Essentials in Immunology

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Module No. # 19

Lecture No. # 36

Transplantation immunology

Hello, welcome to this lecture on Transplantation and how the immune responses are involved in graft rejection or graft acceptance reactions. To understand this lecture, we will go through some basic studies that were done earlier and were also covered the class on major histocompatibility complex and how inbred strains were made.

Later on, we will go briefly into some of the ways that these rejection reactions can be controlled, in order to facilitate the acceptance of skin graft. After all as we realize now, the availability of different grafts whether organ or skin grafts, depends upon the availability of suitable donors. And donors are very hard to combine, even if they are presented a particular time and location. The matching of the tissue is very important in order to facilitate acceptance of skin grafts. Therefore, there seem to be a tendency for taking grafts from cadavers, rather than from living donors although living donors are preferred.

So, some coverage of some of the basic aspects of why tissues or organs are rejected will help us understand, some of the strategies that are being employed for enabling the graft acceptance, so that more grafts can be, organs can be transplanted to those who require it.

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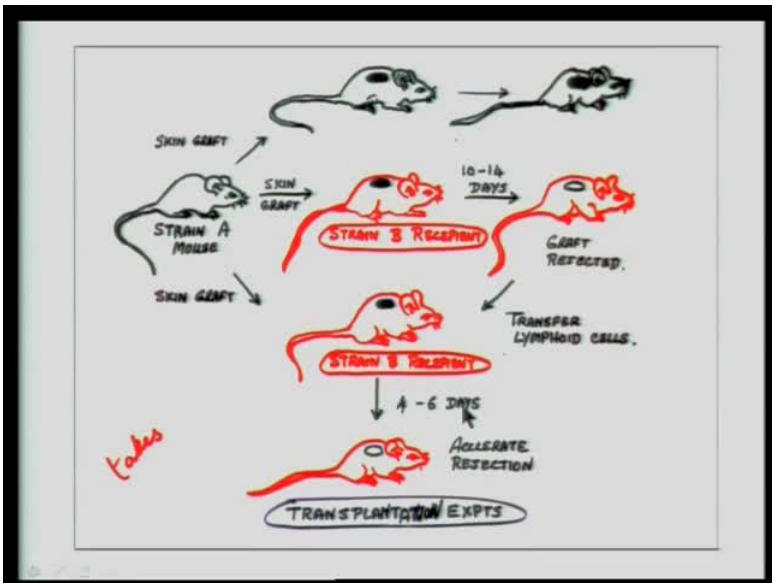

So, basically to recap some of the studies that were done in mice earlier was the fact that different inbred strains of mice, individual belonging to different inbred strains of mice actually rejects skin grafts, if they are grafted on to the back of the mouse. Usually, they use skin on the back or a piece of tail, that has been got from the tail and then graft it onto the tail of the other inbred strains of mouse. I mean, the studies or the lectures, that were given about inbred strains of mice must be reviewed in order to understand the slide. And basically here, what I show is a black mouse, an example of a black inbred strain of mouse is c 57 black 6 and then you have a red colored mouse here, let us call it as the white mouse or the albino mouse, which is characterized by BALB c or NIH mice as inbred strains of mice.

So, if a piece of black skin were to be grafted onto the white mouse, here it is called as the strain B recipient. Recipient is one that receives the graft and a donor is one that donates the graft, whether it is a skin or whether it is an organ. So, when the strain A mouse donates this graft and this is sutured onto the back of the white mouse, normally this black piece of skin actually a word that is used takes, just one moment.

So, they say the graft takes which means that, the graft gets fused onto the other piece of skin, the circulation is re restored and therefore, the cells inside the graft actually start to proliferate and then actually fused onto the background skin.

Now, after a period of 10 to 14 days, something ensues which is mainly a T cell mediated response, where the T cells are come to recognize that this piece of skin is expressing foreign antigens. Foreign with respect to the major histocompatibility complex, that is because the recipient cells or the antigen presenting cells have actually taken up some of these antigens that are there in the piece of skin that was grafted and they have been presented via dendritic cells to the T cells of the recipient.

In addition to that, there are other aspects that are recognized by T cells, which will go into later on in the coming few slides and that has to deal with the recognition of the major histocompatibility complex class 1 MHC. Once this graft fuses onto the background skin, the T cells that are now activated that are actively proliferating and have recognize the presence of this foreign skin start to react against the skin and activate inflammatory processes, which cuts off the circulation to this piece of skin.

Inflammatory processes are generally the same of kind of processes that occur, when you have inflammation going on during infection or other kinds of immune responses. So, within a period of 10 to 14 days, this graft because the circulation has been cut off, the graft loses its circulation and becomes dry and slowly starts to die and necrose. And after a period of time, it becomes dry and the whole piece of skin just falls off from the back of that of the grafted mouse leaving an empty area and exposing the muscle underneath. Therefore, this period of primary graft rejection takes about 10 to 14 days in the mice, which is due to a primary anti graft response.

Now, if one takes lymphoid cells, specially T cells from this mouse and adaptively transfers it, in other words take these cells and inject it via the tail vein into a naïve mouse or a mouse that has not been exposed to this black skin, after which you take the same piece of skin from the black mouse and graft it onto the back of this white mouse. You find that instead of 10 to 14 days, a graft is rejected within 4 to 6 days. Reminiscent of our lectures signifying that, this is the memory response and this memory was actually transferred because these lymphoid cells, especially studies have been done to show that a majority of these reactions are due to the transfer of T cells. Although antibodies that are raised against these piece of skin, can also mediate graft rejection reactions by accelerating complement mediated lysis as well as other kinds of reactions.

So, this acceleration of reaction is due to a secondary response. So, this is the basic graft rejection response that is studied in mice and these are the studies that were used in order to characterize the H-2 complex or the MHC complex in the mouse.

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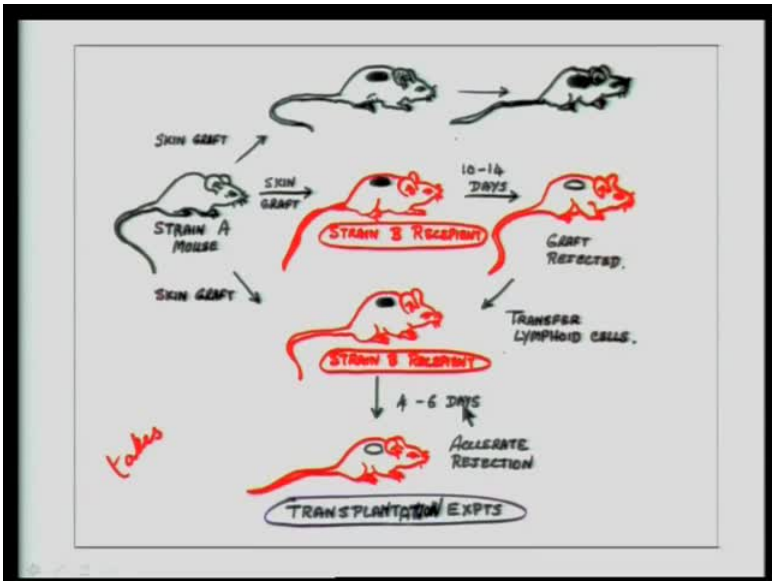

Now, all this lead to the concept of course, that the self is recognized within the body's immune system and therefore, these cells when they recognize something coming from outside is considered as foreign. To the concept of foreign, self and non self now needs to be defined in a different way and that is something to do with the MHC per say as well as a pathogen coming in. And we all know that, the pathogen that infects an animal can infect can be presented in two different ways and that is the class 1 mediated response and the class 2 mediated antigen presentation.

So, a class 1 mediated antigen presentation involves endogenous virus or pathogen replication within a cell. So, if a virus invades or infects a cell, and the virus proteins are made within the cell, these cells are processed and presented by the antigen presentation machinery via the class 1 molecules. Just to summarize it, the viral antigens are broken up into smaller peptides and these peptides are transported onto the MHC groove of the class 1 MHC, which is expressed on the cell surface and these peptides are usually 9 amino acids in length.

