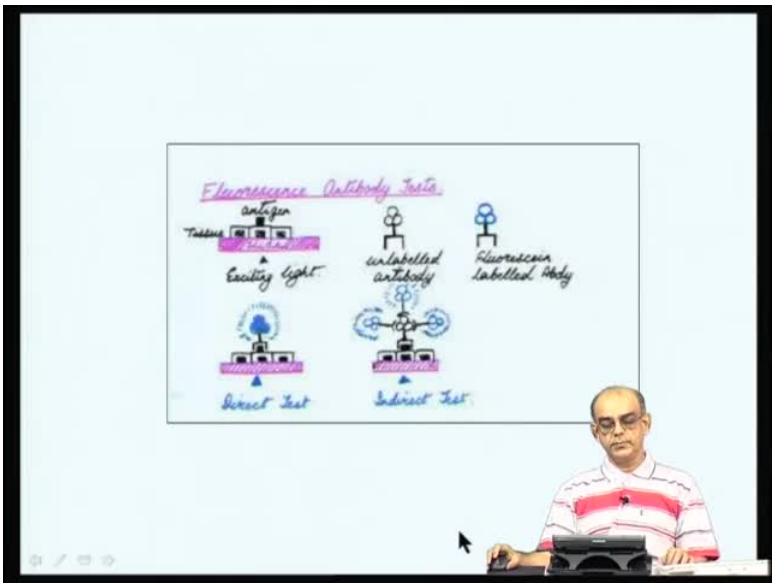

So, noting these facts about antibodies, going a little bit detail into this florescent antibody technique, one can use these antibodies in order to light up certain molecules that are expressed on tissue cells or for that matter, tissue sections. Let us say that these tissue cells are expressing a certain protein molecule against which you have generated an antibody that will specifically recognize, for example, on the surface, this square, black square on this tissue section. So, this antibody, you can use in different ways. So, if you have an antibody that has not been labeled with any chemical, this antibody being specific to this antigen will go and bind to this antigen, when incubated along with this particular histological section.

Now, this antibody, commercially, you have reagents that will recognize this antibody. For example, if you have used a mouse IgG that recognizes this antigen, you have commercially available, for example, sheep anti mouse immuneglobulin that is conjugated to a fluorophore.

So, if you emit, if you excite this particular section under in a microscope by UV light and you use this secondary antibody or this double antibody, which is fluorescently tagged, which will go and bind to this primary antibody because it is directed against the mouse antibody, you will get fluorescence, when you shine UV light from underneath. similar This is called as an indirect test. You can also use a direct test, which utilizes a fluorophore coupled primary antibody itself without the need for a double antibody or a

secondary antibody that has been raised against the primary one in a different species, animal species. So, using such fluorescent tests, you can also detect molecules that are expressed on the surface of cells. For example, B cells or T cells express certain specific proteins that are expressed on the surface.

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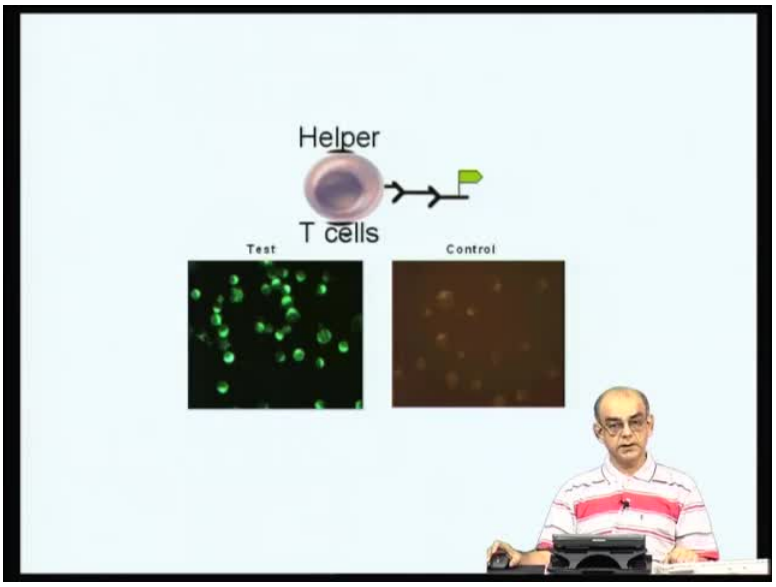

So, these are called as markers. For example, in helper T cells, there is a marker called a CD4, CD stands for cluster of differentiation; so, that is depicted over here in this small line over here. This can be detected by anti CD4 antibodies that in turn can be detected by fluorophore conjugated anti antibody molecules.

For example, if this is an anti CD4 immunoglobulin that has been raised in a different species, for example, mouse and this being a human CD4 molecule or a human helper T cell, you can use secondary antibody that is directed against this mouse anti CD4, which is fluorophore coupled and if you were to shine, if you were to look at it by stimulating them with UV light, this is the type fluorescence that you will get on these cells as compared to a control section or a control cell, where it has not been incubated with this anti CD4 antibody. Therefore, you can look at these fluorescence cells and then say that these cells are positively expressing CD4 molecules.

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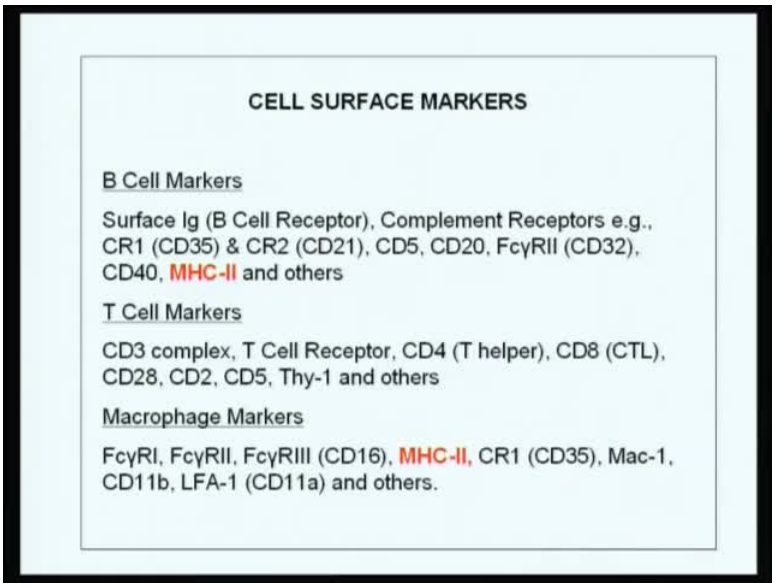

So, using these techniques, these cell surface molecules have been characterized as being present on B cells, T cells or for that matter, macrophages and also several other types of cell specific markers on different other kinds of cell types, which we will not be going into in the immunological classes. So, for example, B cell markers have a very specific marker called as the surface immunoglobulin molecule, which is also called as the B cell receptor, which is expressed on the surface of B cells which is the one that recognizes the incoming antigen.

