Essentials in Immunology

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Lecture No. # 29

T cell survival

For today's class, we will be looking into different aspects of T cell survival, and what this means is, what are the pathways involved in T cell survival and which involve obviously T cell that because survival and death are sort of interlinked.

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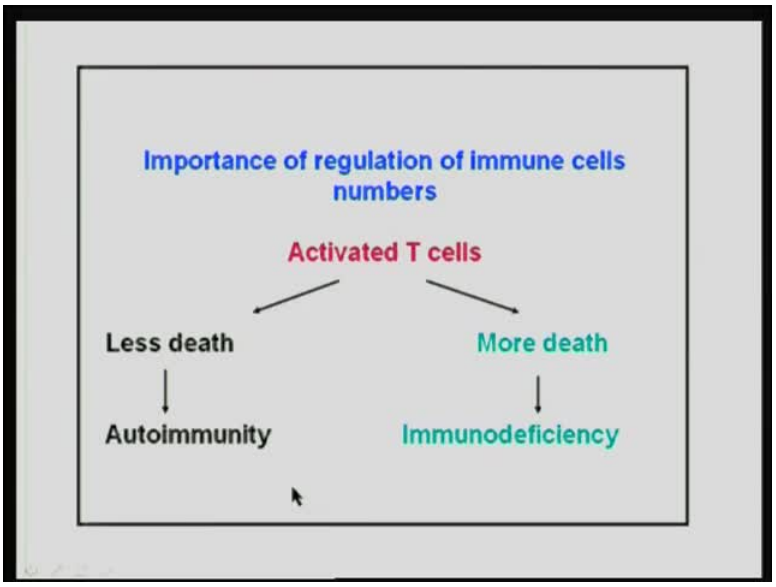

So, why is this aspect important? If we just look at the slides and see the importance of regulation of immune cell numbers; so once T cells get activated, they increase their cell numbers, well for that matter B cells or T cells, once lymphocytes get activated, their numbers increase, and that those numbers have to be controlled, and the reason for this in the previous classes too I informed you that if upon activation the T cell numbers continue to be high, it results in immunopathological problems for the host, and therefore, their numbers need to be controlled.

Now, here there are scenarios ,which is what I have shown over here; you have activated T cells, and for example, if there is less death, that means, the cells that ought to be removed after activation, they have done their job, they now need to be removed; if they are not removed, then it results in autoimmunity, and we will see examples of this; so, for example, in humans or in people with mutations in a Fas cell, you are increased numbers of activated ion T cells, and it causes major problems, so it causes autoimmune, like syndromes in these mice and people, we will be discussing some of that aspect.

On the other hand, if there is enhance death, and then it results in immunodeficiency, so then the cell numbers of immune cells need to be tightly controlled, and these involve death pathways, and it is important for us to understand what this is all about.

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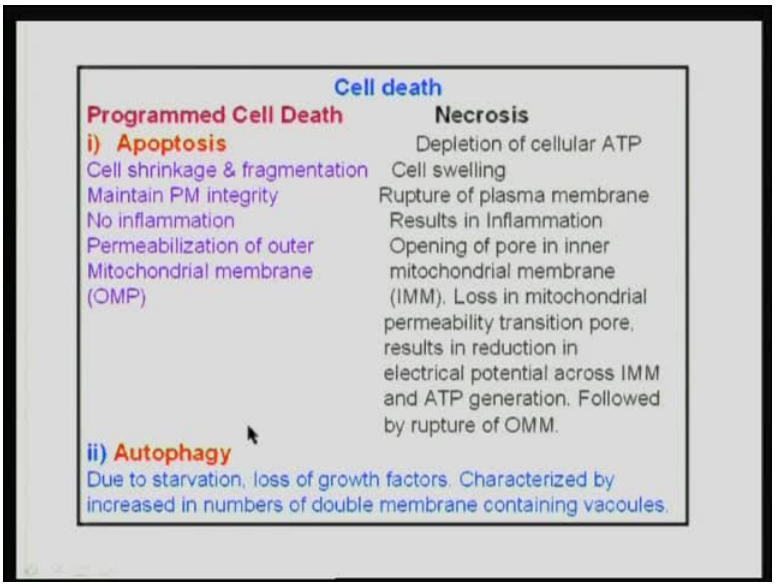

Now, in general, a cell that is a phenomena, that is not linked only to immune cells, all our body cells undergo homeostasis, and their cell numbers are regulated for, so all the cells, they are born, they live for some time, they do their jobs, and then they get aged, and so they need to be removed, and how does this process occur? It occurs by a process known by different ways, and cell death, there are two main types, one is in which you have programmed cell death, which means, its controlled the cell, death is controlled, and there are two main types of PCD or programmed cell death, the first one is apoptosis and the second one is autophagy.