As oppose to viral toxins or viral antigens that are circulating in the serum are taken up by antigen presenting cells by micro pinocytosis or endocytosis or during phagocytosis

of the pathogens, so that they are treated as exogenous antigens and the processing and presentation occurs through a different pathway called as a class 2 antigen presentation pathway, in which these different antigens are again clipped into smaller fragments which are then loaded onto the class 2 type of MHC molecules.

In other words, the class 2 type of antigen presentation involves exogenous antigen presentation, because the antigen is derived from outside and whether its class 1 or class 2 mediated antigen presentation, the ensuing immunological events are different in terms of activation of different kinds of T helper cells and the kind of pro or anti inflammatory responses or cytokine release that happen later on due to this stimulation.

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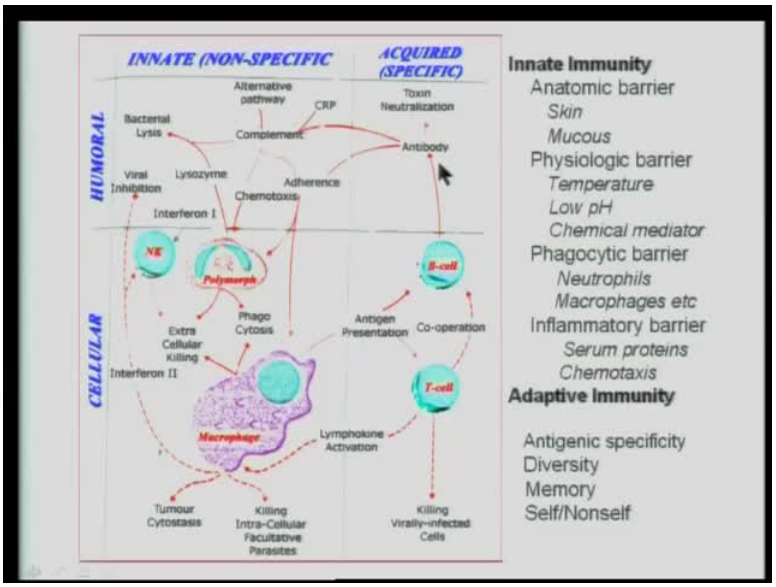

So, just to recap some of the lectures that were done earlier or to recap some of the inflammation, that is required to understand this lecture is that, the adaptive immune response or the acquired immune response requires T B cooperation and this T B cooperation requires this antigen presentation. Antigen presentation of course requires the participation of MHC molecules, which are yourself signature molecules and distinguish self from non self.

So, these presented antigens then tell the B cells to make or activated B cells to make antibodies against different kinds of antigens. So, how does a virus or a virus infected cell get recognized by this sort of acquired immune response?

So, when there is a transplantation process going on or in grafting procedures, one has to take into account these sort of responses that the T cells orchestrate to the foreign MHC or the foreign incoming organ and of course because this sort of acquired immune response is involving a variety of cytokine release as well as chemotaxis of various kinds of cells, one has to look at how these kinds of processes can be inhibited so that the graft survives.

In fact, later studies or modern days studies have revealed that, immunosuppression is a better way to go about trying to make the graft survive, rather than to try and find a 100 percent matching organ for the recipient, because 100 percent matching is only possible in identical twins and this is not possible with any other donor; although siblings and relatives can be better donors, than other kinds of donors who are not relatives. So, this is the concept of tissue, typing will come to that in a little while.

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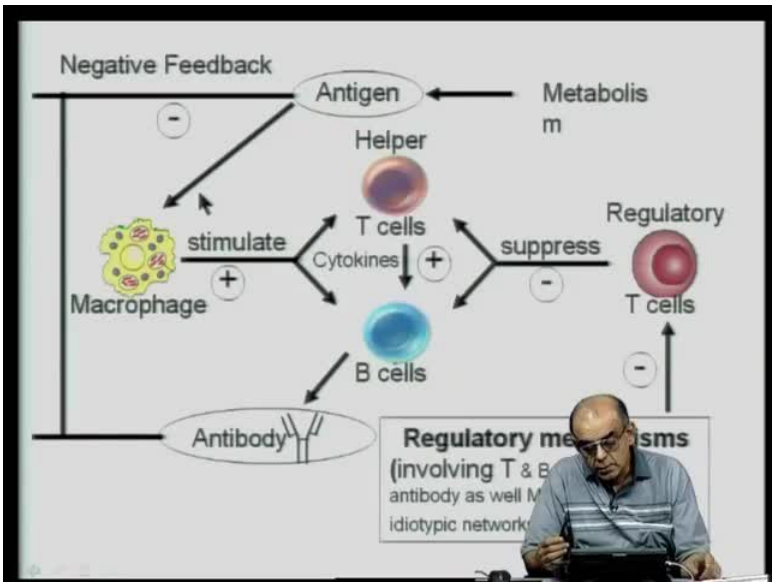

So, if you look at how T cells and B cells cooperate, they cooperate by the secretion of cytokines. Therefore, if you alter the secretion of the cytokines, then try to use agents or chemicals that will suppress the formation of cytokines that activate T cells and B cells. Then you have a muted response to the incoming organ, so that is one way of trying to combat organ specific allotransplantation reactions within the recipient.

The other way of course is the discovery of regulatory T cells or T reg cells, can these T reg cells somehow be stimulated in order to suppress ongoing T cell, B cell interactions

as well as T helper responses. So, these are the two ways by which these regulatory mechanisms can be tinkered around with in order to enhance the period of survival of an incoming graft when it is not a 100 percent match.

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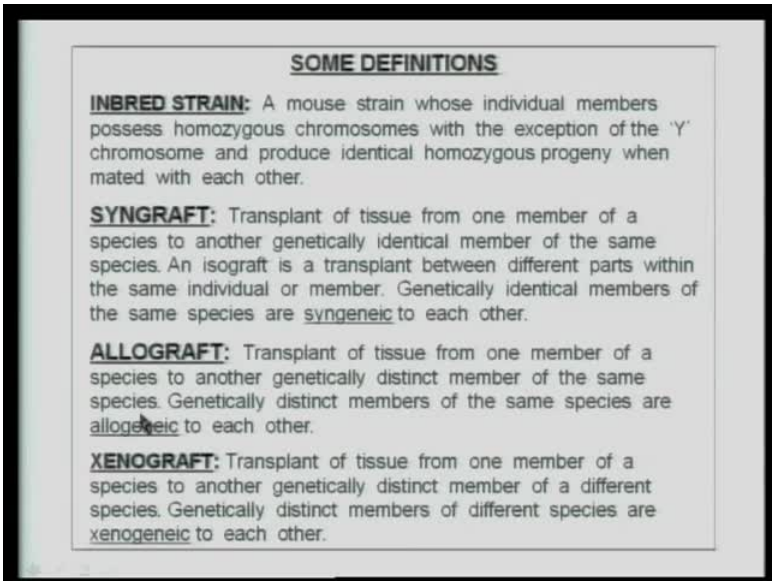

So, some of the definitions are in order and as we saw earlier, a mouse strain whose individual members possess homozygous chromosomes that means, all the chromosomes are identical with the exception of the Y chromosome. Now, these inbred strains produce identical homozygous progeny, when they are mated with each other and these inbred strains of mice can be obtained by brother sister mating, which is based upon a statistical principle of mating more than 10 to 15 time brother sister matings.

As oppose to an inbred strain of mice, whose chromosomes are identical except for the Y chromosome, wild mouse populations as well as human populations cannot be are not inbred and therefore, there is a variety or a wide variation in how in different kinds of the sequences that are present within the genome of individuals and therefore, they cannot be considered as inbred.

So, in order to understand this graft because we are going to deal with grafts, we need to understand, the terms that are used with regard to grafts. Here we see, what is a syngeneic graft? A syngeneic graft is the transplant of tissue from one member of a species to another genetically identical member of the same species.

An isograft is a transplant between different parts within the same individual or member, in other words, if I take a piece of skin from one from my left hand and put it onto my right hand, then it is an isograft. This is one of the procedures that is followed in burn victims, so when patients coming with serious burns, they take the piece of skin from the unburnt area of patients and then, try to grow it within the lab on synthetic media in order to increase their mass, if that is not possible, they take that piece of skin and put it onto the area, where there are burns. This is what plastic surgeons do, in order to see how beautify the area which is burnt.