In addition to that, we have other receptors called as complement receptors called as CR1 or CD35, CD21, CD5, CD20 and this Fc gamma receptor stands for fragment constant region of the molecule and gamma stands for the immunoglobulin class; so, gamma - IgG. So, you have Fc receptor II being expressed on the surface of B cells and therefore, the immunoglobulin can also stick to the B cells via the constant regions of the antibody molecule and as well as, if there is an antigen, it will stick via the variable portion of the antibody molecule.

So, this is the CD32, where the Fc is used to bind to the Fc receptor; CD40, MHC 2 as well as other markers that are not covered in this slide. Now, T cells are recognized by the expression of a marker called as a CD3 complex as well as the T cell receptor, which recognizes, which is one of the most important molecules that recognizes the antigen.

Now, the CD4, we just now went through, which are characterizing the T helper cells. The CD8 molecule, which are present mainly on cytotoxic T killer cells and these really do not help the B cells. So, these are called There are very different kinds of killer cells like CTL, which express the CD8 marker.

In addition to that, you have the CD28, the CD2, the CD5; Thy-1 is also called as a pan specific T cell marker because all different kinds of T cells express this Thy-1 molecule on the cell surface as well as other kinds of markers that are present on T cells. Incidentally, it is interesting to note that these are the molecules, CD4 and CD8 bearing T cells are the ones that get the CD4, especially are the ones that get effected or get depleted when patient suffer from aids

Now, the macrophage markers constitute again different types of Fc receptors. For example, Fc gamma receptor 1, Fc gamma receptor 2 and Fc gamma receptor 3; this Fc gamma receptor 3 is also known as CD16. MHC 2, we will come to MHC 2 in a little while; that is reason why I have marked this in red. Complement receptor 1 like CD35 as was mentioned earlier, the Mac-1, the CD11b, LFA standing for lymphocyte function associated molecule, LFA-1, which is also known as CD11a and others.

Now, in this slide, I have noted this MHC 2 in red because we are now going on to the classes that will cover the molecule called as MHC, which also stands for major histocompatibility complex; histocompatibility because these are the antigens or the locus that determines how grafts are rejected.

Now, everybody is aware that for when kidney fails, you need to get a donor, who will give you a kidney for when where and the doctors, the surgeons will operate and put in the kidney to replace the abnormal kidney that is not functioning. Now, this cannot be done from kidneys that that are obtained from any patient or any cadaver.

This has to be done only after typing, which means, this term typing means that you are, when I say self, this self molecule is what is determined, when you type an individual. What is the nature of the self molecule or self signature molecules? It is something similar to the finger prints that we have that is very unique to each one of us. So, the self signature molecule is also a polymorphic molecule that is expressed on the cell surface of individuals which are unique to that individual.

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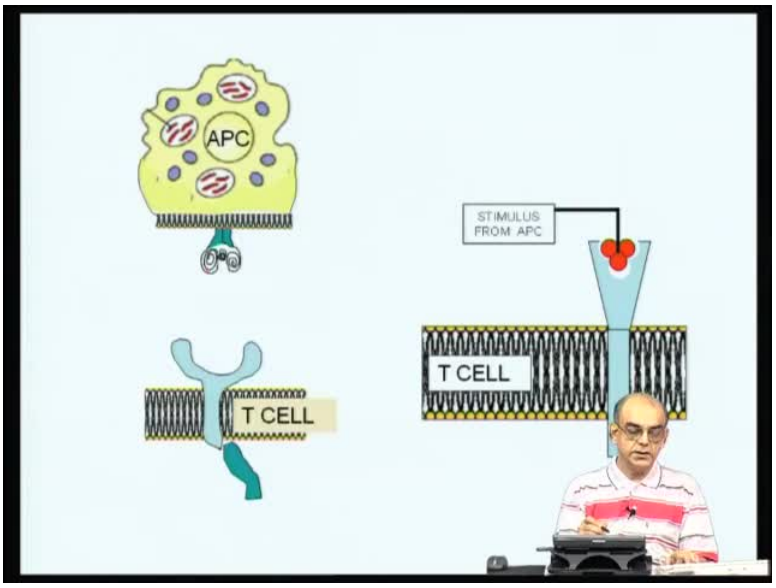

So, in order to learn more and more about this, we need to go back to the previous classes and take stock of certain transparencies or certain figures that we considered. Now, you see here, in the first or the second lecture that was delivered, we recognized the presence of what is called as a stimulus. We did not categorize this as anything, but we mentioned this as a stimulus that is coming from an antigen presenting cell or APC.

Now, the following classes that come later on are actually dealing with this particular stimulus. So, we said that this stimulus actually goes and stimulates the T cell and we went on to say that this binding of this stimulus to the T cell receptor or a receptor that engages this stimulus results in a consequence that activates the T cell and this activation of T cells could be followed by the liberation of a lymphokine called as interleukin, either by measuring the IL 2 by ELISA techniques or by measuring the consequences of IL 2 action. For example, proliferation, IL 2 induces the proliferation of T cells and therefore, one could add radioactive thymidine, which will go into the DNA and follow this particular activation of T cells. Basically, you are looking at a downstream action of IL 2 functional aspect of IL 2, which will result in T cell proliferation.

So, going on further, what is the nature of the stimulus? So, if you look at the stimulus, actually I told you that this stimulus comes from an antigen presenting cell. An antigen presenting cell for us at this stage now, this preliminary stage of this immunological class could be just a macrophage. This macrophage you have seen in earlier slides.

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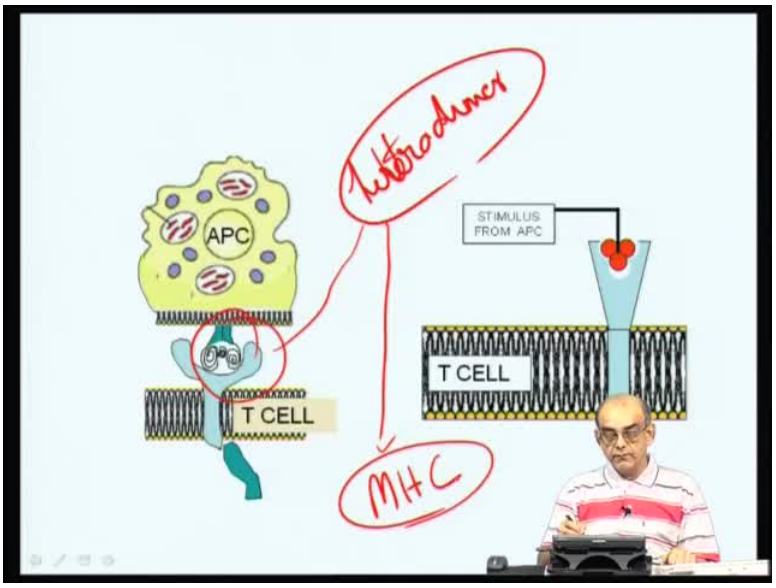

This macrophage on the surface put forwards a molecule, which is actually a heterodimer meaning that it is consisting of two chains or two subunits, two protein chains that come together and this is the one that actually stimulates the T cell. So, what we are going to do now in the following classes is to try and identify and to learn more about this heterodimer. What is the nature of this heterodimer?

