Essentials in Immunology

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Module No. # 09

Lecture No. # 18

Cancer

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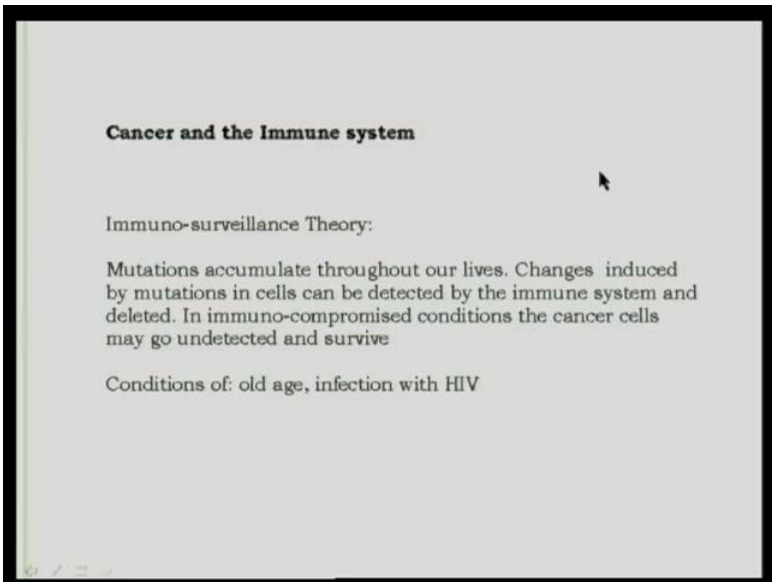

So, I will continue with my lecture on cancer in the immune system. We have already discussed a few aspects of antigens and the immune response to cancer cells, but I will go on now, more towards the management of cancer. Now, just to recapitulate your memory, let me say a few words with regard to the immune system and why do we study cancer under deficiencies of the immune system. This is based on the fact that it is only when there is, not only, mostly when there is an immuno compromised condition, cancer cells tends to proliferate or establish themselves.

So, this is supported by or this supports the immuno surveillance theory. The immuno surveillance theory says that our cells are constantly undergoing mutations. They could be point mutations or more deleterious mutations. There are always changes induced by mutations in cells and these changes, especially if they are on the cell surface can be

detected by the immune system very efficiently and therefore, they can be deleted because of the change on the cell surface, these cells can be recognized as non-self. Also, changes in the on the cell surface can also be recognized by NK cells, which you probably have already studied by now.

So these there is an effective immune response against these changed cells, cancer cells. Now, the same thing also is true of virally infected cells. Virally infected cells alter on the cell surface because of the expression of some viral antigens. So, these are also thereby I mean you can have an analogous situation with cancer cells as we have discussed in the previous lecture that carcinogens as well as say, UV radiation induces changes on the cell surface.

Now, this can happen, you know that mutations accumulate throughout our lives. So, this of course, even we know that cancer is not a disease which occurs because of 1 mutation, but because of accumulated mutations. So, one can ask a question, why these accumulated mutations which would reflect in the change and drastic differences in the cells which have undergone this change, as apart from normal cells which would not be recognized as non-self, why then there would be no deletion of these cells.

So, it is believed under the immuno surveillance theory that individuals, when they are in an immuno compromised conditions, these mutated cells are not recognized as non-self or there is a weak immune response. Therefore, they go undetected and because cancer cells have a better opportunity for cell division and growth, they soon form a mass of cells and once this mass of cells keeps growing, then of course, cancer is established.

Now, is there any evidence for what I have said just now? Yes, there are 2 examples that I can quote. Conditions of old age - I have discussed this long, you know several lectures ago that efficiency of the immune system starts to decrease with age. Now, it has been seen that not all, but several cancers appear beyond a particular age. Therefore, we know the correlation between old age, decrease in efficiency of the immune system, thereby mutations which would lead to cancer, such cells cannot be recognized or even if they are recognized as non-self, the immune response that is generated is not adequate to delete and therefore, yes, cancer you know takes hold.

The second example, I can give which probably all of you all would know is infection with HIV. Human immuno deficiency virus, we know is the AIDS virus. Now, which are

the cells that the virus infects? It is the CD4 cells. CD4 cells, as you know by now are the central regulators. They regulate the B cell proliferation and differentiation as well as the cytotoxic T cell proliferation, which would mean that now with this helper cells or the CD4 cells, now coming down with the infection with HIV, which would now decrease the numbers as well as efficiency, it has been seen that individuals were infected, you know AIDS patients, who already started showing symptoms of immuno compromised state, now start to appear with cancers and there are certain cancers say, of the skin which otherwise are not very common. You know these uncommon cancers start to appear in aids patient. So, here is the evidence one can give that in fact, decreased immune system or immuno compromised conditions can allow cancer cells to go undetected and survive.

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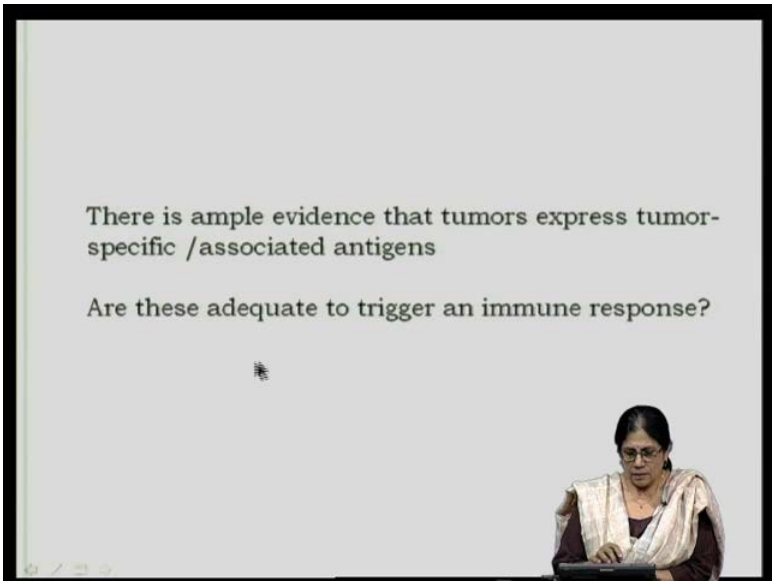

So, there is any way from the previous lectures there is one can say that there is ample evidence that tumors express tumor specific or tumor associated antigens. You do remember in alpha beta protein as tumor associated antigens and there are tumor there are experiments that have shown that most tumor cells express tumor specific or associated antigens at least to begin with, you know at least when they are established, may be not in the course of their development of the disease itself, but initially, yes. So, the question is, in spite you know though they have antigen expressed tumors, are these adequate to trigger an immune response. Do we have evidence?

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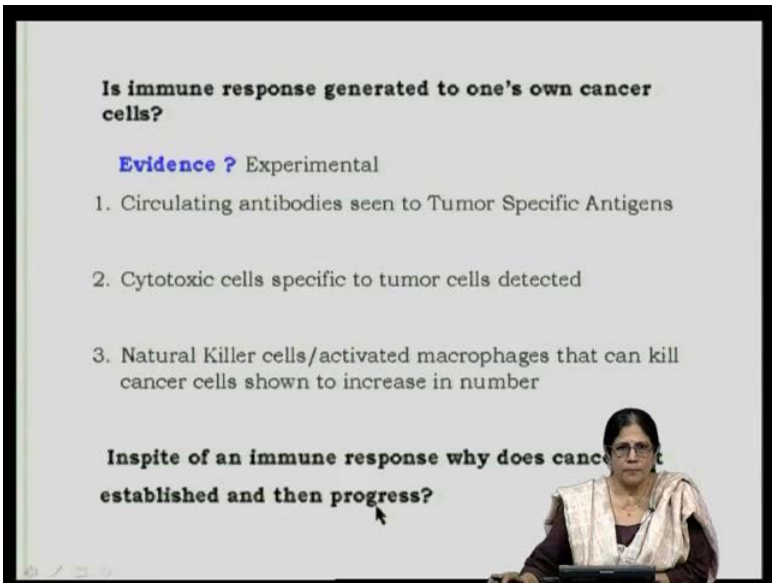

So, the question again I can put it in another way. Is the immune response generated to one's own cancer cells? Is there an immune response? Is there any evidence? Again, there are very large number of large number of literature which gives this evidence, but let us talk about the experimental ones because here we can actually, you know in mice, one can induce formation of tumor and one can look at when the antibodies or the cytotoxic T cells appear.