In other instances, during a bypass, where you take the veins that are from the leg and then put it on to the place, where it is required in the heart in order to takeout those portions which are blocked and therefore, restore the coronary circulations so that the heart can keep pumping.

The other term that needs to be understood over here is what is called as a allograft. What is an allograft? Allograft is a transplant of tissue from one member of a species to another genetically distinct member of the same species. And this is where organ transplantation in humans comes in. For example, if one takes heart from my body or a piece of kidney organ from my body put it onto another person, then you have allograft. So, these allografts are usually expressing our self signature molecules, in fact the class 1 MHC antigen is ubiquitously expressed all cells of the body except some of them, some of the cells like the reproductive organs and the brain.

So, genetically distinct members of the same species are allogenic to each other. So, we in the human population are actually allogenic to each other and therefore, grafts that are taken from individuals are allogenic.

Now, the other one that is comes needs to be defined is the xenograft. Xenograft is the transplant of tissue from one member of a species to another genetically distinct member of a different species. For example, mythologically we always celebrate Lord Ganesh festival and there you have actually a xenograft, the head of an elephant on top of a godly human, let us say.

So, these are actually genetically distinct members of different species, they are said to be xenogeneic to each other. The reason why this term needed to be defined is that, nowadays the medical fraternity is resorting to more and more of trying to see, if you can

take organs from pigs and graft it onto humans because pigs in many respects are supposed to be closer in respect of molecular expression, surface expression or different kinds of molecules, they are supposed to be closer humans. In which case, immunosuppression becomes a very important part of transplantation, also they are resorting to pigs again because trying to get a tissue match, trying to get a donor organ all the time when you require it is always not possible. So, the idea is to try something that is xenogeneic like from the pig and enhance the life of that individual for how many ever years that the medical people can.

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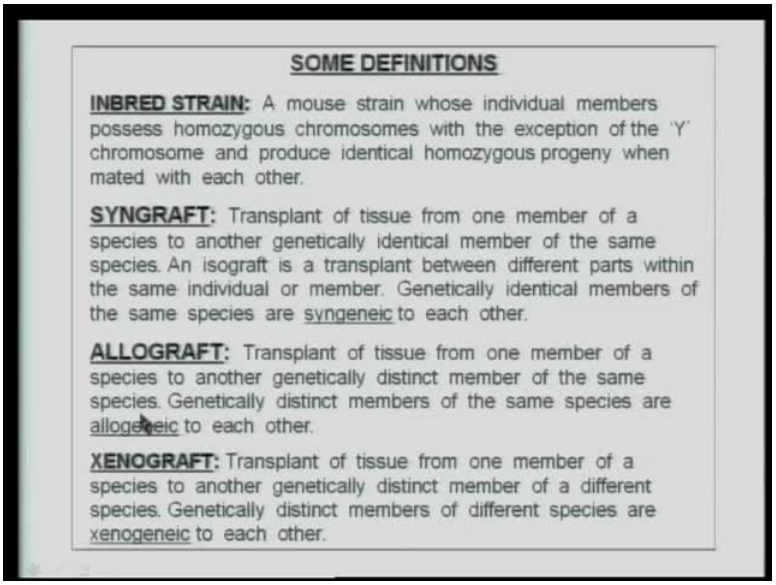

So going on, some other points that we need to consider is that, the MHC is polymorphic. The presence of strain specific antisera has been these are all reagents that were used to show the presence of cell surface antigen and they showed that this cell surface antigen is actually highly polymorphic. In other words, their alternatives form of the gene or alleles and this is the concept of the cell signature molecule of the MHC complex. And if one were to compare, the primary amino acid sequence of the MHC molecules that are present within two different individuals or human individuals, you would find that this primary amino acid sequence as well as the primary nucleotide or the nucleotide sequence of that gene is going to be very different and that is what makes the MHC polymorphic and it being self signature molecule.

So, the important concept arises now in order to understand, the concept of anti graft rejection reactions. That is the expression of these molecules on the cell surface is codominant. Codominant meaning is that, we need to remember that we inherit half our traits from our parents, half from the mother or maternal and half from the father or paternal.

So, you see even in the MHC, the MHC molecules half of them are derived from your father and half from the mother. And both of these molecule the MHC alleles that are derived from the father and the mother is actually known to be expressed right from very early embryonic stages, somewhat later than 8 cell stages. However, the fetus is never rejected or not subject to harmful immune reactions by a variety of mechanisms which is a field in itself.

So, going back to codominancy, which is meaning of course that since you derive your MHC antigens from your father and mother, your antigens are both the types of antigens that are coming from the father and the mother are expressed codominantly. In other words, both of these molecules are expressed on every cell surface. So, if you have different class 1 alleles, then these different alleles are expressed on your cell on every since they are ubiquitous, they are expressed on more cells of your body.

So, both maternal and paternal gene derived proteins are expressed on the same cell, this is the concept of codominant expression. In outbred populations, the progeny are heterozygous, whereas in inbred strains of mice the maternal and paternal H-2 loci are homozygous because their genetic sequences are identical. Therefore, the MHC molecules that they express will have identical structure as oppose to outbred population or and a population that is inbred. In fact, this is how the MHC function was discovered because in the strains of mice were bred.

So, bough primary, secondary and tertiary structures could be slightly different, in different individuals. Primary of course definitely being different, but there is overall as an MHC NIS to the MHC molecule and that is where you have what you call as an allo recognition phenomenon, which will come into in the next few slides.

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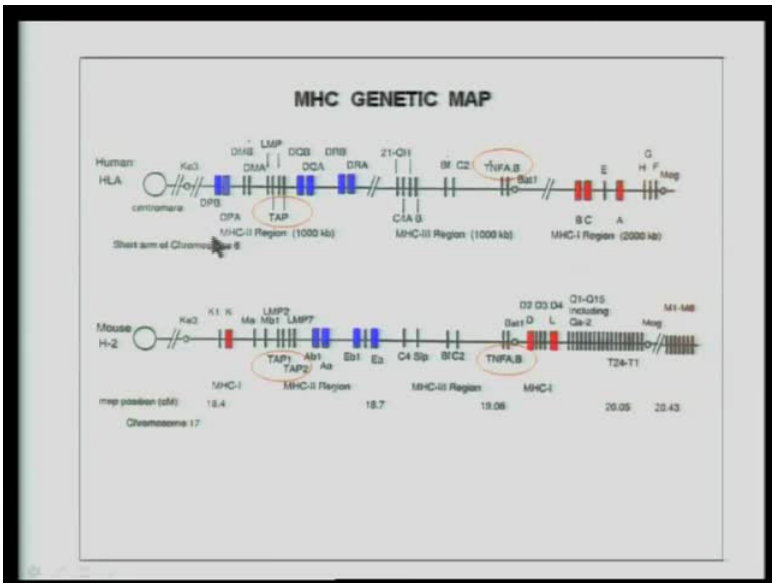

So, going to the MHC genetic map, you find of course this genetic map shows you that there are different kinds of MHC antigens as I told you, class 1 and class 2 antigens. Class 1 being signified by the red rectangles over here called as the A, B and C, so HLA A, HLA-B and HLA C are class 1 molecules. As oppose to class 2 molecules which are shown over here, so you have HLA DP, DQ and DR, so you have DQ over here and you have DR and DP. So, you have different alleles, so there are different there are polymorphic, since they are polymorphic, they are going to have different sequences and therefore a different alleles are going to be expressed on the cell surface.