How was this heterodimer discovered? So, in order to go along into this particular aspect, we need to know that this heterodimer is actually what constitutes the MHC complex or the major histocompatibility complex, which I have also told is the self signature molecule that is unique to each one of us.

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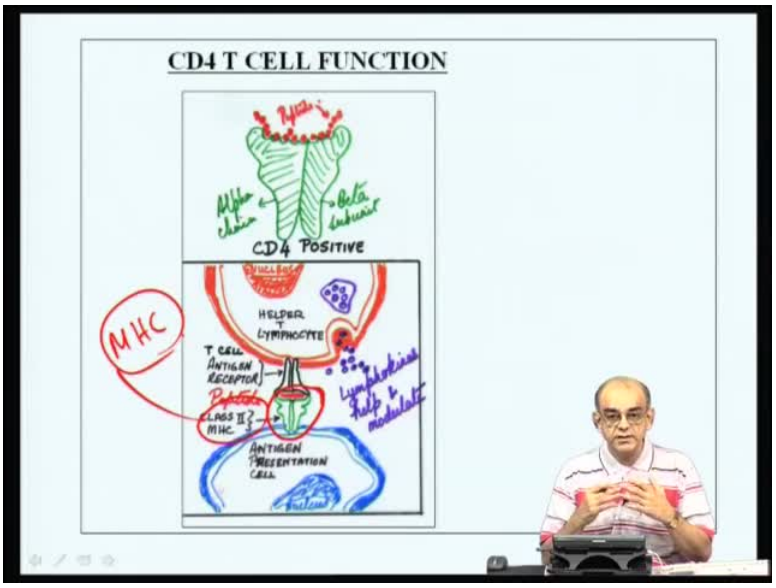

So, going on further, this particular heterodimer is what is depicted in an earlier slide, which I am showing you again which was consisting of two subunits over here and this subunit was is also the these two subunits are also the one that actually bind to the peptides that are generated within the macrophage lysosomes by protease action.

So, this is the structure that we are now going to discuss, which is actually binding the peptide antigen that is derived from the antigen that was endocytosed by the macrophage. So, you have these two subunits which are depicted in green over here. It is the same two green subunits and we call this and this as the class 2 MHC, class 2 because there are different types of major histocompatibility molecules. There are two types: class 1 and class 2.

So, before we try and understand what this major histocompatibility complex or MHC antigen is all about, we need to understand some of the historical aspects of how MHC was discovered. If you understand the historical aspects of how MHC was discovered, then you will understand the nature of self, anti-self reaction of the immune system.

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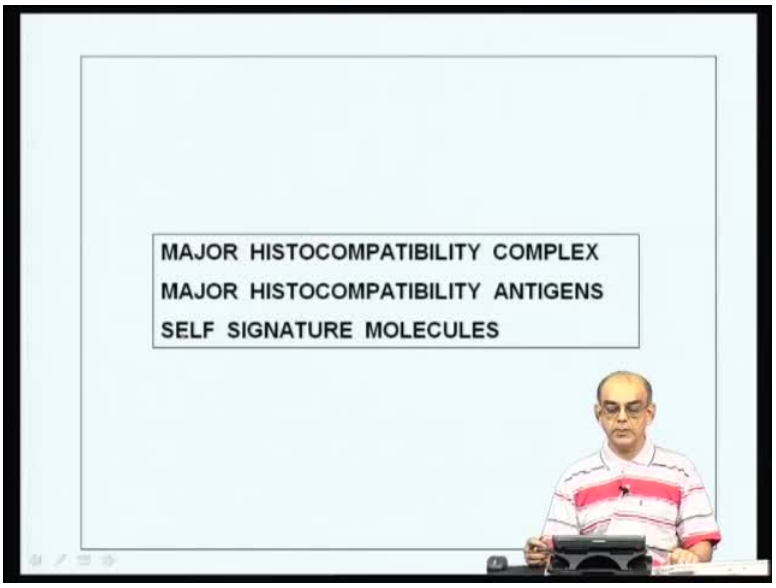

So, we come on then to these classes that are going to look into the major histocompatibility locus or the MHC genes, which actually give rise to the major histocompatibility antigens. These major histocompatibility antigens are the ones that are expressed on the cell surface of all of us, on all individuals, which are the self signature molecules. That means the very polymorphic nature, these antigens being polymorphic in nature also decide that they are the ones that are unique to each one of us and therefore, signify what is self in the body.

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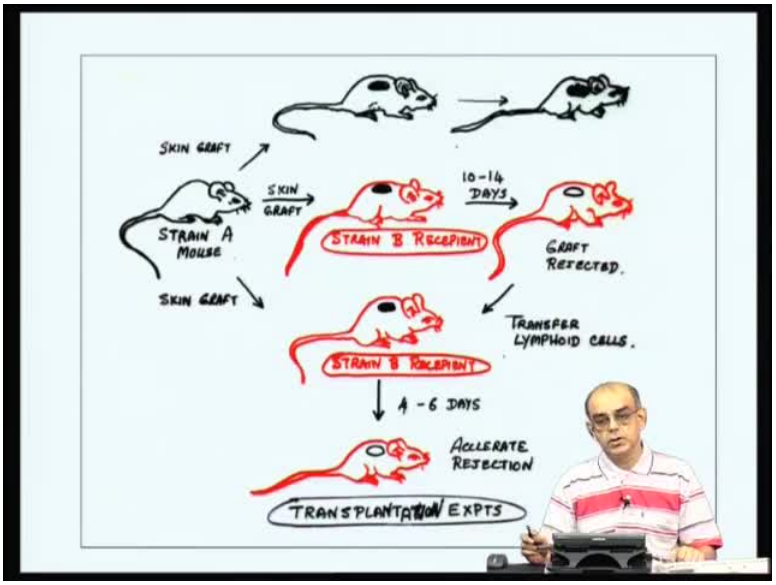

So, in order to understand this, we need to go back in history to understand some of the experiments that immunologists were doing. Now, as immunologists came to recognize the presence different kinds of cells in the immune system in the body humor or the humeral immune system and for that matter, the cellular immune system. So, they also saw that all these different types of animals that they were breeding in their laboratories or in their animal houses also had in many cases developed different kinds of tumors and tumors of course, an age old subject and age old phenomena. So, they were trying to find out how these tumor cells were being combated by the immune system.

So, what these immunologists were doing is used they used to take tumor bearing animals and some of the tumor's names that you perhaps have heard about is what are called as myelomas and which is actually the prelude of the hybridomas that are made in getting monoclonal antibodies, which you probably have heard about.

So, these tumors were being isolated from different mice or for different animals and they were trying to culture it in the lab by using different types of synthetic media that they were doing. For example, Ehrlich was establishing a tumor called as Ehrlich ascites tumor model. So, they used to take these tumors and inject it into mice and then see how the ascites or peritoneal fluid would contain the same kind of tumor cells.

So, in their efforts to try and find out, how these tumors grew in different mice, they found that the there was a genetic component or a genetic basis or a genetic way by which these tumors were actually growing in different types of mice. In other words, if they took these ascites that were forming in certain mice and injected into different kinds of mice or different other members in the colony, they would find that certain members of that colony would actually grow the ascites again and therefore, being a source again for getting more of these tumor cells, but several other members in the same colony would not grow these tumors. In fact, they would be cured of this injection of tumors.