And yes, experimentally, it has been shown undoubtedly that there is immune response generated to one's own cancer; there are circulating antibodies seen to tumor specific antigens. Now, this of course, is also been seen not only under experimental condition, but also in human that there are cytotoxic cells specific to tumor cells detected. Now, this again experimentally, has been proven undoubtedly and in both cases, let me finish the third one - natural killer cells. Now, natural killer cells are part of the innate immune system to some extent because they recognize non-self and they evoke an immune response immediately by killing the cells.

Natural killer cells as well as activated macrophages, they can kill cancer cells and these cells increase in number, when there is a tumor. So, all these three have been shown not only to get activated, these cells, you know do and they can recognize specifically cancer cells, not only that, they are able to now kill cancer cells specifically, cytotoxic T cells as

well as NK cells and by direct cell to cell attack in case of circulating antibodies, antibodies, if complement is provided, antibodies also can kill.

So, we know then that yes, there is establishment of cancer, which is a non-self-cell. Now, cancers are recognized as non-self by the immune system and there is a response generated. So, in spite of this, why is the immune system not able to delete the cancer cells? Why is the cancer allowed to get established and then progress is a question mark.

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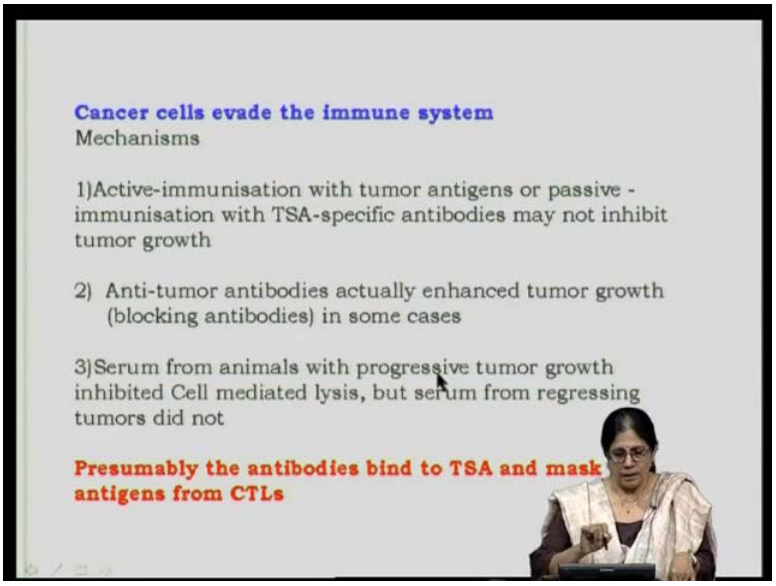

So, that is because cancer cells can evade the immune system. Now, is there evidence to say that? There is evidence. Again, there is huge literature which actually discusses on the mechanisms by which cancer cells can evade the immune system. Let us go one by one with these. How have people shown that? Now, the first one which is with respect to antibodies actually, was quite surprising. Now, what people were doing earlier is they establish a tumor in an animal model.

Now, resect the tumor, take it aside and grow the cells out in culture. Now, take the serum sample from the same animal as well as the cytotoxic T cells from the same animal and now, look in vitro outside the animal, whether antibodies can do anything to the tumor cells. Well, it would be again [here self] and build the cytotoxic T cells. Are they able to kill the cells? Now, in such a system, almost 100 percent of the time there was killing. However, interestingly, when the tumor is in the mouse and well, even the serum and antibodies in the mouse, it has been seen that in the mouse in fact, even

though, there is good level of tumor specific antibodies, there is no inhibition of tumor growth in several types of tumors. not only that So, that means the antibodies are doing nothing, not only that, it was also seen in several instances that anti-tumor antibodies actually, enhance tumor growth.

You know how do they do that? Now, they had mice which they have induced tumors in and there are they are checking for the progression of the tumor and checking simultaneously for production of antibodies. Now, they found that though there is the production of antibodies specific to the tumor keeps on increasing, the tumor itself starts growing much more than there are antibodies. Now, these were called blocking antibodies. Why should antibodies block? Now, especially when there is in the case of a solid tumor, we have a solid tumor and where will the antibodies bind? They would tend to bind to the cells, which is in the outside of the tumor. First, tumor penetrance may not be very good with respect to antibodies; antibodies are large molecule.

So, let us say that they bind to the outer surface. Just binding of the antibody to the cells would not do anything, especially in case of a solid tumor. If they are cells, which are in case of leukemias, it might be a different thing, but let us say, in case of solid tumor, the antibodies bind. Now, for the antibody mediated lysis to take place, there should be complement activation. Now, complement should be there. Complement components are present in circulation in the blood, but complement may not necessarily be adequately present around the tumor for the triggering of the complement cascade to take place.

So, therefore, now, anti-tumor antibodies may enhance tumor growth because the antibodies block. How? Apparently, it is believed that the blocking in fact, the antigen is being to some extent protected from cytotoxic T cells. So, the blocking antibodies are blocking the cytotoxic T cells from getting to the tumor cells. Now, is there again evidence, further evidence that says anything about these blocking antibodies? Yes of course, experimentalists have gone on further to show the mechanism.

They have taken serum from animals with progressive tumor growth. Now, they have taken this serum out and they have done in vitro CTL assay. That means target cells being killed by cytotoxic T cells, in the presence of serum from animals with progressive tumor growth or serum from regressing tumor. Interestingly, they found that serum from

animals with progressive tumor growth always almost always inhibited cell mediated lysis. So, obviously, again these could be blocking antibodies.

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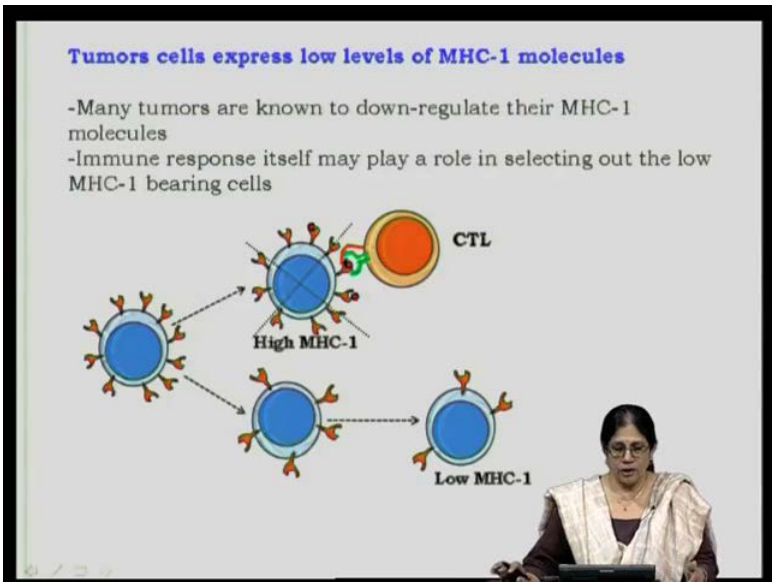

So, we know now, the different ways so far, how tumor cells are able to evade the immune system. Remember, there are antigens, but there are blocking antibodies, which can inhibit the generation of an effective immune response. There is another mechanism. Tumor cells express low levels of MHC-1 molecules. By now, you would have studied the way CD8 cells or the cytotoxic T cells can bring about target cell killing. In fact, T cells recognize peptides from antigen or antigen peptides in the context of class 1 MHC or class 2 MHC molecules.

All nucleated cells have MHC class 1 molecules. Now, in case of cancers, if the cancer cell has to be recognized as non-self, then there should be these cancer specific antigens which are loaded onto class 1 molecules, which can be recognized by cytotoxic T cells for them to effectively kill. So, what happens to MHC-1 molecules on the tumor cells? It has been reported that tumor cells start down regulating their MHC molecules. So, what is shown in this picture is that original tumor cell here has the same number of MHC molecules which a normal cell would have.

Now of course, tumor cell is usually larger. So, by that logic, one would have more MHC molecules, class 1 molecules I am talking about, per cell. Now, if you have a good number of these molecule, there is no reason why the MHC-1 molecules would not

express the antigenic peptide on the cell surface, which would be recognized by the cytotoxic T cells of the same individual, which can recognize cell MHC and thereby the peptide in the context of cell MHC and bring about killing. Now, I have not shown how the killing? It is because of perforin and granzymes.