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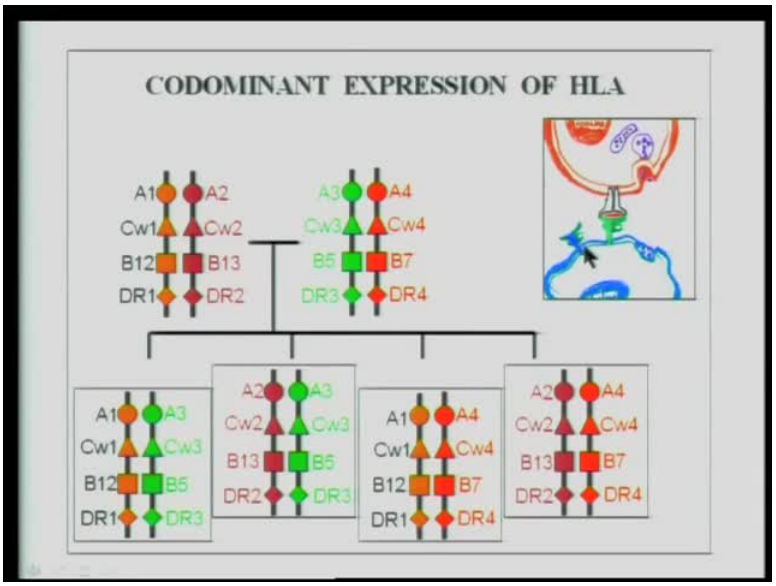

So, again trying to explain this codominancy in expression, so these are the parents let us say, the male and the paternal, and the father and the mother and you see that, I put here for this is just for explanation sake, you have HLA-A, HLA-B, HLA C and HLA DR. So, these are all the numbers that are given to various alleles that have been discovered and known to be present, so these numbers signify a certain nucleotide sequence in the gene of that particular allele.

W stands for workshop, before it has been recognized as a definitive allele. So, if you look at these two sequences or these two alleles that are present in the father and the mother. Now, you have children, these children can have a combination as you know from mendelian inheritance. For example, here I have designated A1 from the orange color is combined with the A3.

So, A1 and A3 or it can be A2 and A3, which is given over here. So, these are all tightly linked loci, so many of these are actually coming together, so you will see that A2 Cw C 2 B13 and DR2 come are inherited as a complex because they are tightly linked. Occasionally of course, many times of course, this sort of lets there could be a recombination within the MHC instead of DR2, you can have DR1 or if the parents are somewhat different having a different allele, they can have different alleles coming in. And so, you have a recombination within the MHC complex, but will not go into that just to show you to drive home the concept that, this sort of inheritance of these alleles of the MHC alleles, let us see what happens at the level of the cell surface.

So, what you see over here is, I have designated this green as the A3 allele (Refer slide Time: 26:33). So, if you take the parent over here, you find that the A3 allele is expressed as this is a class 1 molecule, this being the beta 2 M and this being the heavy chain, both of them put together of course hold the peptide bound to its MHC binding groove and this entire complex is recognized by the T cell receptor of the incoming or the recognizing T cell.

This of course is the antigen presenting cell or the antigen bearing cell, which are whatever you can consider as, if it class 2, it is antigen presenting cell and in the case of class 1, it is also known as antigen presenting cell, but many of these cells expressed these MHC molecules on the cell surface.

So, what you see over here which is now, I have put it in a different color is trying to represent that, this allele is different from the one in green. So, based upon what sort of allele is there, let us say for example, if you take this parent A4, this designates A4. So, you have both A3 as well as A4 class 1 molecules being expressed on the surface of the cell. And therefore, the peptide that is bound to this different MHC class 1 molecule of this different allele, will also be different and a T cell receptor from a non self individual or let us say, you have a T cell receptor that recognizes A3 as self, then the same T cell receptor will recognize A4 as non self.

So, you see how the self and non self recognition comes into play over here. So, in a certain individual like for example in this parent A3 and A4 are expressed on the surface of a single cell, the thymic differentiation has educated the T cells within that body to recognize A3 and A4 as self. Therefore, when A3 and A4 present peptides, the T cell starts to react and recognize the peptide.

In addition to recognizing this peptide associated MHC class 1, the T cell receptor also recognizes the alpha helices on the sides of this class 1 and therefore, it's recognizing MHC 1 also. In fact, that is the way it tells whether, the MHC 1 is actually is self or non self. A non self MHC molecule, which is now an allo MHC molecule, with its bound peptide is recognized as some things as a different MHC molecules, that is bound to the peptide by self T cell receptors.

So, that is the concept of an allorecognition, in which case, the allorecognition of course stimulates T cells in a much better way. Therefore, these are the different kinds of alleles that are present within the children of these parents and they are codominantly expressed. This is an example that have shown you only for the A HLA allele, but the same thing holds true for the other HLA alleles, whether it is BC or DP, DQ and DR.

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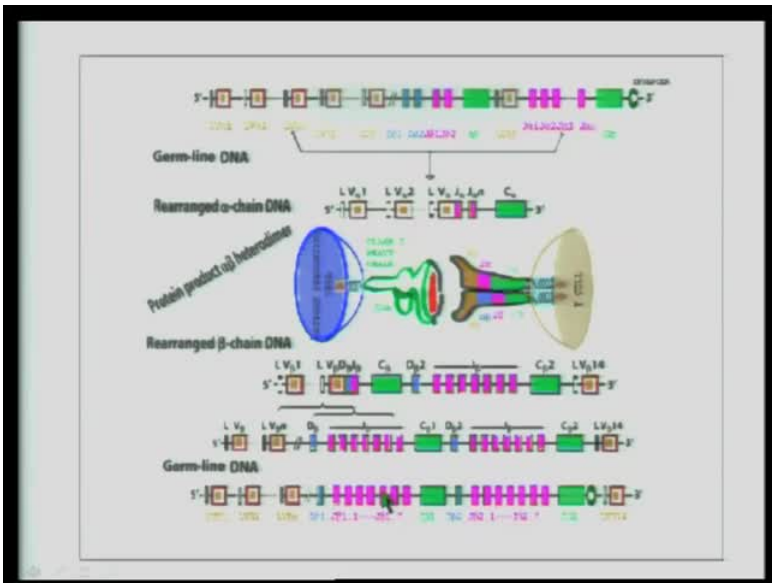

Now, this is just to show you, how the peptide as I told you earlier, is recognized by the variable regions of the T cell receptor, which recognizes this entire MHC class 1 molecule or the class 2 molecule, if the class 2 mediated presentation.

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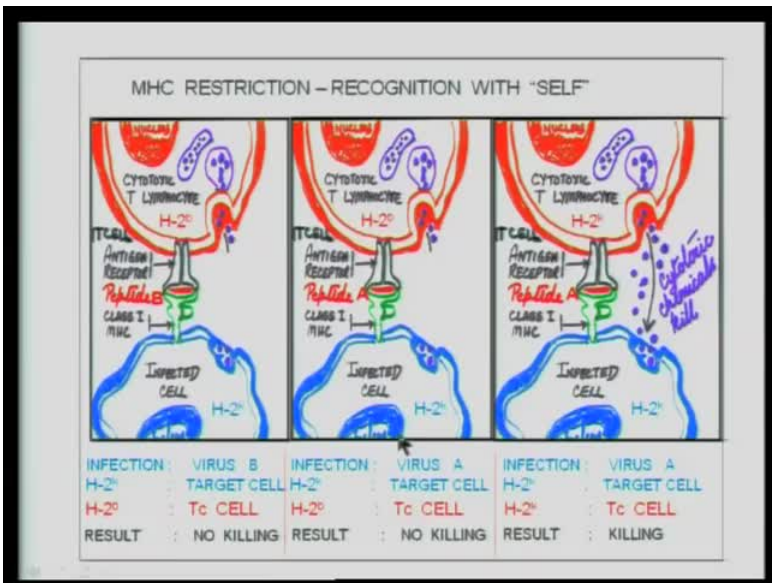

So, this is just to show you, how the self is very important for recognition. So, if you have the same haplotype bearing self cells, recognizing an infected viral antigen, then there is a killing, if there is no match within the MHC, then there is no killing.