So, therefore, they were taking such animals and trying to look at why these animals were not coming down with these tumors and there were breeding these animals and found that the some of the progeny that they obtained from these so called tumor resistant mice were also found to be resistant and therefore, the thought that there was a genetic component to it.

So, there were doing these experiments and they found the need for because it was a genetically determined or they thought it was a genetically determined phenomena, they came to the conclusion that they needed to have a model system, where different members of the mouse colony were genetically identical.

In other words, all the 40 chromosomes that are available in these mice had to be homozygous except for of course, the sex chromosome because the female has the XX and the male has the Y chromosome. So, again to repeat all members of a mouse colony had to be genetically homozygous. That means all the chromosomes had to be identical; all the genes had to be identical in their sequence of nucleotides. At that time of course, all that was available was outbred mice - mice that were actually got from people who are using mice as pets. They were they used to take They used to capture a mice from the from the fields. These wild type mice used to be cultured in laboratories or for that matter, for people who like them as pets.

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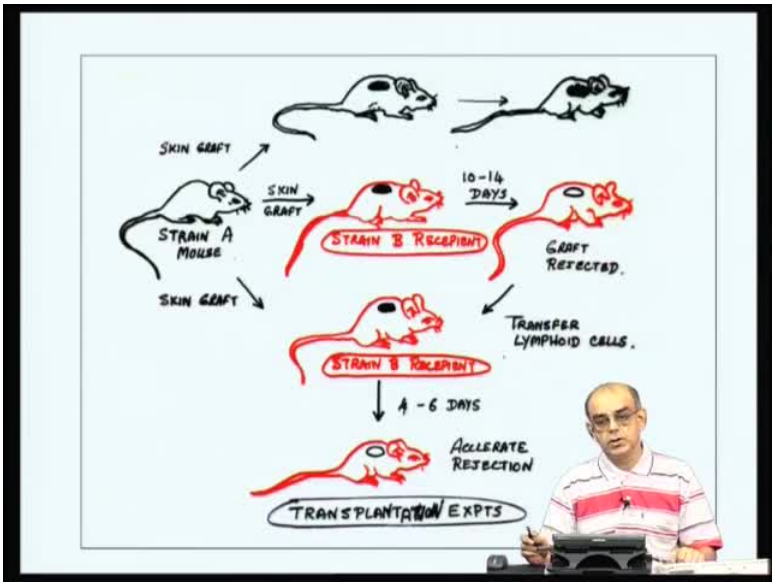

So and to There is another aspect to understand the concept of major histocompatibility. Why is this called as histocompatibility? So, this tumor graft, which they were using to inject into different mice, is also a kind of a graft Just like you just like in human they are giving kidney graft to people who do not have functional kidneys. You had experiments that were looking at graft rejection, means they used to do experiments which entailed the cutting of let us say, a piece of the tail and then putting it on the back of a different

mouse and then trying to see whether that piece of skin would actually survive on a different mouse, for example, in this case given by red. So, there were trying to see if tail pieces or tail grafts or for that matter pieces of skin that were taken from the back of these mice when they were sutured onto a different on the back of a different mouse, would they be functional or not.

Now, just to cut the story short, if you had different strains of mice in the laboratory and you did this experiment, where you took from a black strained mouse, which in this case I have put as a strain A mouse. If you took a piece of skin and sutured it on to either the same mouse or another black mouse in the same colony, you find that if it is the same strain of mouse that this particular strain, this particular piece of skin would actually get, it would merge with the other skin, the sutures can be removed and the circulation would be reestablished and that piece of skin would actually merge with the surrounding tissue and therefore, the mouse would not have any patch.

As opposed to the same piece of skin or a black skin being put on an albino mouse, they found that for a certain period of time the circulation would get established. This black skin would actually merge onto the white background or the white skin or the white fur, but after about 10 to 14 days there was something happening and this piece of skin would dry out and fall off.

This is basically a cell mediated phenomenon because of the rejection reactions that is orchestrated by this white mouse's lymphocytes. So, summarize it to say that this the lymphocytes in this white mouse would recognize this black skin as being non-self or foreign to itself and they would react against this and this reaction would cause the stoppage of circulation that had already been established in that 10 to 14 days and this piece of skin would fall off.

Now, if you were to do this again into for the same thing to this mouse, let us say a mouse that has rejected this black piece of skin and you again put sutured on another piece of skin onto this mouse, which had already rejected this black skin, you would find an accelerated response. In other words, there would be a immunological memory; remember, we went into this, when we said that patients who had suffered from small pox and had got over it would never ever again succumb to small pox. This was an established fact immemorial, from immemorial times.

So, this is a phenomenon of memory, which is very characteristic of immune responses. So, what they found was that such mice, which had rejected this particular skin, would have an accelerated rejection in a period of 4 to 6 days or about 7 days. So, instead of 14 days, in 7 days the skin would be rejected.

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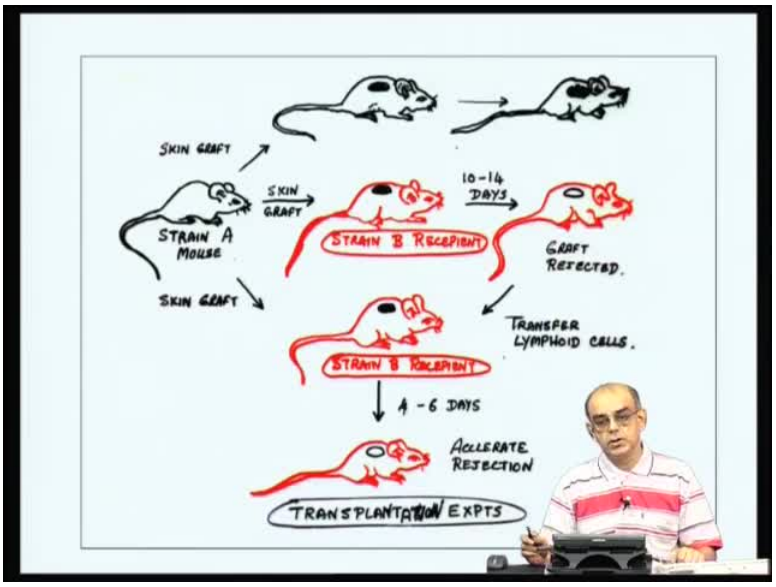

Now, the same thing would happen, if one was to take out lymphoid cells, which you can take by taking the spleen from these sacrifice, these white these white mice that were rejecting the black skin or you can bleed these mice and get the cells, but you will get a very small proportion of cell because 1 ml of blood is very difficult to take from a mouse; it would actually kill the mouse.

So, the number of cells that you take from 1 ml from these mice would also be very less. So, the best optimal way was to sacrifice this mouse and take the spleen cells; take the spleen, squash the spleen and in the spleen, as you saw in the earlier slide, had a lot B cells and T cells in it.