Now, what would happen if the MHC molecules start getting down regulated? CTLs can no longer recognize and therefore, low MHC bearing tumor cells can go undetected. In fact, the immune response itself may play a role in selecting out the low MHC bearing cells because the strength of binding of the CTL to the peptide in the context of class 1 molecules definitely starts to decrease with decrease in the number of MHC. Now, all the cells which have tumor cells with high MHC expression would of course automatically get deleted. So, thereby the immune response is actually is allowing or selecting those cancer cells, which have low MHC molecules.

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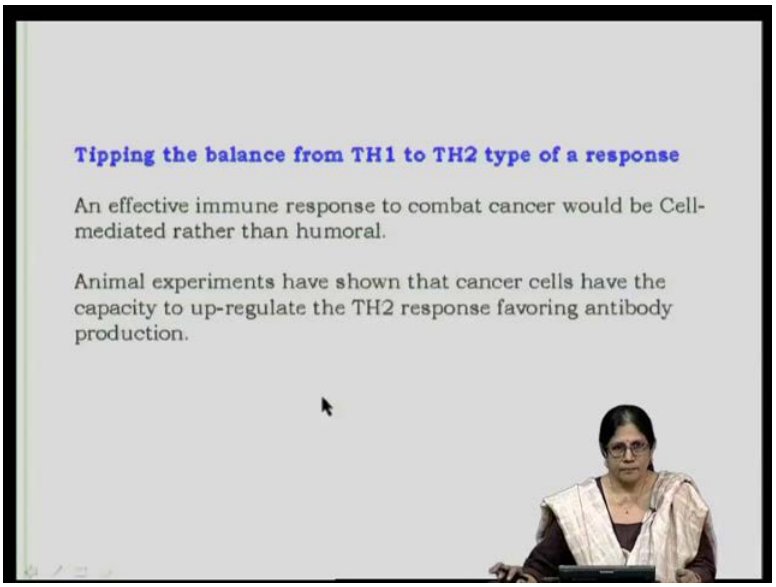

Now, this has been of course, shown in a number of cases that there is down regulation of the MHC molecules. Another important mechanism by which cancer cells evade the immune system is tipping the balance from TH1 to TH2 type of a response. I have not taught or lectured on the T cells, but by now again, you would have, you know about the 2 types of helper T cells, TH1 and TH2 and TH1 secretes cytokines, which help the cytotoxic T cells to proliferate and TH2 help the B cells to proliferate. TH2 therefore,

would secrete cytokines such as interleukin4, which is required for B cell proliferation soon after activation.

Now, during cancer or Well, I can give you an analogy with respect to cancer pregnancy and the foetus. Actually, the foetus is similar to a cancer. During pregnancy, the mother's immune response has to be modulated to an extent that it does not attack the foetus and you know the foetus is an allograft, 50 percent father 50 percent mother. In pregnancy also, it has been seen that the TH2 type of response gets elevated and concomitantly TH1 goes down. In fact, there is a very good balance between the TH1. That is, the TH1 cytokines, which help the cell mediated immunity versus TH2 which help the humoral immunity; now, there is always a perfect balance.

However, in certain cases like as said in pregnancy, TH1 gets down regulated so that TH2 gets up regulated. The balance is always, it is automatic. You have TH2 going down; you have TH1 going up. So, it is like a see saw. So, during pregnancy, TH1 has to get down regulated because the T cell responses or cell mediated responses to the foetal allograft needs to decrease.

Cancer and That is of course, under so called natural conditions. There are of course, large number of factors which do this, you know tipping the balance in the favour of growth of the foetus. In case of cancer, it has been seen similar situation takes place. There are some factors which have not been really fully identified, but there have been factors which are synthesized by the cancer cells such that there is an up regulation of TH2. Now, as I have already mentioned a little while ago, presence of antibodies to the tumor specific antigen does little to eradicate the tumor.

In fact, these antibodies can enhance. So, therefore, TH2 up regulation would only help the tumor to grow better. So, in this case, especially in case of the tumor, an effective immune response to combat cancer would be cell mediated rather than humoral immune response and it has been shown adequately in animal experiments, animals which have tumors or those that have progressively increasing tumors have an up regulation of the TH2 type of cells and down regulation of TH1. So, again one more mechanism is that the cancer can tip the balance from TH1 to TH2.

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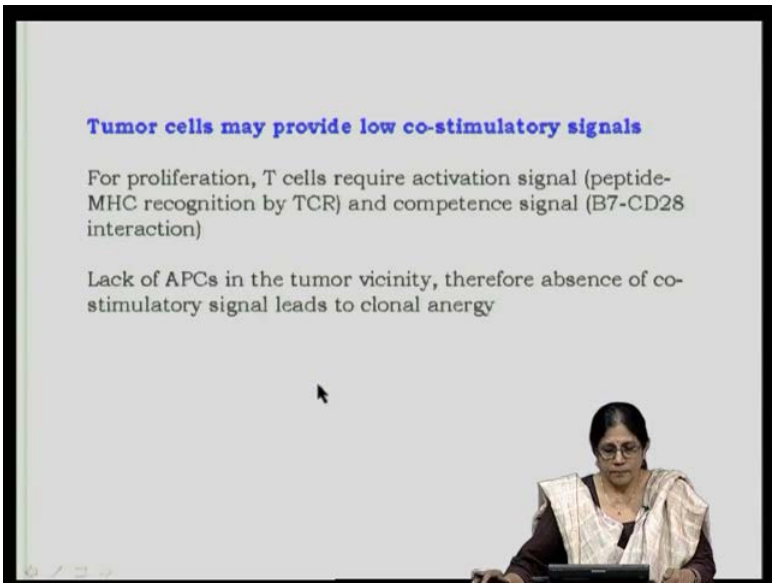

Now, this is not something, which probably the tumor cells do it actively like in the previous cases, but the tumor cells may provide low co-stimulatory signals, but this could be a mechanism by which tumor cells can escape the immune response. So, for proliferation of T cells, you might remember, for proliferation of T cells, apart from activation signal, which is through the peptide MHC recognition, through TCR or T cell receptor, there is a competence signal required.

So, for activation, it is only the recognition, but for proliferation, a competence signal B7 on the antigen presuming cells and CD28 on the T cells, this kind of an interaction is required. If there are no APCs or antigen presuming cells in the tumor vicinity, there would also be absence of co-stimulatory signals; this would lead to clonal energy. So, this is again a way by which tumor cells which have already started to decrease the MHC class 1 molecules, these go undetected.

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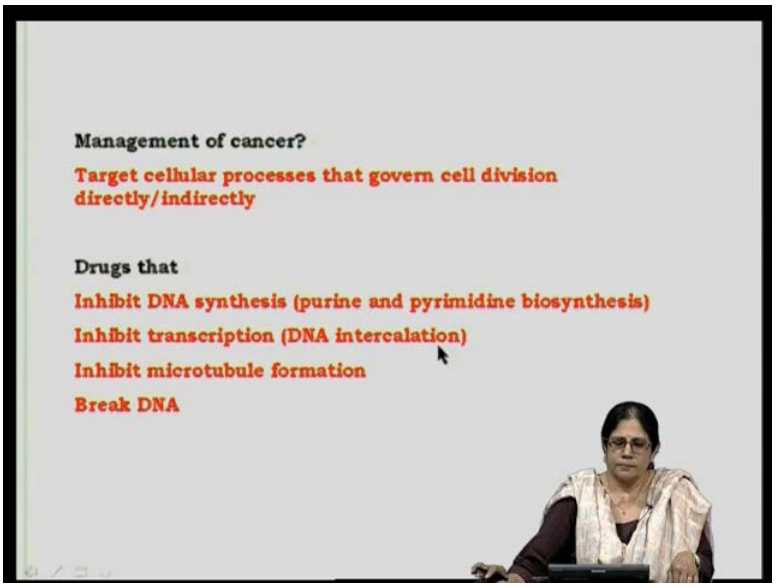

Now, let us come to management of cancer. We have talked about antigens and the way immune response is generated against cancer. We can look at now, management of cancer from the view point of immuno therapy, but before we do that let us look at how cancer is managed. That means what are the target molecules in the cancer cell, which can delete or kill the cancer cell. So, the cancer therapy targets cellular processes that govern cell division directly or indirectly. We do know that cancer cells are fast growing cells, which do not confirm to the dictates of hematopoiesis or you know the inhibition of cell division, when there are adequate number of cells that are already formed.