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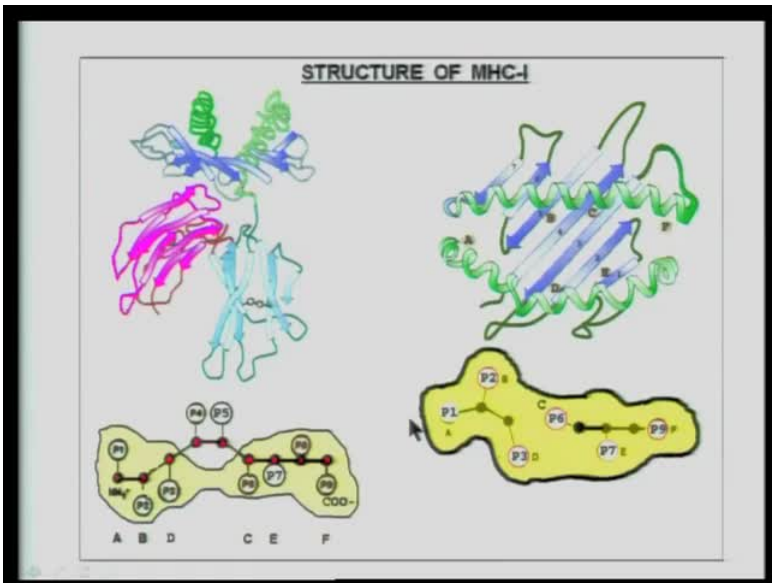

So, in addition to all these, you have of course other antigens playing a major role or minor role in organ transplantation. So, as you see over here, this is the area where there are a lot of differences in the different alleles and of course, within the floor of the MHC molecule, you have differences in how different amino acids are replaced in different alleles. And these are of course, just to show you, how the peptides sits in that groove and this peptide is actually contacted by the T cell receptor, that comes from the top and that is how the T cell receptor signaling occurs because it is activated.

So, this non self MHC molecule is able to act as a self MHC molecule, that is conjugated or bearing a pathogenic viral antigenic peptide, so this is called as an alloresponse to reiterate.

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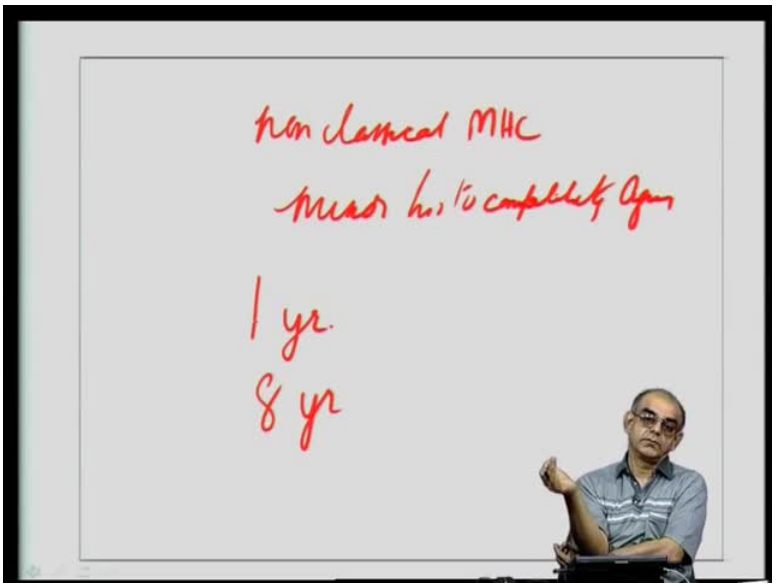

So, in addition to all these kind of major histocompatibility reactions, you also have minor histocompatibility reactions. What are these minor histocompatibility reactions? In addition to these major histocompatibility molecules, you have various other kinds of what are called as non classical MHC molecules. And it is practically impossible to match and try to tissue match, the tissue type for all these different kinds of antigenic a major histocompatibility molecules. It was called as the major histocompatibility molecule, because it is the major site or the major complex of antigen that determines skin graft or graft rejection reactions.

So, these non classical MHC molecules can also have different kinds of alleles, in fact amino would be as polymorphic as a classical MHC molecules but, they are polymorphic nevertheless. In addition to this major histocompatibility non classical molecule, you have what you called as the minor histocompatibility antigen. So, these minor histocompatibility antigens are nothing but, different kinds of antigens within the body. For example, we have several enzymes, they may have different kinds of isoenzymes or isozymes, so many of these isozymes, may have small differences in their sequences from person to person.

So, these are different kinds of antigens that are that could presented by the self T cell presented by the self APCs or the antigen presenting cells and you can have a reaction to that presented antigen. And therefore, you will see that, many of these graft rejection

reactions are usually evaluated majorly for 1 year, beyond which of course if the graft is accepted very successfully, the studies that you will see in the literature is goes on for about 8 years. But subsequently, you do see certain kinds of reaction setting in subsequent to 1 year, which is basically because all the different loci cannot be matched. And these are then controlled by different chemicals, which we will see as we go on to immunosuppress the ongoing inflammatory or activated inflammatory responses. The better the medical means by which you can suppress immune responses, the better will be the prognosis of grafting procedures.

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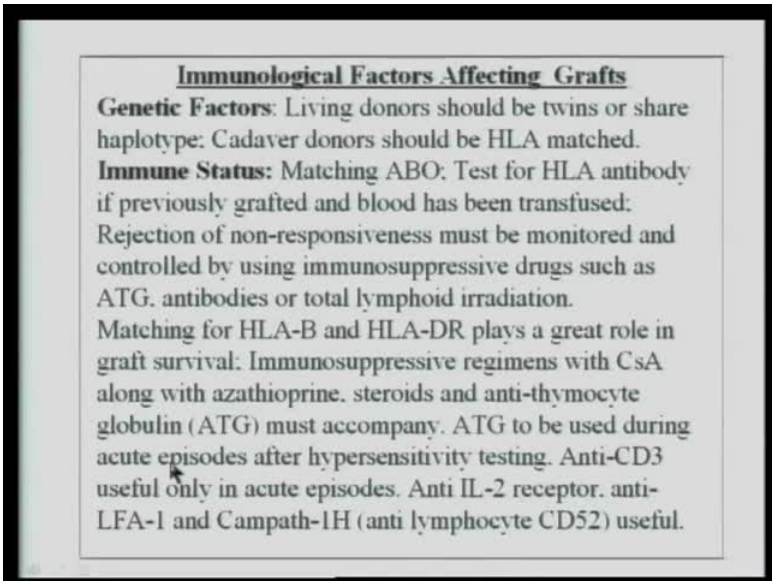

So, what are all the different factors that affect skin grafts? So, you see some of the genetic factors like for example, living donors normally twins, they are the best match that one can get, they share their haplotype and therefore, there could be a perfect match. But, how many of us can get twins as donors or in the situation of accepting or taking skin graft.

So, cadaver donors are preferred, so these cadaver donors should be HLA matched, for obvious reasons, as I just now indicated to you. So, what is the immune status of what one does during this whole procedure?

So, in addition to testing for the tissue type or trying to test what type of HLA molecules are there between these two organs or the cadaver and the recipient, who also have to type for the A B O blood groups, we will come to that in a little while. Also one has to

test for the presence of antibodies to the incoming HLA molecule or from the recipient organ. So, if the person already has for some reason antibodies, that can recognize some glycosylated portion of the incoming HLA molecule from the graft, then you will have an immediate reaction against the graft.

So, one has to pretest the individuals or the recipients for anti HLA antibodies. So, perhaps some of these patients might have been grafted earlier for some reason and therefore, this becomes very much necessary.

In addition, if they have undergone blood transfusion, people who undergo multiple blood transfusions actually develop antibodies to the small quantity or small numbers of T cells that have gone in with this blood transfusion. After all blood, the RBCs are isolated within these medical facilities, but all these procedures cannot make them 100 percent pure, there will be a few T cells, that will leak across and will be transfused to the person whose undergoing the transfusion.

But of course, some of these T cells are not a major problem, when you are undergoing blood transfusion or requiring blood, but they may cause problem because they will have ellicitated anti T immune responses over a period of time and then, when one has to get an organ then, you will have this T cell responses playing a major inflammatory role.

So, the rejection of non-responsiveness must be monitored continuously in grafted individual and controlled by using immunosuppressive drugs. What are these immunosuppressive drugs? This ATG will come to the azathioprine will come to that in a little while, antibodies against T cells or total lymphoid irradiation. That means you irradiate the individual in order to inactivate the T cells from proliferating. So, in somewhat you cut down the extent of the immune response because you have stopped the proliferation of T cells, but of course, if there cytokines can cause some damage. But, this total lymphoid irradiation is actually now not being preferred as much as they were in the earlier days.