So, if you took this cell suspension, spin them down and inject it into a strain B recipient. It is called as a recipient because it is receiving these donor cells. After injecting the cells into this strain B recipient, which had not undergone any skin grafting, that means it was a naïve white mouse and into this you have injected cells that were derived from a white mouse that had rejected a black piece of skin.

So, you took such a recipient and then grafted on the black piece of skin and he found the same phenomena of accelerated rejection. In other words, that this kind of immunological memory that we spoke about was being transferred by these lymphoid or lymphocytes or splenocytes that were being injected into this mouse and therefore, they came to know that the skin graft rejection, skin the term is related to histo histocompatibility.

Therefore, major histocompatibility locus was something connected with the immune system and therefore, these are all the transplantation experiments that actually came about to say there was a genetic basis to it and this genetic basis was actually due to a locus called as the major histocompatibility locus or the major histocompatibility complex. It is called as a locus because they later on found that this particular rejection reactions was actually due to not just one gene, but a but due to a complex of genes or a complex of protein antigens that was being coded for by different number of several genes and therefore, it was termed as the major histocompatibility locus.

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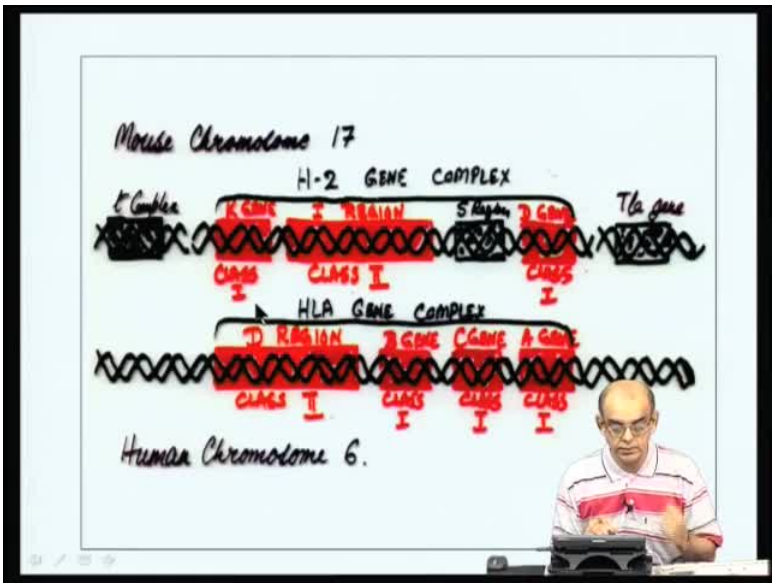

Now, in order to understand, what these antigens are, let me give you a little introduction before we go on to the history of how MHC antigens were discovered by breeding inbred strains of mice. What do you mean by inbred strain? Inbred strains of mice are these mice strains, where all the genetic material, that means all the chromosomes were homozygous. So, if you were to take different members in a colony, let us say in a mouse

cage, which was holding different inbred different mice of the same inbred strain, and checked their DNA, you would find that all the DNA would be the same. So, they were all homozygous; that is what is an inbred strain of mouse.

Before we go onto that as to how they made these inbred strains of mice, we will just tell you a little about this major histocompatibility locus. This major histocompatibility locus in the mouse is present in chromosome number 17 and as I told you, in the mouse, there are at least about 40 chromosomes and in the human, it is found in human chromosome number 6 and it is just a small little region in human chromosome number 6 or small little region in mouse chromosome number 17 and in humans, the number of chromosomes are 46.

So, what you must realize is that all the history of these immunogenetics experiments, actually identified this particular small little region that is found in one chromosome; in humans that is number 6 out of this 46 chromosomes and therefore, you need to go into all the details of the history of immunogenetics, in order to understand, better understand how MHC was discovered. So, this locus actually consists of different sub loci, which is why you have two types of MHC antigens as I told you earlier.

In the mouse, this MHC is written as H dash 2. This is the way in the mouse it is written and in the humans, it is called as HLA standing for human lymphocyte antigen gene complex and in the humans also, there are two types of HLA antigens or HLA genes that code for them, called as the class 1, which is these three one these three genes, these three loci over here and the class 2 which is shown over here, which is also called as HLA D region as opposed to HLA A, B and C which form the class 1 complex or the class 1 type of MHC.

In the mouse, it is called as the K region and the D region, which forms the class 1 molecules and the class 2 locus that is made up by what is called as the I region. So, suffice it for now just to indicate that all the next series of slides, we will try and show you how they identified this small little region, which is called as the MHC locus that is present on one chromosome, out of the number of chromosomes that are there either in the humans or in the mouse.

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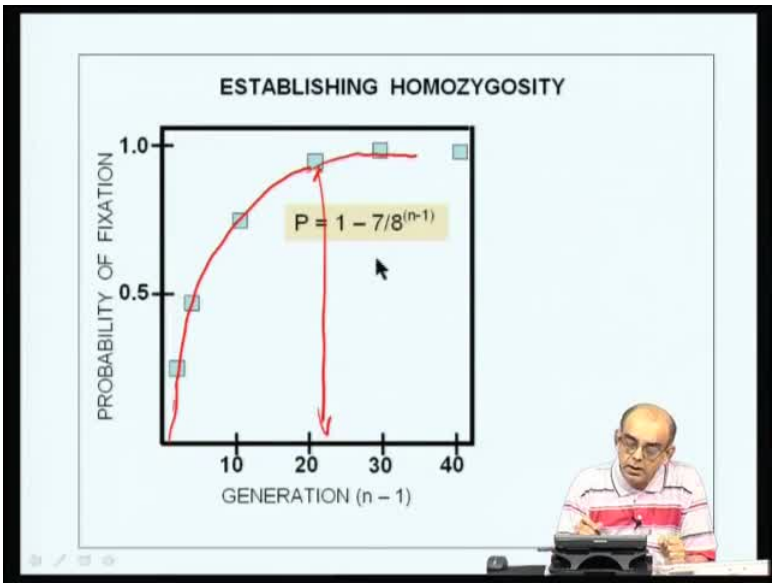

So, in order to understand this, we need to understand certain statistical principles, which were looked at during those times, when they said that there was a genetic basis to skin graft rejection or for that matter, tumor graft rejection, tumor rejection as I told you in the case of ascites. So, what they since they knew that there was a genetic component to it, they looked at statics and they said that there is a way to establish homozygosity, if you were to trap a wild mouse population.

So, you had have the male mouse, male wild mouse and the male female wild mouse. So, you made them and then tried to see, look at the progeny. Every mouse pair will give rise to at least some ranging from 5 to 10 pups or 5 to 10 progeny, which are called as mouse pups.

So, one could actually breed these mice and the basis, the statistical basis upon which they did was that if you went on doing a brother, sister mating; that means, a pair gives rise to 10 pups and out of those 10 pups, they are all brothers and sisters depending upon whether it is a male or a female. You took these brothers and sisters and mated them again and so on and so forth.