So, what are these molecules? Of course, DNA synthesis itself, drugs that inhibit DNA synthesis, drugs that inhibit transcription, drugs that inhibit microtubule formation and drugs that break DNA; all these are the basis of management of cancer. There are of course, others and there are newer ones, but these are all the therapies that are still in practice and for several long years, are those that in fact do not allow transcription and DNA synthesis, thereby cell division itself is inhibited.

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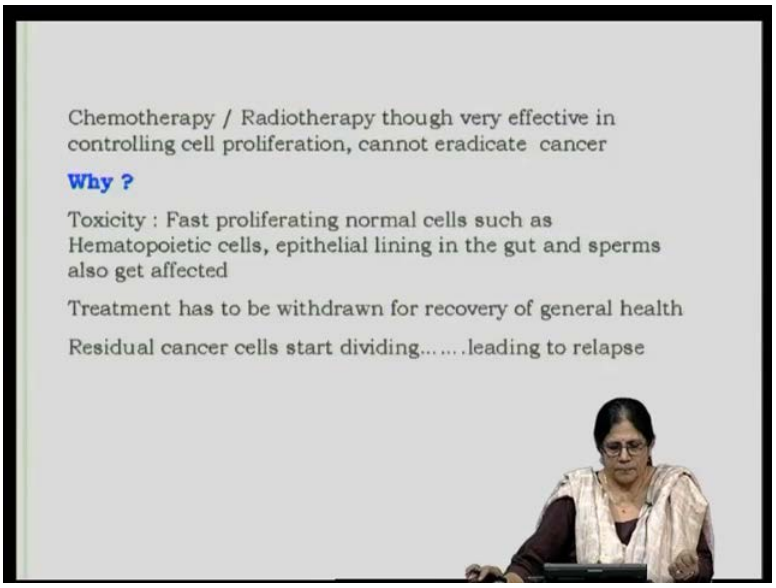

Radiotherapy and chemotherapy, both these are of course, still being used to control cancer because these are effective in controlling cell proliferation. However, there is a reason now, though chemotherapy and radiotherapy are extremely efficient, in inhibiting cell proliferation, why cancer still persists? Because radiotherapy and chemotherapy, both of them target fast proliferating cells. Therefore, the normal cells of our body, which also undergo fast proliferation, they get affected and very seriously.

For example, cells of the hematopoietic cells, epithelial lining in the gut as well as sperms, hematopoiesis itself is affected. No blood cells, no RBCs, no WBCs, all numbers very, very low. Therefore, when especially chemotherapy, now radiotherapy of course, is little bit, you know radiotherapy is given focused on the cancer itself. So, may be things are little better, with but chemotherapy affects the entire body. They are agents, I mean medicine that is taken in the body. So, treatment therefore, has to be withdrawn for recovery of general health.

Because you cannot try to kill the cancer cells completely, if the individual is going to die because of anemia. Therefore, treatment to eradicate cancer is done and withdrawn as soon as there are alarming signals of low blood count and of course, toxicity because of the epithelial lining getting disturbed. Now, when the treatment is withdrawn, there are residual cancer cells left, you know the ones we have talked about, that down regulate the MHC molecules and I must add that cancer cells can also, down regulate these tumor

specification antigens with time. So, these residual cancer cells start dividing and this leads to relapse.

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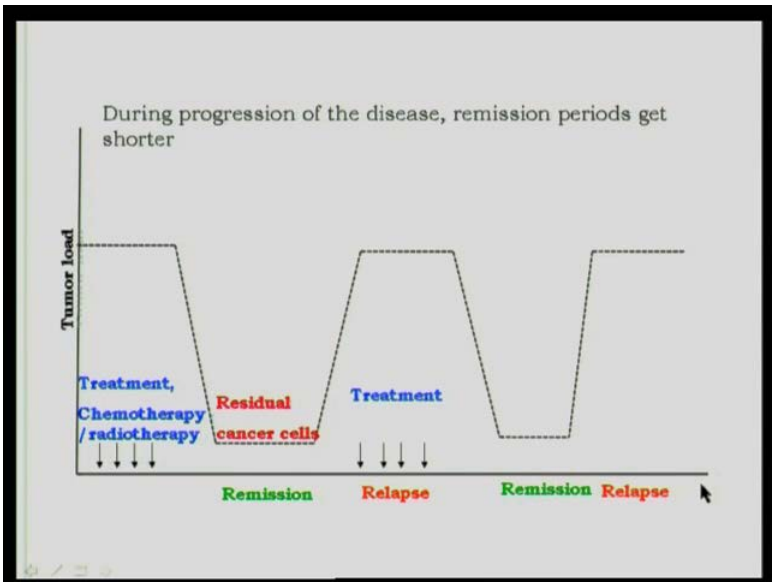

Now, this has been shown. Well, I try to show this in this diagram. Typically you know This is Y-axis, shows the tumor load and X-axis is the time. So, when the tumor load is very high chemotherapy or radiotherapy is given constantly, in fact, there is a regiment of this, the tumor immediately starts to decrease; the tumor load goes down. The patient now is known to be in a state of remission. There are residual cancer cells which cannot even be detected very easily. They are so low in number that even if you have biomarkers here, you will not be able to detect.

So, you know that this is the phase of remission. There are residual cells here and we know that there are residual cells because the moment that chemotherapy is withdrawn and has to be withdrawn here because of toxicity. Toxicity, which is seen by way of lower number of blood cells, seen by way of the gut lining getting totally destroyed, that leads to bleeding and well several other problems. So, now though the person is in remission, soon after, number of tumors cells increase. Again there is treatment given and the tumor load comes down; again there is a phase of remission.

But you know the phase cannot go further, remission cannot be better because of the toxicity. Once again, now the tumor starts to proliferate. There would be of course, slight difference between the tumor cells here verses tumor cells here. In fact, the tumor cell

starts to get a better and better with respect to managing, to evade the immune system better and better. You can see also here, the first phase of remission is much longer than the second phase and what you cannot see here that subsequently, if the same cycle continues, the remission gets smaller and smaller and relapse occurs faster and faster. Therefore, chemotherapy also has to be ultimately stopped.

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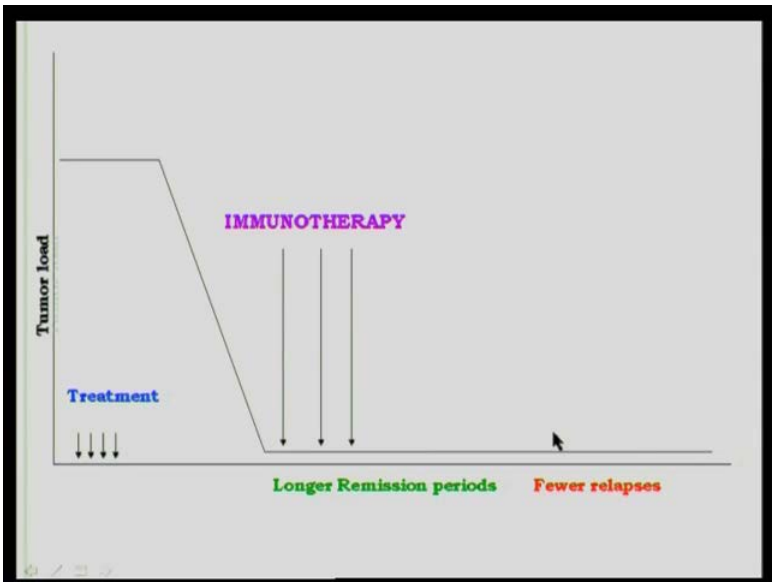

Now, immunotherapy would be the method of choice here, to try to take away the residual cells and this chemotherapy, in fact has started to gain importance. Now, of course, immunotherapy becomes difficult with regard to, you know every tumor, every cancer cell is different, but, you do know that chemotherapeutic agents as well as radiotherapy would now act on every cancer cell. Immunotherapy has to be specific. So, let us look at where immunotherapy is given? Can immunotherapy be given in the beginning, when the tumor load is high? It will not help too much.