So, matching for HLA-B, HLA-DR play a very great role in graft survival; so, relative to HLA A and C, HLA-B and DR need to be considered much more. Immunosuppressive regimes which involves drugs like cyclosporine, along with azathioprine, which is a in this ATG, steroids and anti thymocyte globulin must accompany these procedures.

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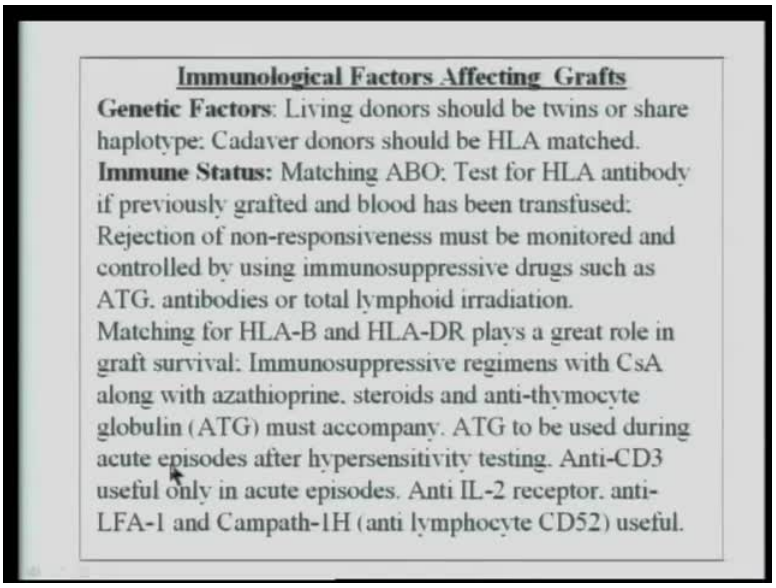

So, these kinds of interventions they are to be used during acute episodes, acute episodes meaning that as soon as one has the graft, you see that rejection reactions because of some problem. So, there has to be hypersensitivity testing for allergic reactions to the incoming graft, because that is one part of inflammation. Anti CD3 is useful only in these acute episodes, now anti CD3 is the anti lymphocyte antibody because CD3 is the T cell receptor. T cell receptor has the CD3, so if you have antibodies to the T cell or the anti CD3, it cuts down on the function of these T cell.

By using this anti CD3, you can also deplete these T cells because of complement mediated reactions. But basically, it inhibits the function of T cells by using these anti CD3 and this is possible, anti CD3 is known or documented only to the very useful, when there are acute episodes rather than a chronic rejection episode that is occurring over a long period of time.

Anti IL-2 receptor, anti this IL-2 receptor is very important for T cell proliferation. So, if you were to use antibodies to this IL-2 receptor, you can cut down on the proliferation of T cells and therefore, less T cell help to the T cells that are reacting against the graft.

Some the adhesion molecules like LFA-1, so anti lymphocyte function associated 1 molecule, you have antibodies to that, you can actually inhibit the migration of many of these cells and therefore, they do not go into the proper location. Now, Campath 1 is an

anti lymphocyte CD52 antibodies, which have been used with a fair degree of success in transplantation reactions.

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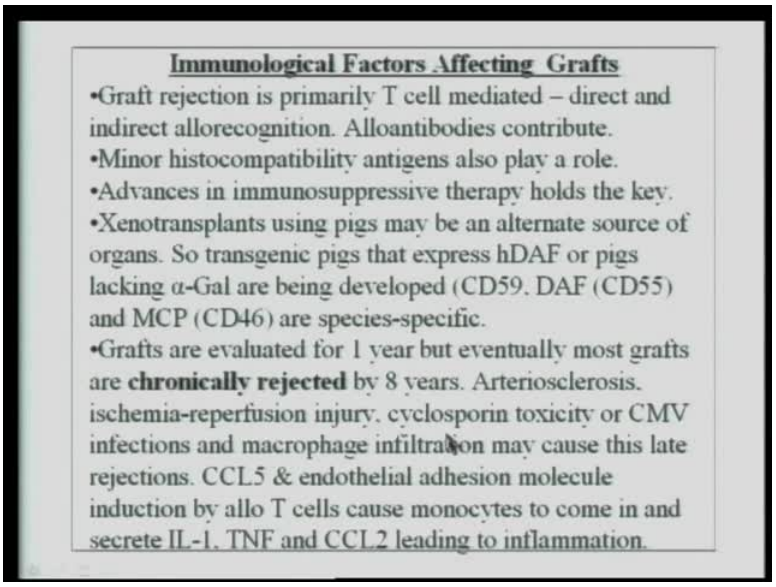

So, when you look at other immunological factors of course, total lymphoid irradiation using X irradiation was used for Hodkins's disease. It is more useful in the case of kidney allografting, but is now less widely used. Anti class 1 antibodies cause hyperacute graft rejection HGR, this is in fact a term that is used in transplantation studies. So, if there is anti class 1 antibodies already present in within the individual before the grafting occurred, then you have very rapid graft rejection reactions.

Anti class 2 is less severe in causing hyperacute graft rejections, so anti lymphocyte, auto antibodies of course can be present but, they do cause problem but, not as much problems as anti class 1 antibodies. So of course, you have to screen for anti monocytes and anti endothelial antibodies which are poor prognostic and indicators of good skin graft acceptance.

As I told you earlier, cross matching is a must; cross matching for antibodies is a must and it must be countered if there is presence of these antibodies, it must be countered by intravenous immunoglobulin or what you called as IVIG treatment.

Now, in renal disease, one can make out if this is a hyperacute graft rejection, if there is anemia and of course, it is treated by recombinant erythropoietin and blood transfusion

and the blood transfusion has shown to be beneficial to decrease graft rejections for a variety of reasons.

Now, if you look go on further, as I told you earlier, minor histocompatibility antigens play a very great role. So, graft rejection is primarily is a T cell mediated reaction, now advances in immunosuppressive therapy actually holds the key. Xenotransplants using pigs may be an alternate source of organs, so transgenic pigs that express human decay accelerating factor or pigs lacking alpha gal are being developed, in order to see how these different kinds of organs can be used in human transplantation. Because in humans, antibodies prevail against this alpha gal moieties and therefore, they are preventing these sort of organs being used from the from the pigs.

Now, the grafts are evaluated as I told you earlier for 1 year, but eventually most grafts are chronically rejected chronically means slowly being rejected over long period of time by about 8 years. Now, much of the many of the reasons for graft rejection are arteriosclerosis, meaning blockage of blood vessels. Ischemia reperfusion injury that occurs that occurs when you are actually suturing the graft inside because you are cutting the blood vessel and then suturing them. So, ischemia means, blockage of blood supply and then you open the blood supply again and that is reperfusion which means, now the blood starts to flow ischemia itself or reperfusion itself are known to have a lot of deleterious effects within the body.

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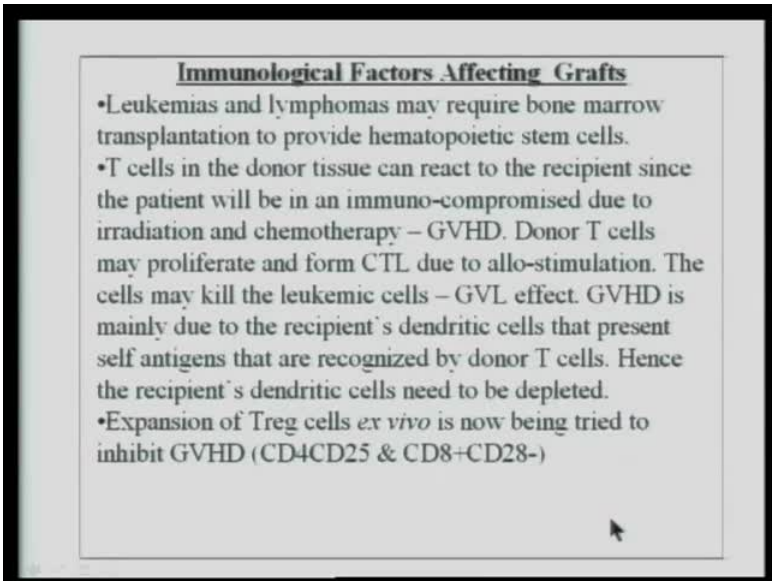

Cyclosporine is an immunosuppressive drug, but it has its own toxicity and of course, you have cell mediated reactions, macrophage infiltrations into the graft, which causes all these different kinds of graft rejection reactions and of course, there are different kinds of chemokines like chemokine ligand all of them participating in these inflammatory reactions. For volt of time, I would like to skip some of these, some of this information but, they will be available for you in the slide.