So, according to genetics, you will have F1, F2, F3, F4, all these generations will come as you know will happen in genetics. So, when you do these such a sort of brother sister mating, this is very, very important in establishing an inbred strain of mouse. If you did this brother sister mating, they found according to statistical principle, you had the

probability of fixation. Fixation, which is put on the y axis and the number of generations, that means the number of times these pairs were bred, brothers sisters were bred; so, they put this on the x axis and they had a formula, statistical formula that told them that if you went on doing this with brother sister mating, you could establish homozygosity in all the chromosomes, in all the genetic material except for the sex chromosome by this particular graph, which is given by this particular graph.

So, you see probability of fixation means establishing homozygosity - fixing a locus to become homozygous or becoming same in different members of the same inbred strain. So, this comes out to be something like this. So, you see that it becomes or it reaches a plateau by about 20 generations. So, you will see about greater than 20 generations will reach a probability of one, 1 being the homozygous point. So, if you took a pair of mice wild type mice, male and female bred them, took their progeny, brother-sister, mated them for 25 generations, you would make all the members in that colony homozygous in nature.

So, what was this formula that is given over here indicate. So, this formula indicates P standing for probability of fixing a locus or becoming homozygous. P equals 1 minus 7 divided by 8 to the power n minus 1, where n is the number of generations that you have bred these mice. So, how was this formula calculated and how this formula was derived? So, in order to do that let us go into the next slide.

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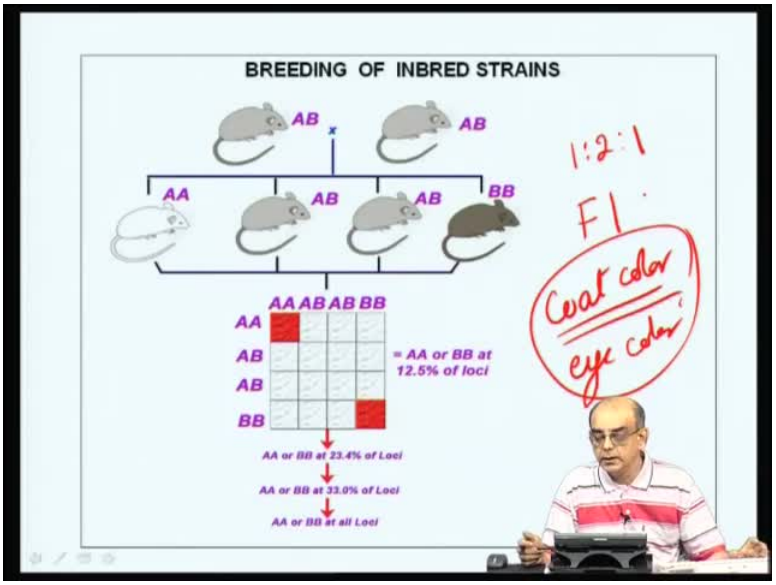

So, this slide now, depicts just for convenience sake, just two particular characters or two particular loci that you would like to follow, like you do in genetic text books. So, if you were to take wild type mouse, they would be heterozygous for these particular characters. So, you have A and B just to just for an example. So, this is also heterozygous because they are wild type mice. When you bred them, these characters would segregate in a mendelian ratio, which is 1 is to 2 is to 1 and therefore, you would have this 1 becoming homozygous AA, and AB, being the heterozygous ones, so 1 is to 2 is to 1, which is the other homozygous partner to that.

So how was this? Now, if you were to take, this is actually the F1 generation. So, this is the F1 generation and in the F1, you have a ratio of 1 is to 2 is to 1. Now, another important point that one follows in all these breedings is the coat colour. So, all these breedings of inbred strains actually, looked at the coat colour of these mice. So, you could look at how you bred white mice with albino mice and look at how these colours segregated; not only coat colour, they were also monitoring the eye colour. So, you had the eye colour being determined by these various kinds of breeding and therefore, these loci determining these phenotypic characters

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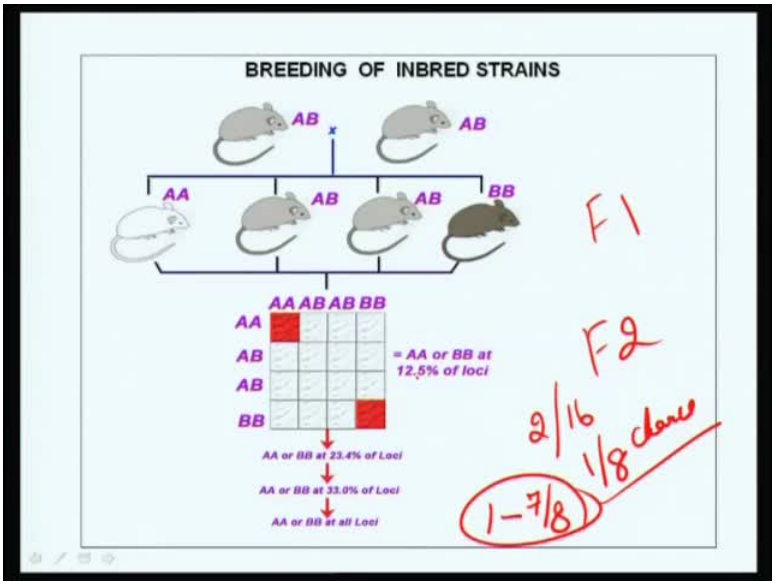

So, what happened during these generations? As I told you earlier, that this is the F1 generation. So, you took brother and sisters from this F1 generation and mated them

together. When you mated them together, you had this checker board, which all of you know about in genetics classes.

So, you had this AA, AB, AB and BB. So, you put them on the top and you had of course, on the left side, the same thing and when you looked at this mating, you would have homozygosity over here and homozygosity over here and all the rest of them being heterozygous.

So, how is this brought about? How is this now to be designated? So, you had two of these squares; out of it total of 4 into 4. So, you had 2 out of 16 becoming homozygous and therefore, this is actually a 1 by 8 chance that you have of making this particular mating or the progenies that is derived from them, becoming homozygous.

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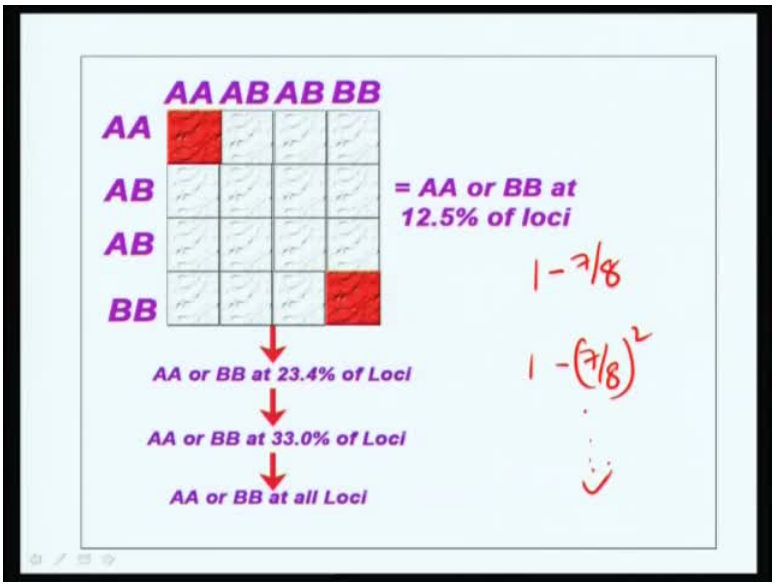

So therefore, it was 2 of 16 and therefore, when you have fractions, you actually designated as 1 being the total. So, you had 1 and the rest being 7 by 8 or 12.5 percent of the loci every time you bred it. So, going on, you went on further to look at this. So, you had 12.5 percent in the first F1 generation. You took members of this F1 generation and mated them again. So, therefore, the probability of more loci becoming homozygous increased, which went to, this is actually 1 by 7 by 8. Now, it became 1 7 by 8 to the power 2 and so on and so forth.