However, it has been seen it is beneficial to patients, when the treatment to get down the tumor's load is by chemotherapy or radiotherapy and once the tumor load is very small, now, give the patient immunotherapy so that the small number of cancer cells over here can be effectively recognized by the immunotherapeutic agents, whatever one is given and destroyed and it has been seen that with such alternating kind of therapy, chemotherapy and then immunotherapy, there are longer remission period and fewer relapses; so, it is beneficial.

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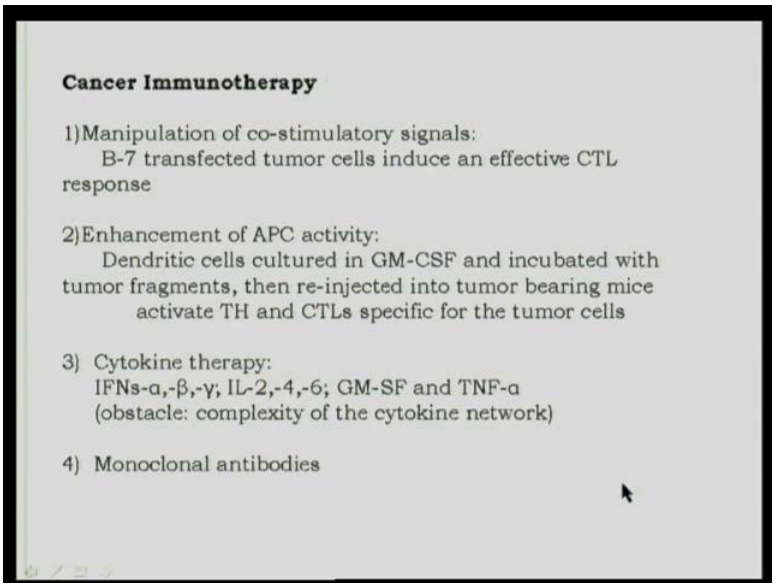

So, what are the cancer immunotherapy that are being utilized now and where research is going on? Maybe to some extent, even in patients, it is still at the research stage. One is manipulation of co-stimulatory signals. I have already told you that the lack of co-stimulatory signals is in fact, what does not make an effective CTL response of cells - cytotoxic T cell response and we do know that the cytotoxic T cells or the cell mediated immunity which is better in eradicating cancer than antibodies.

So, what people have tried to do is transfect one's own tumor cells. Let us go back to, you know make it little simpler. Now, there is a tumor in tumor load, There is a solid tumor in a patient. So, this tumor is regressed, it is taken out, excised. Now, the tumor cells, you can make single cell suspension, transfect these tumor cells with B7 so that now, there could be an effective, you know B7 binds to CD28 of the cytotoxic T cells and this way, you can have an extremely good cell mediated lysis.

Secondly, enhancement of APC activity - antigen presenting cell activity I told you is very important and this is what lacks in the vicinity of several tumors. So, what people have done, taken the patient's dendritic cells, they are cultured in cytokines such as GM-CSF - granulocyte, monocyte colony stimulating factor and also incubated with tumor fragments. So, tumor fragments would have antigens which are now taken up by the dendritic cells, then re-injected into tumor bearing mice and this activates the TH and the cytotoxic T cells specific for the tumors.

So, you are activating the dendritic cells and by GM-CSF as well as tumor fragments, re-injected effective APC activity. Cytokine therapy is quite well known. Cytokine therapy, in fact, is in use in human. Gamma interferon alpha, beta; interleukin2, which would, you know heighten the cell mediated immunity; 4, the B cells; 6, differentiating B cells; GM-CSF, I have already talked about; TNF alpha, which brings about lysis.

The only negative thing about cytokine therapy is that each individual has to be tested for several of these before one or a combination can be given because the cytokine network itself is so complex that you know one starts to treat with one particular cytokine, it would have repercussions on the entire network and what would be the outcome could be anybody's guess, but any way anything to try to manage cancer. Fourth, in cancer immunotherapy and which probably, you all must have already read quite a lot about are monoclonal antibodies.

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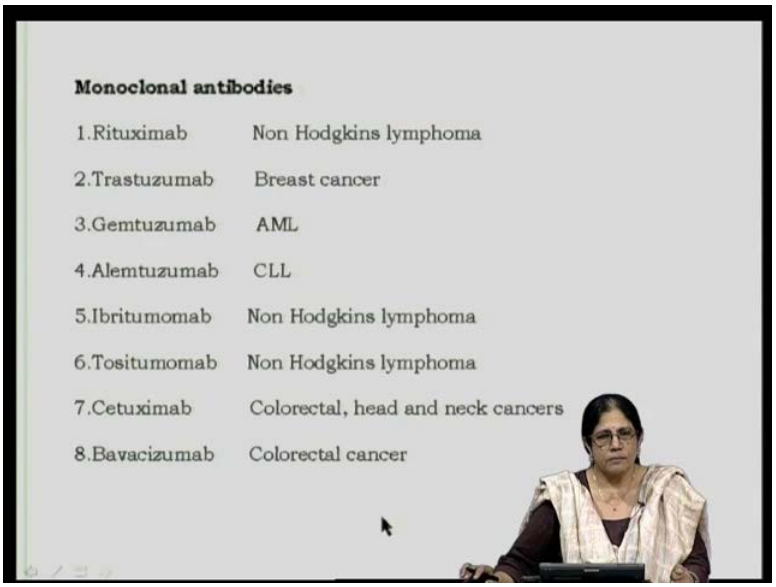

There are eight different monoclonal antibodies that are being used which are commercially available and are being used to treat cancers. Let us just quote. Rituximab to treat non hodgkins lymphoma; Trastuzumab - breast cancer; Gemtuzumab - acute myeloid leukemia; Alemtuzumab - chronic lymphocytic leukemia; Ibritumomab - non hodgkins lymphoma; so, also Tositumomab - non hodgkins lymphoma; Cetuximab – colorectal, head and neck cancers; Bavacizumab - colorectal cancer.

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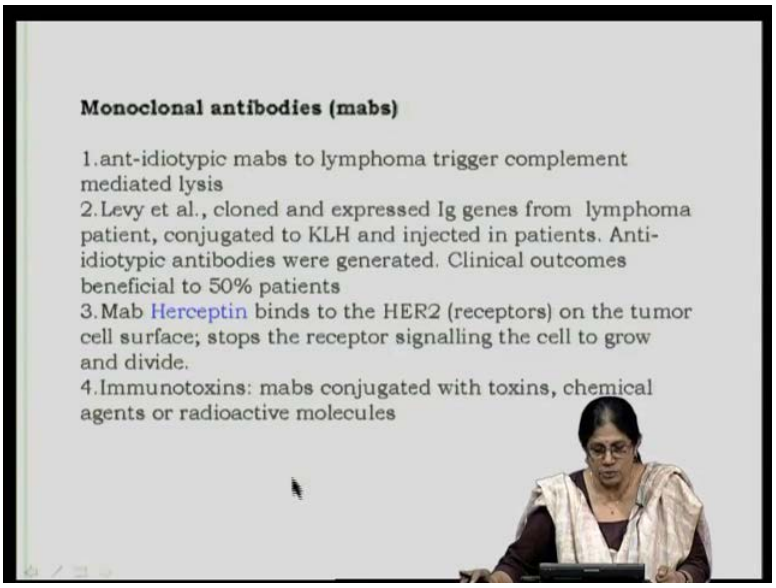

Now, what do all these Monoclonal antibodies do? Why Monoclonal antibodies? Because they are specific to one particular epitope and one particular let us say, receptor or protein on the cell. These antibodies can be, they can inhibit. For example, it is known that Trastuzumab, this inhibits cell division because of inhibiting a particular signaling cascade. The others inhibit ligand binding. There are those that can be used in combination and let us look at that.