Now, some of the important another interesting feature or important feature to be considered is that leukemias and lymphomas, they are blood cancers. They require bone marrow transplantation, this is how they actually try to address or combat people who have leukemias and lymphomas because they are highly proliferative lymphoid cells. Of course, there are different kinds of leukemias, this bone marrow transplantation is actually done to provide hematopoietic stem cells. So, these hematopoietic stem cells, proliferate within after giving the bone marrow, they proliferate and then repopulate persons immune system by providing them with new lymphoid cells, of course these hematopoietic stem cells have got to be normal.

In fact, nowadays, they are trying to see how stem cells therapy can replace many of these kinds of medical treatments because stem cell itself does not express MHC molecules. So, you have stem cell then differentiating to form different kinds of blood cells as these blood cells are differentiating tolerance sets in and that could perhaps mean that, there could be a longer time for a particular graft to survive.

T cells in the donor tissue can react to the recipient, which we are studying in this lecture, since the patient will be in an immuno-compromised stage due to irradiation and chemotherapy. So, during this whole procedure, in order to prevent the rejection of the graft, the patient has been treated with immunosuppressive drugs, be its steroids or cyclosporines. So, it gives a chance for this bone marrow, the bone marrow has lymphoid cells and therefore, may have T cells.

So, you have T cells reacting against the recipient, so the donor T cells now starts to recognize the host and starts to react against the host because the host because the host is immuno compromised. The host can also be immuno compromised, if you irradiate the host because the host or patient is got a tumor like leukemia, so this is called as a graft versus host disease, GVHD.

So here, donor T cells proliferate and they form cytotoxic T killer cells, due to allo-stimulation because these donor T cells recognize the host T cells as foreign, but the host T cells cannot react to the donor T cell, because they are immuno compromised due to irradiation or immunosuppression.

The cells may kill these leukemic cells, this is called as the GVL - Graft Versus Lymphoid effect, which is in the medical field. They understand, what this GVL effect is, which is basically that the T cells coming from the donor graft they kill the leukemic cells because they can recognize the leukemic cells. For example, NK cells natural killer cells can recognize tumor cells specially leukemic cells, which are decreased MHC antigen. In fact, that is one of the ways by which NK cells recognize and kill their target cells. So, they can kill, so these are little beneficial effect of these donor T cells, which have been activated from the donor grafted tissue.

GVHD is mainly due to the recipient's dendritic cells. So, the host dendritic cells, take up some of the antigens from the grafted tissue and present it to the donor T cells. Hence, the recipient dendritic cells need to be depleted. So, these are all the strategies that one follows during this kind of transplantation, bone marrow transplantation procedures.

Expansion of T reg cells as I told you earlier, regulatory T cells are immunosuppressive T cells, they suppress cytokine release. So, many of these T cells, T reg cells if they are expanded, then you have a good prognosis. So, they take out this, they expand them ex vivo that is now being tried to inhibit graft versus host disease.

Now, these T reg cells can be purified based upon the expression on the cell surface, which are the CD25, so they express CD25 on the cell surface. So, CD4, CD25 positive cells can be purified by a variety of techniques, grown in vitro or using tissue culture and then reinfused into the patients.

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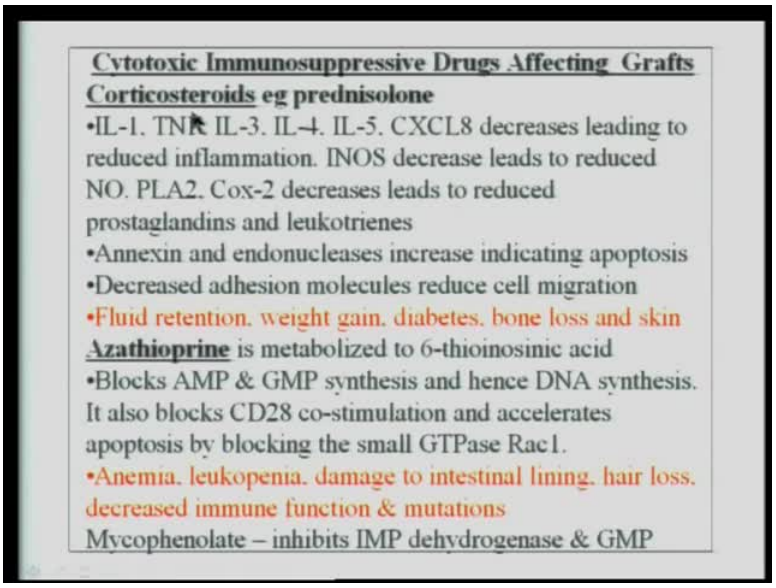

So, what are these immunosuppressive drugs? So, corticosteroid that is known to be all of us knows that they are used for immunosuppression. So for example, prednisolone, so you see IL-1 TNF or tumor necrosis factor IL-3, IL-4, IL-5, CXCL8, they are all decreases when corticosteroids are administered. Therefore, this decrease in these molecules actually leads to reduce inflammation.

Another molecule called as INOS nitric oxide synthase, it also decreases when corticosteroids are injected this leads to decrease NO production and therefore, decreased inflammation. In addition to PLA2, phospholipase a 2 cyclooxygenases they are all decrease when corticosteroids are injected and it leads to reduced prostaglandins and leukotrienes and therefore, decreasing the ongoing inflammatory response against the graft.

Endonucleases which cleave up the DNA, they increase indicating apoptosis. So, corticosteroids actually increase apoptosis of many cells, they decrease adhesion molecules, therefore lymphoid lymphocyte trafficking also decreases, reduces cell migration. So, macrophages coming to the vicinity of the inflammatory site will be decrease, some of the negative aspects of course are fluid retention, weight gain, diabetes, bone loss and skin problems.

Azathioprine is a chemical, it is metabolized to 6-thioinosinic acid, and it blocks AMP and GMP synthesis. In fact, that is how it proliferation of treated cells and hence, DNA

synthesis, it blocks CD28 co stimulation of T cell immune responses T cell in addition to the primary respond. Primary stimulation, it requires the co stimulatory signal coming from CD28 and it accelerates apoptosis by blocking the small GTPase calls as Rac 1.

They derogate bad effects or anemia, leucopenia, damage to intestinal lining, hair loss decreased immune function and mutations, which is very bad aspect of azathioprine treatment. Similarly, mycophenolates is used to inhibit IMP dehydrogenase and GMP, which ultimately leads to blockage of proliferation.

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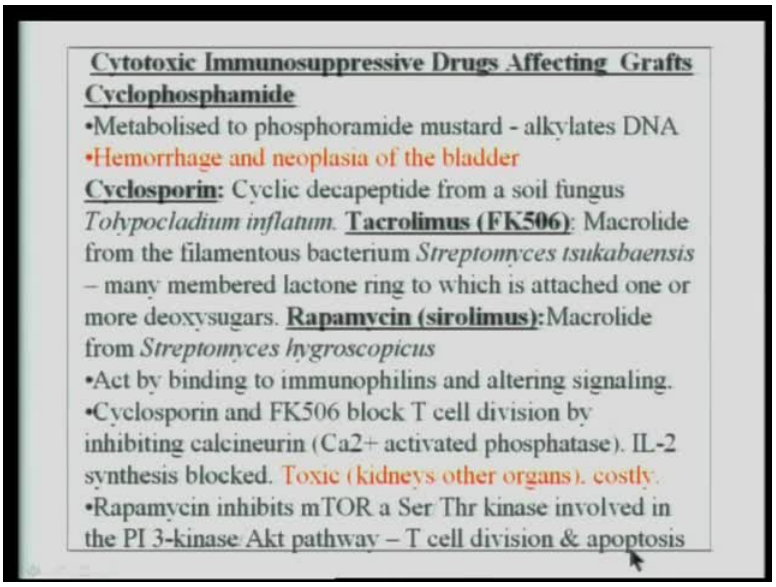

Cyclophosphamide is another drug, which is metabolized to phosphoramide mustard which alkylates DNA. The bad effects are hemorrhage and neoplasia of the bladder. Cyclosporine which is the most important or most popular drug that is used for immunosuppression, it is a cyclic decapeptide. It is actually got from a soil fungus called as tolypocladium inflatum, it is named as tacrolimus. FK506 is the other name, this it is a macrolide from the filamentous, bacterium, streptomyces, tsukabaensis. It is a many member lactone ring to which it is attached one or more deoxysugars.