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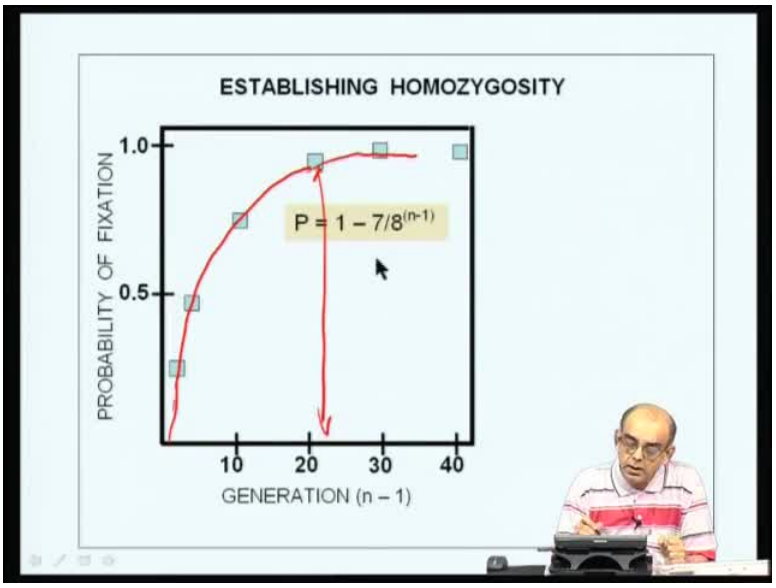

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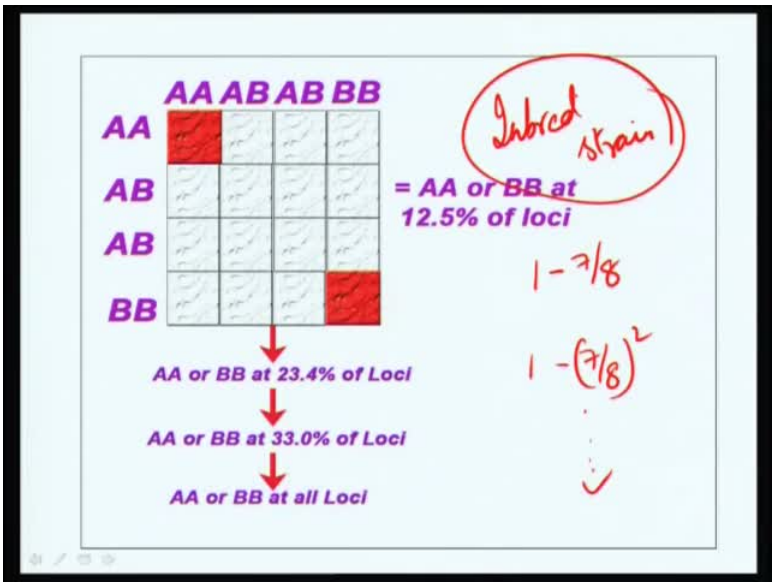

So, this actually worked out to the formula that you saw in the earlier transparency here, which is actually, 1 minus 7 divided by 8 to the power n minus 1 generations. So, as you went on, according to this principle, by 25 generations, you had all the members becoming homozygous within each other. So, this was actually called as the inbred strain of mice.

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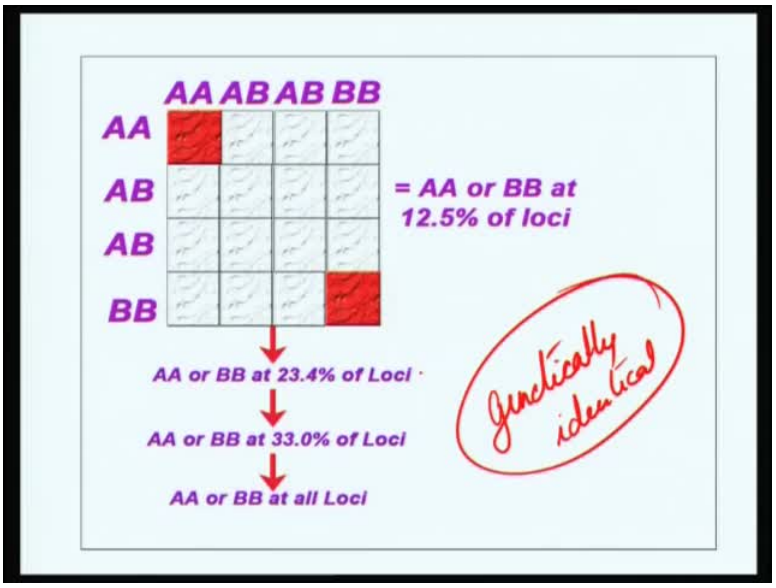

So, if you were to do this with a wild mouse pair, you would establish an inbred strain colony. So, what is this inbred strain? This inbred strain is nothing, but a mouse colony where they are genetically identical, where different members of that colony are genetically identical between members of the same species, as I told you, except for the sex chromosome or the Y chromosome.

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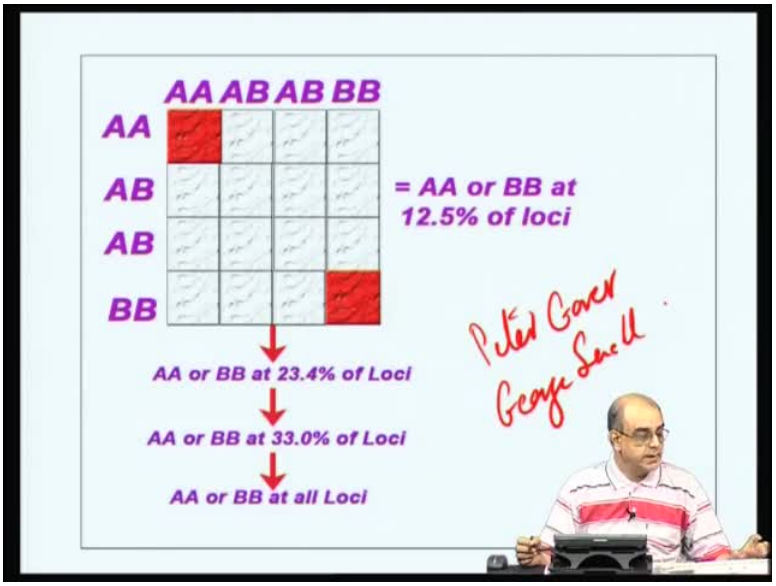