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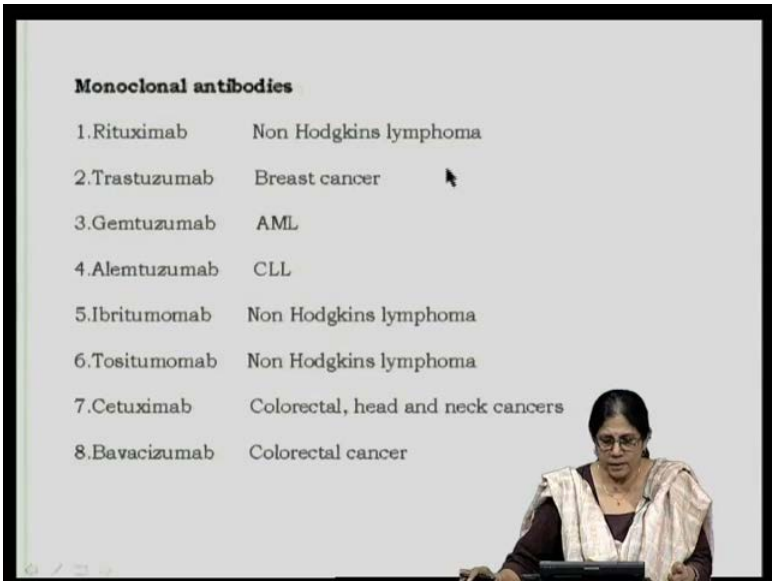

Now, apart from that in case of lymphomas, now, why are they three or well, three to non hodgkins lymphoma and two to leukemias. Now, why monoclonal antibodies would be there are, 5 out of 8 are two leukemias and lymphomas because again lymphomas are also well, they are solid tumors, but not solid tumors as one would think in terms of carcinoma. It is loosely associated mass of single cells, it is a lymphoma, but penetrance of the tumor is very, very high.

So, monoclonal antibodies also can penetrate. So, these antibodies are specific to receptors, which are hyper expressed on these cancers and these can evoke or fix complement rather easily because like I told you in case of solid tumor, it is difficult to have tumor mediated lysis, but leukemias and lymphomas much, much better and in fact, all these monoclonal antibodies have shown to be good with respect to management of cancer.

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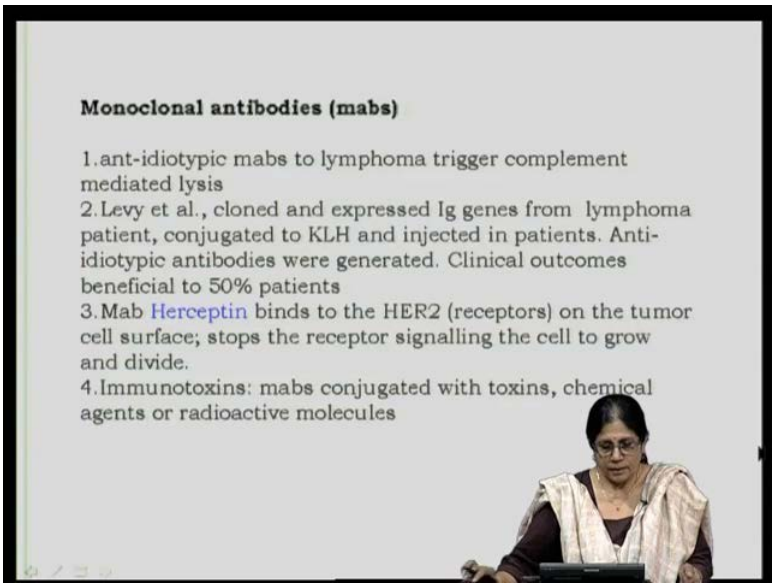

So, anti-idiotypic mabs to lymphoma trigger complement mediated lysis; that means these antibodies can bind to those specific receptors. there are people who have Now, this is another type of therapy that people have tried to do and quite interesting really. Now, Levy et al cloned and expressed immunoglobulin genes from lymphoma patient. Now, let us look at how this would help. Immunoglobulin genes from lymphoma patients, now, that lymphoma would actually be a monoclonal.

I have told you that most cancers arise from signal cell and therefore, are monoclonal. Therefore, the lymphoma of one patient would have only one type of immunoglobulin hyper variable region. So, this research group, they have cloned and expressed immunoglobulin genes from lymphoma patients. These immunoglobulins would be specific to that lymphoma and so, conjugated to KLH - keyhole limpid haemocyanin so that the immunogenicity is increased and now injected into patients.

They are large number of, if I remember correctly, there was something like 40 cases or more studied and it was seen that anti idiotypic antibodies were generated to their own immunoglobulin hyper variable regions in 50 percent of the cases and this thereby inhibited the tumor growth and about 50 percent of this patients actually, the clinical outcomes are very beneficial, quite interesting. This is of course, not a monoclonal antibody; it is monoclonal with respect to the immunoglobulin genes that I express on the lymphoma patient cells because the lymphoma is monoclonal.

Mab Herceptin - this binds to HER2 receptors on the tumor cells. This HER2 receptor are growth factor receptors. This stops the receptor signaling and the cells to grow or divide. So, antibodies can also inhibit, if they are specific to a particular growth factor. Fourth is making immuno toxins. That is, now, you have monoclonal antibodies that can bind to tumors. Now, this might be quite helpful in case of solid tumors; for example, where there is no antibodies bind to specifically tumor specific antigens on the tumor cells, but there is no lysis because of lack of adequate complement.

So, instead, one can have monoclonal antibodies that can recognize specifically, tumor cells or bind more to tumor cells. These antibodies can be conjugated with toxins or chemical agents or radioactive molecules. Now, toxins are elaborated a little bit more. Let us go to chemical agents. Chemical agents, why? Because if you just inject chemotherapeutic agents, I have already told you that these are going to affect also the normal cells and those that are rapidly growing, for example, the cells of the bone marrow. Instead, suppose this chemical reagent is conjugated to the antibody, then the antibody delivers specifically the therapeutic agent. Antibodies can also be conjugated to radioactive molecules so that there is specific delivery.

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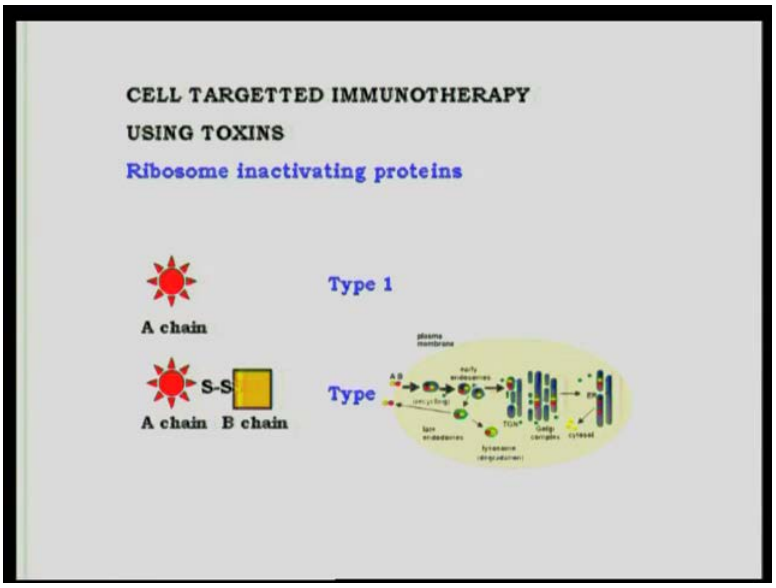

Now, I would like to go to cell targeted immunotherapy using toxins. One reason for why I would like to elaborate this is because we are doing research in this particular area. Cell targeted immunotherapy using toxins - there are a large number of toxins which are used. Bacterial toxins or plant toxins are well known. I will talk about plant toxins called ribosome inactivating proteins or RIPs in short, which can be either of type 1 of type 2. The type 1 has only the A chain, which is the toxin and type 2 has A chain, which is conjugated to the B chain. Type 1, which has only the A chain does not in any way kill a cell, eukaryotic cell I am talking about; so, therefore, mammalian cells as well.

The type 2 on the other hand, allows the entry of the A chain because the B chain is the lectin. It binds specifically to sugars. In case of the one that we are using in my lab, the B chain is a galactose specific lectin and eukaryotic cells have large number of these galactose on the cell surface. So, any receptors which have terminal galactose, the B chain binds to the entire molecule; therefore, gets internalized. A few molecules escape the regular pathway, the lysosomal degradation and they go through a retrograde transport, where the molecule goes first to the Golgi and then to the endoplasmic reticulum.

Here, there is cleavage between A and B chain because they are single disulphide bond which gets cleaved in the ER. The B chain because it is a lectin, again gets retained on the glycoproteins, which are present in the ER and the A chain is thrown out in the

cytosol, where it binds to a specific it binds to the loop structure in the 28S ribosomal RNA, depurinates it, thereby inhibits the elongation factor from binding and protein synthesis is completely stalled. The toxin, whether it is an A chain of the type 1 or A chain of the type 2, this is deglycosidase; it is an enzyme. So, being an enzyme one single molecule has been reported to inhibit 2000 ribosomes per minute. So, that is really extremely fast, you know highly efficient enzyme; protein synthesis stalls and the cell dies.