The other drug is rapamycin, which is called as sirolimus, it is also a macrolide from another streptomyces organism. All these act by binding to what are called as immunophilins and they alter signaling within cells, trans membrane signaling events. Cyclosporine and FK506 block T cell division by inhibiting calcineurin, calcium activated phosphatase, calcineurin is a calcium activated phosphatase. IL-2 synthesis is

blocked and therefore proliferation of T cells are blocked, the toxicity of cyclosporine is actually shown in kidneys and other organs and one of the most problems with using cyclosporine is of course, it is very costly.

This another the other drug rapamycin inhibits mTOR, mTOR is a serine threonine kinase, which is involved in PI 3 kinase Akt pathway, which is operative for T cell division and apoptosis.

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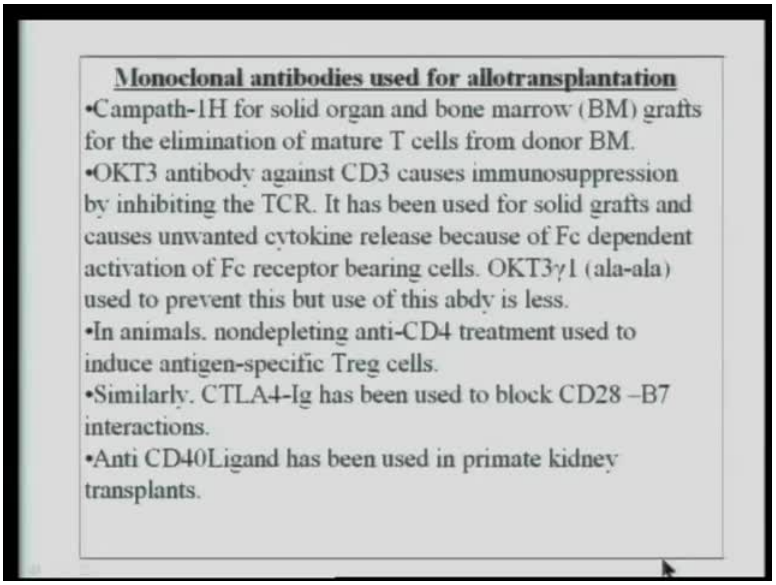

Monoclonal antibodies have also been used in transplantation like for example, campath H as I told you earlier, it is been used for solid organ and bone marrow transplantation grafts and for the elimination of mature T cells, because it binds to T cells from the donor bone marrow. So, a GVHT can be inhibited, OKT3 antibody against CD3 causes immunosuppression by inhibiting the T cell receptor, it has been used for solid grafts and causes unwanted cytokine release because of Fc dependent activation of Fc receptor bearing cells. So, they have used a mutant, which is a mutant where alanine has been replaced in the normal sequence of the CD3, this is used to prevent this, but use of this antibody is less frequent.

In animals, non depletion of anti-CD4 treatment is used to induce antigen specific T regulatory cells. So, non depleting meaning, that this anti CD4 antibody has the property of not causing toxicity or not being cytotoxic to the CD4 cells. Apparently this sort of antibody enhances or induces T reg cells.

Similarly, CTLA4 is participates in the co stimulatory signal of T cells, this CTLA has been used to block CD28-B7 co stimulatory interactions and that is how it causes blockage of T cell stimulation.

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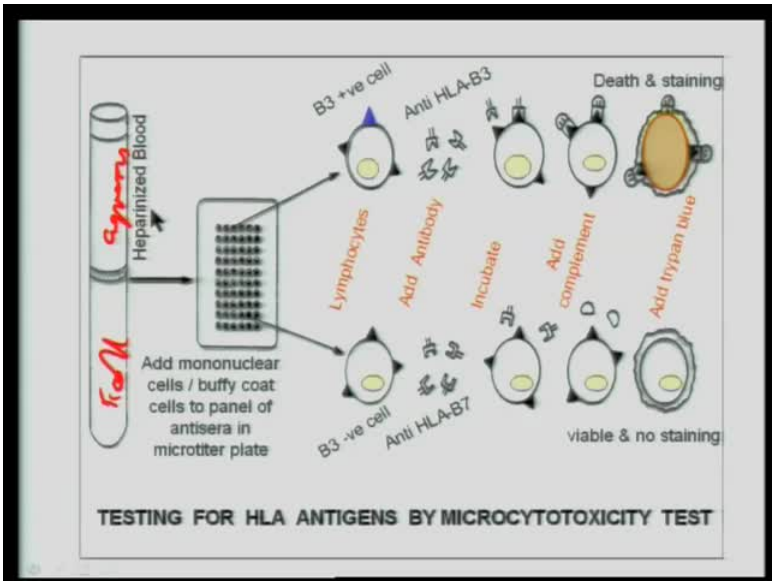

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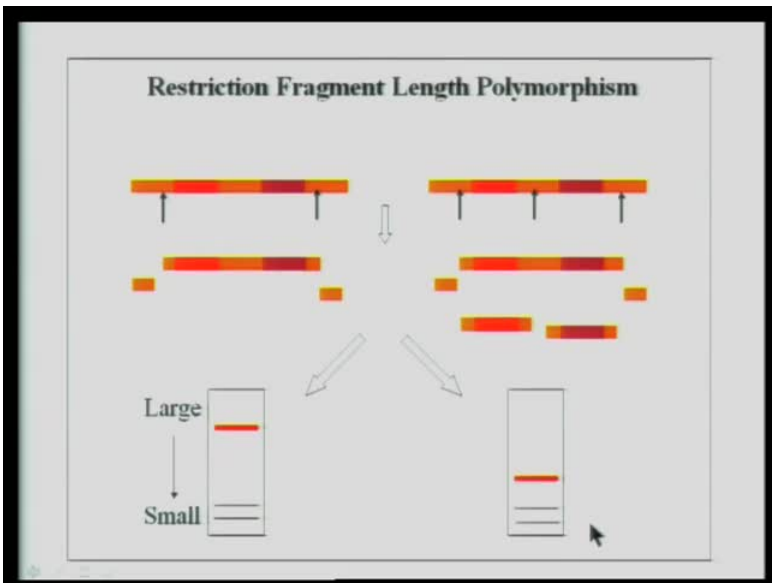

Anti CD40 ligand is another very important strategy that has been used in primate kidney transplants. Of course, all this needs to have the HLA being typed and this HLA typing procedure uses antibodies to see whether, there are allele specific antibodies, so you have specific anti sera that recognizes specificities and using these plates, you can look for

staining of the cells by using different kinds of dyes, which has been enumerated in the MHC class. And these various kinds of HLA typing procedures, this is one of looking at the micro cytotoxicity, then you have what is called as RFLP or Restriction Fragment Length Polymorphism using different restriction enzyme to cut up the DNA of different individuals and then trying to prohibit with a particular probe, that will bind to a particular HLA allele.

So, to summarize all these studies, you find that all these graft rejection reactions, rather than trying to explain the different procedures that are involved in graft rejection reactions and how they are evaluated because that is more clinical in nature, I have tried to focus on some of the basic principles that are involved in trying, explaining, why an allotransplantation reaction ensues if the graft has not been typed or matched.

So, basically then , if you look at graft transplantation reaction, one has to type the tissue and this typing of course is done to match the HLA types on the incoming or the donor receiving the graft, so both of them have to be HLA typed. And of course, nowadays since there is more difficulty in getting this match to tissue grafts available, one resorts more and more to try and see how you can improve the techniques, that will immunosuppress the ongoing immune responses. Thank you very much, we will go on to other lectures in the future.