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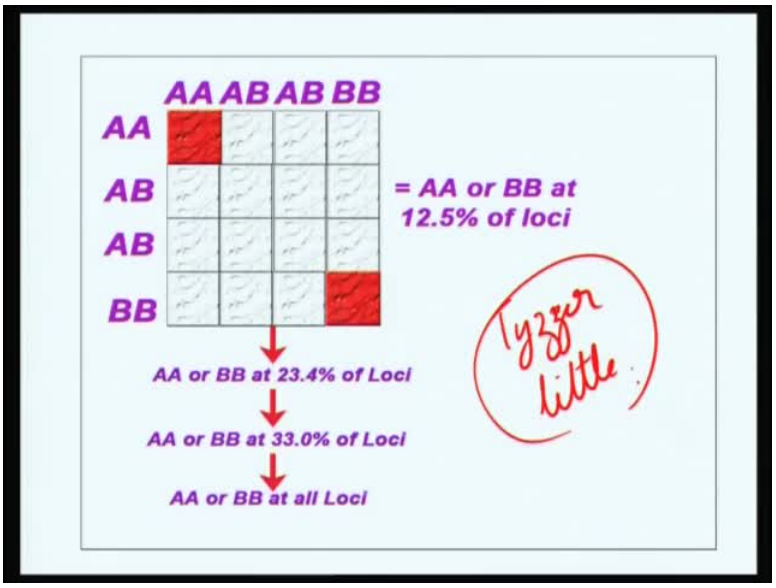

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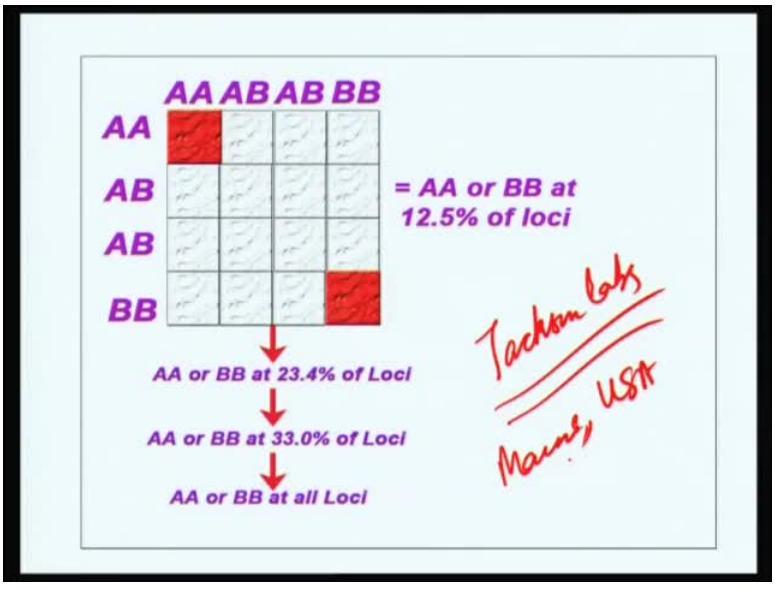

So, what experiments were did? This was actually set about in the 1940s. Peter Gorer is a very big name in immunogenetics. Peter Gorer and George Snell, also you have names like Jean Dausset and Baruj Benacerraf, but the actual experiments of breeding were started by two scientists called as Tyzzer and Little. How did they go about doing this? So, they went about by getting these mice that were kept as pets by different mouse dealers, not only in the United States, but in different regions of the world. By doing all these different kinds of experiments, they actually setup what is called as the Jackson

Labs, which has become very famous these days for the supply of different strains of mice which is located in Maine, USA.

So, this is the lab or Jackson Labs are the ones that are actually responsible for distributing these different kinds of inbred mouse strains and of course, all over the world, nowadays, you have other centers, which are having these inbred mouse strains.

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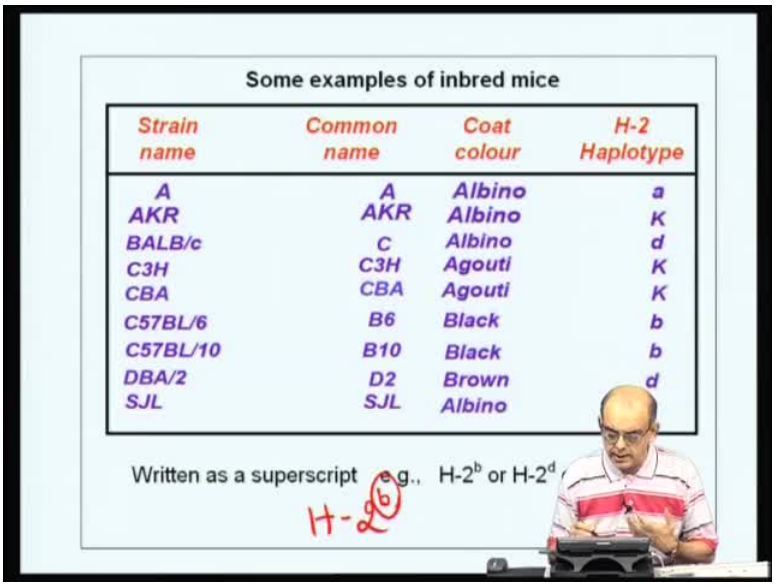

So, to go on further, some of the mouse examples of some of these different kinds of mouse strains are designated over here. As I told you earlier that this particular the way you write these particular inbred strains of mice is by writing H dash 2 and you put a subscript along superscript on just by the side of H 2. These alphabets were actually named or derived as the breeding process went on.

So, during this breeding process, the experiments were being done to see, whether these different members of these species or the strain that were there, all of this done was done with of course, the mouse, and these different strains, where they rejecting skin grafts and other types of techniques that people were doing. And as and when they made a homozygous strain, they would put different alphabets to this and in small caps. So, you had H 2 b and you had H 2 a, H 2 k, H 2 d and so on and so forth and as I told you, coat colour was one of the important criteria by which they used to follow these mice also.

So, these are some of the common strains that are available nowadays. These C57 black 6 and black 10 are black in color. So, you see here and then DBAs are brown in colour, brown fur and of course, there are albino mice which is BALB c, which is the normal type of mouse that you make monoclonal antibodies with and these are all the different kind of what is called as a haplotype.

A haplotype is nothing, but a letter or something that designates the genetic component to that particular mouse strain. So, you say that it is a particular genotype or a particular haplotype and all these means is that when you say b over here, it means that the primary amino acid sequence of the MHC antigens that are there on the cell surface is of a certain sequence, which is very much different from a different haplotype, for example, a.

So, suffice it now to tell you that we have begun to try and see how the MHC was discovered and one of the aspects of this particular sequence of lectures is to try and understand that MHC was discovered only when inbred strains of mouse were made because you needed to have genetically homozygous strains of mice, in order to say that skin graft rejection depended upon a certain locus in the different chromosomes and the next series of breeding experiments will tell us how they identified the actual region, the MHC locus that was responsible for skin graft rejection.

So, we will now leave it at that and go on to the next class later on and to see how these inbreeding experiments actually, told scientists as to what this H 2 locus was or MHC locus was and how they identified the nature of the MHC antigen. Thank you very much.