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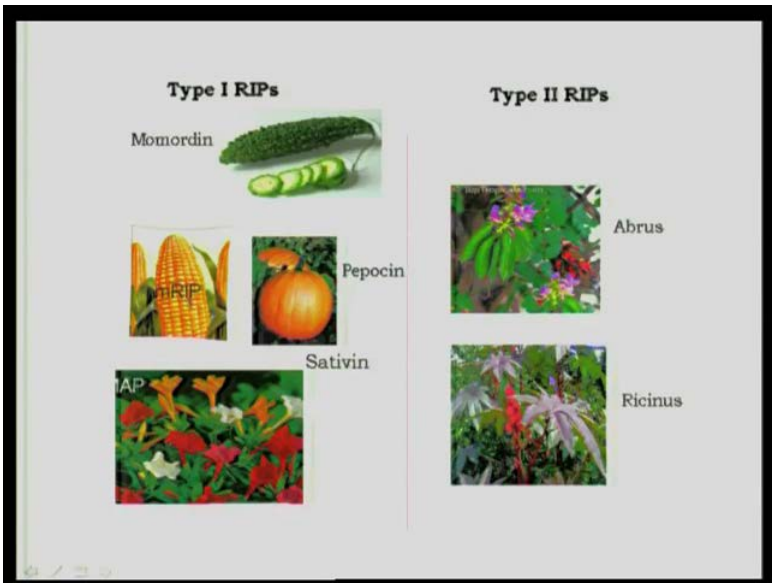

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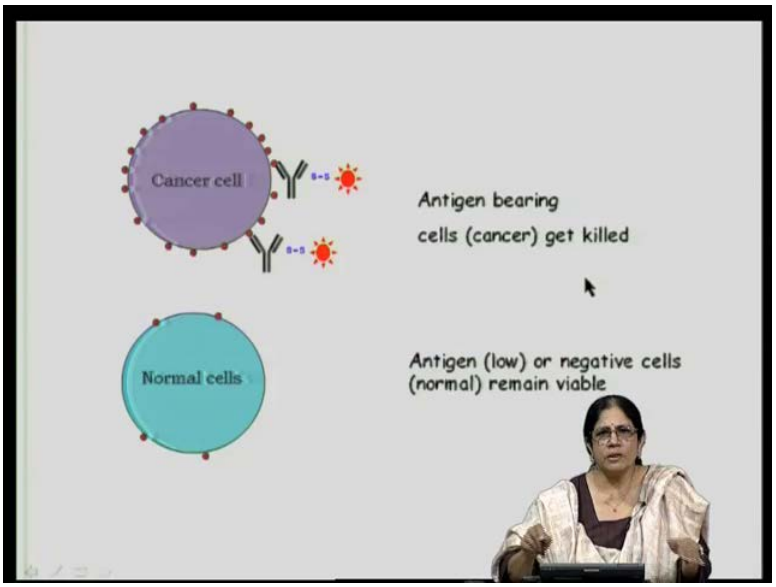

Where do you find type 1 RIP and type 2? You know interestingly these are found in vegetables [FL] momordica charantia, this has toxin called momordin. So, one can conjugate directly the type 1 RIP and in case of type 2, you take away the B chain and you replace the B chain with an antibody molecule. The antibody is specific and it would target only those cells, which have the antigen to which the antibody is directed. Normal cells

Now, I have shown two situations. In case of a tumor specific antigen like for example, those that are induced, that the tumor cell is induced by let us say, a virus. Then of course, you would have those only on the cancer cells; you would not have those virally induced antigens on normal cells. So, the normal cells are not touched at all. However, in tumor associated antigen, there is a chance that you can have some normal cells also having those antigens, but the number of antigens on normal cells would be minimal. So, you have now, an antibody molecule which can recognize a cancer cell, only if it has x number of antigens on the cell surface because of its affinity.

So, in such a situation of course, the antibody molecule would no way bind to the normal cell. So, you have now, antigen bearing cancer cells, which are now being recognized by the antibody, which is to the specific antigen. These antibodies conjugated to the A chain, the A chain, either from type 1 RIP or the A chain from type 2 RIP, there is a single disulphide bond, which is introduced, which is present here. So, the antibody molecule has to be slightly modified so that you have an S group, a cysteine, which allows a linkage to the A chain.

This entire molecule then is internalized and what we are trying to see, what we have shown already and others have shown that this immunotoxin is a hybrid between the toxin and the antibody kills specifically cancer cells or those cells which have antigens on the cell surface. Now, I will of course, talk about a little bit of details about this. This is too simplified, but in any case, we have shown that it does not kill normal cells; that means it does not get entry. Now, the question will be, will all antigens, all tumor specific antigens upon binding to the antibody bring about internalization of the toxin because of course, the A chain has to be taken in.

So, what is important here for the generation of immunotoxin, such an antibody has to be used, which has to be shown at first after binding to its antigen, there is a internalization

process which takes place, which is in fact, energy dependent phenomenon. It is only when this whole molecule gets internalized, will the toxin be able to do its job. So, this is quite a complex situation, but just see how important beneficial it would be. If all the cancer cells, which have these receptors or specific antigens, specifically singled out by way of these antibodies or immunotoxins and killed; normal cells are left untouched.

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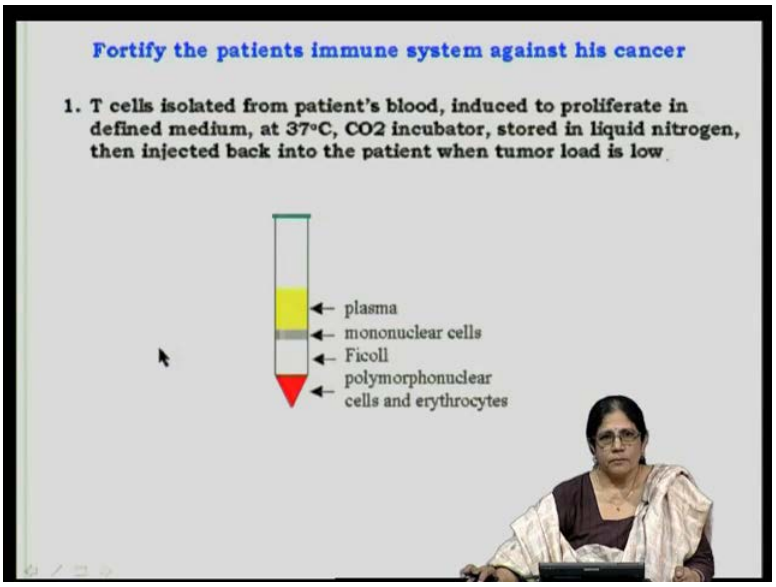

So, the molecule, which is in clinical trials now, is a toxin called risen, which is similar to the one that we are studying in my lab. It is type 2 RIP and people have in fact, started to show that risen bound to antibodies, not only antibodies, also ligands to specific receptors. These toxins are extremely efficient in taking away those residual cells, which remain after the tumor burden is down. Alright, are there any other ways by which an immunotherapy can be utilized? This is again on research stage, but extremely promising.

Fortifying the patient's immune system against his cancer – So, what is done here, T cells, I told you that T cells or cytotoxic T cells would be a better bet to eradicate the cancer, than antibodies. T cells are isolated from patient's blood and they are induced to proliferate in defined medium outside the body. Now, why this? Because it has been shown that patients or it has been shown also in case of mice, tumor bearing individuals seem to have an immuno compromised state, even if the tumor is a solid tumor and not that of the blood cells.

Therefore, their T cells in the blood do not proliferate, cannot be induced to proliferate as simple, if the T cells are isolated, induced to proliferate in refined medium outside in an incubator under of course, regulated conditions of 37 degree centigrade and in a CO2 incubator. After a good number has been generated, then these can be stored in liquid nitrogen.

Then, when the patient can be given chemotherapy or radiotherapy, the tumor burden is now down. Then the same cells, which you believe has a good population of these T cells, would have specific cytotoxic activity, can be injected back into the patient when the tumor load is low. Remember, the dips. So, when the tumor load is high versus when the tumor load has come down after chemotherapy and there are residual cells, now, the residual cells, a smaller number of cells can be effectively killed by the cytotoxic T cells from the patient, which have anyway been induced to proliferate and they are larger in number. So, this shows well, how one can actually purify the mononuclear cells.

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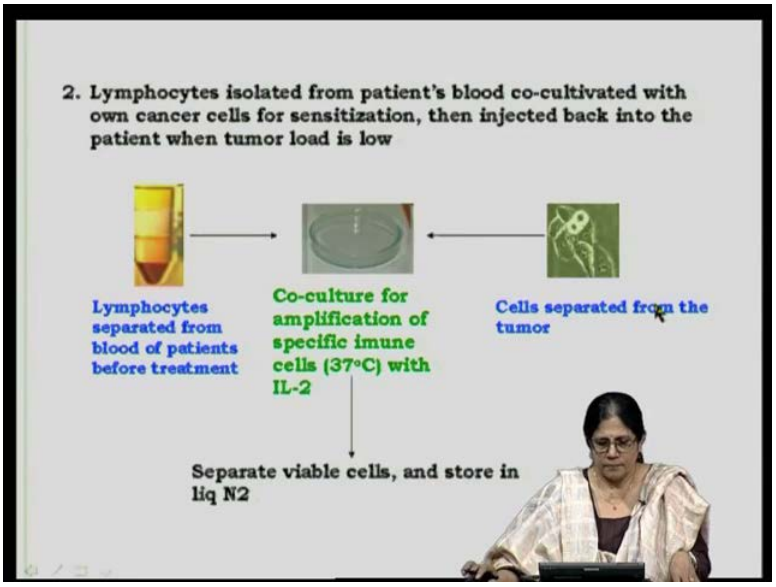

Now, a step forward in this direction would be you know what I talked about so far are lymphocytes which have been taken from the patient, which was sensitized in vivo. They are just been allowed to grow out and then re-injected when the tumor load is low. 1 step better would be to take the lymphocytes taken from the patients the same way now by this gradient centrifugation take the patient blood co-cultivate with the patient's own

cancer cells. so you take the mononuclear cells, lymphocytes in other words and now you put them in culture along with the receptive tumor from the patient.

Of course, one needs to make single cells. These two are co-cultivated. So, in other words, the patient's lymphocytes are being again taught to recognize or get activated to the own cancer cells. There can be amplification or there is amplification of the T cells. One can of course, add cytokines such as interleukin2 and then these can be stored once again. Why store? Because if you inject the patient immediately after the tumor is resected, there will not be much help, but after the patient has gained some slightly healthy condition, then the viable cells, which have undergone training again can be injected and this has to be in there are clinical trials that have gone to show that this has is of great benefit to the patient.

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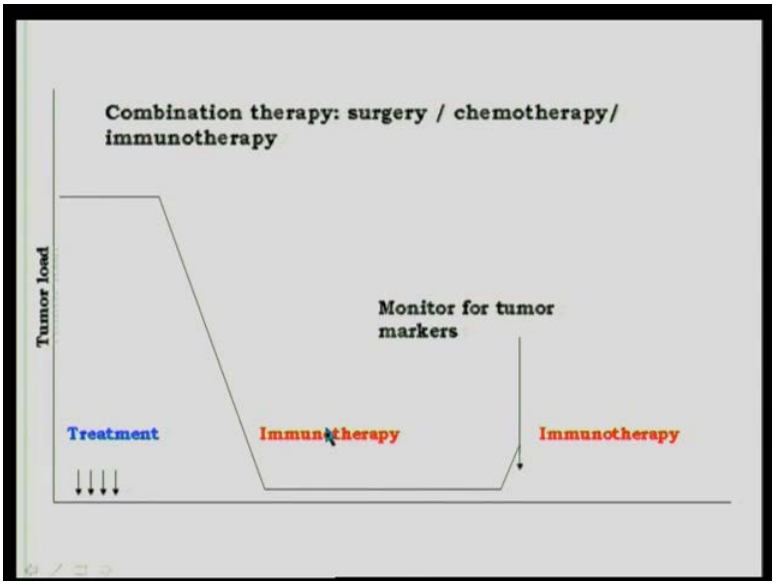

So, combination therapy is absolutely, the method of choice. There is no one therapy for cancer management; combination therapy where surgery, in case of a solid tumor or chemotherapy, in case of lymphomas and leukemias; it can also be radiation therapy. So, first, by surgery and chemotherapy or radiotherapy, the tumor load is brought down to a minimal level.

After this, when the tumor load is very low, this is when a specific therapy can be introduced, either by way of, you know what I talked about the immunotoxins, where there are already antibodies. One should have already had antibodies specific to the

tumor or receptors specific to the tumors, which are not there on normal cells or if they are there, very low. Conjugate to toxin can be injected at this stage or the T cells, either simply proliferated in vitro stored and then injected here or T cells, which have been re-sensitized to the patient's own cells, the cancer cells I mean, re-injected over here.

Look at the progress of the disease. Now, this kind of treatment seems to be working not very well, but at least has lent more years to the cancer patients and also a healthier life. How does one know that now immunotherapy has to be given again? Now, if given immunotherapy, the person is in remission. How one does know when to give any therapy at all? Now, in case of very fast growing tumors may be the patient might require chemotherapy again.

But in case of slow growing tumors, one can keep on monitoring by carrying out immunodiagnostics to find out when the cancers even in spite of giving immunotherapy, has started to come back in circulation. Monitor, therefore, for tumor markers. As soon as these tumor markers, for example, in case of liver cancer, you can look for alpha beta proteins. As soon as the lowest detectable concentration of alpha beta protein can be seen in circulation, immediately without waiting, another lot of immunotherapy can be given. This way, one can effectively curtail cancer.

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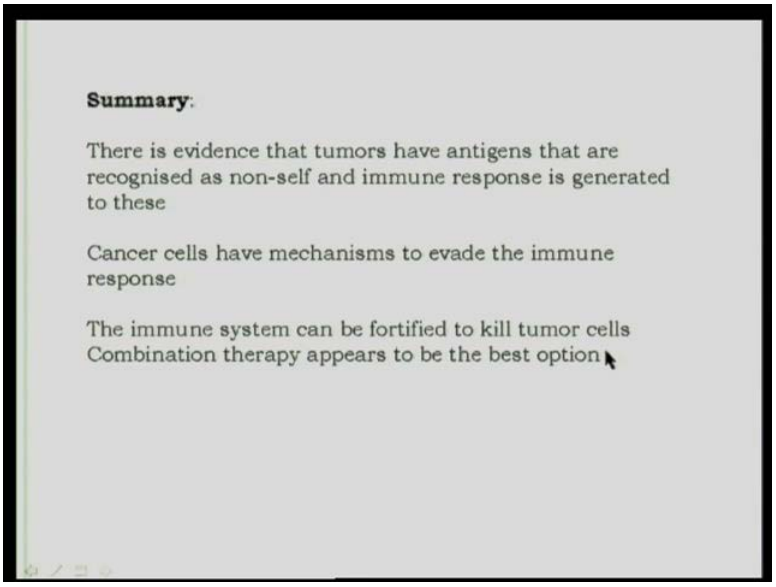

So, just like to summarize here. There is evidence that tumors have antigens. There is evidence that these are recognized as non-self and an effective immune response is

generated to this; there is ample evidence. Unfortunately, in case of cancers, most cancers are unique, especially if they are chemotherapeutically I am sorry, carcinogenically induced. If they are virally induced, then maybe, there would be similarities with respect to tumor specific antigens. However, the negative part about immunotherapy or the immune system, trying to boost the immune system would be, to be able to have unique reagents that recognize those unique antigens.

Nevertheless, we do know that there can be an immune response that can generated to the tumors. So, cancer patient can and is able to mount an immune response to the tumor antigens. Of course, cancer cells have a mechanism to evade the immune response. Therefore, the management of cancer would be to fortify the immune response to kill tumor cells.

And right now, the only way by which one can effectively eradicate a cancer would be to have a combination therapy, where the major part of lessoning the tumor burden is by non-specific mechanisms like chemotherapeutic agents or radiotherapy and then come with the most specific response or specific therapy by way of the immune response, which is allowing the own person's own immune system to effectively recognize and not only recognize, kill the tumor cells. So, I will stop here. Thank you.