Now, in case of apoptosis, you have the cells shrink, and they fragment, but the plasma membrane integrity is maintained, that is a very important highlight, and that is what distinguishes it from necrosis, where you have rupture of the plasma membranes, you have leakage of the intercellular contents, and it causes necrotic conditions, which in which result in inflammation, and we had discussed in the class before that whenever you have inflammatory conditions, it is more of warzone like situation, where you know there is puss, you have cell infiltration, you have a lot of other problems, and apoptosis is a way of, you are removing cells that are not required in a highly regulated manner.

So, for example, in the thymus, where thymic selection takes place, and very few of the cells are selected, majority of the cells are not selected this, and they undergo a process of cell death. If you look at the thymus, you do not see puss, you do not see inflammation, you have major cells, you have a large percentage of cells being removed but everything looks fine, because it is occurring in a very regulated manner and this is the process of apoptosis.

The other important aspect of apoptosis is that, you have permeabilization of the outer mitochondrial membrane, and again, this sort of differentiates from with that of necrosis, where you have opening of the pore in the inner mitochondrial membrane, as a result of which you have a loss in the mitochondrial permeability transition pore, and as a consequence of this, there is reduction in the electrical potential across the inner mitochondrial membrane.

Now, due to this, there is severe loss in the generation of ATP which you understand, and which I am sure, you are familiar with is the energy currency of cells; now, following this, there is rupture of the outer mitochondrial membrane. So, in necrosis you have a, what happens is, you have a plasma membrane rupture leakage of this, and it results in inflammation, that is a very important aspect that you need to be aware of.

The other aspect is in programmed cell death is autophagy, now, in autophagy results due to starvation loss of growth factors, and its characterized by increase in number of double membranes containing vacuoles, finite differences in these three different types will be discussed in subsequent slides.

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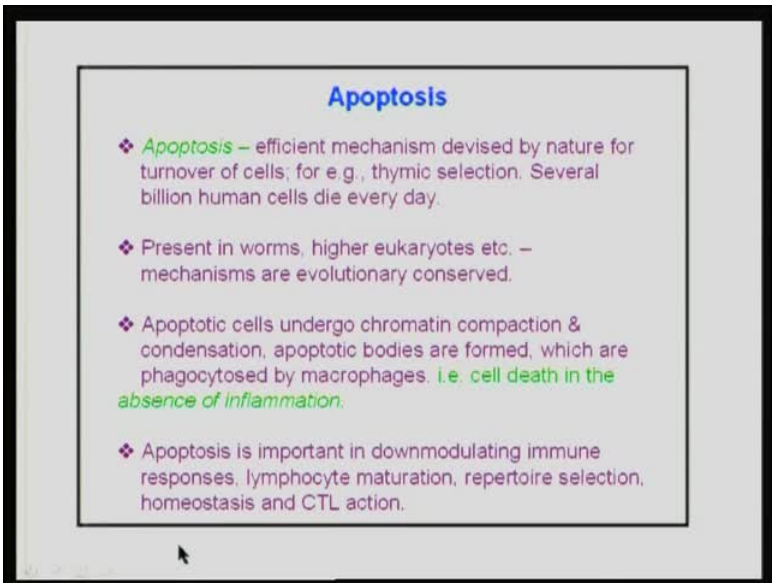

Now, apoptosis, now what is apoptosis, its again efficient method devised by nature for turnover of cells, and these are points that we had discussed thymic selection, and in fact, you have several billion human cells, that die every day, but you know as I said, they died in such, and they are died, and the cellular turnover is highly regulated, that we are, that we do not even realize, that you have such high cell cellular numbers occurring.

Now, this process is present is evolutionary conserved, and it is present in higher in worms, in fact, that is where it was discovered, the genes are important in apoptosis, higher eukaryotes etcetera. Now, apoptotic cells undergo chromatic compaction, and that is very important, because it gives the nucleus a particular morphology, and if you are very good at looking at apoptotic cells, you will be able to pick it out, you have chromatin compaction, and condensation the apoptotic bodies are formed, and which are phagocyte by macrophages. The, as I said one of the hallmarks of apoptosis is that, you have cell death in the absence of inflammation; apoptosis plays a very important role in down modulating immune responses, so once you have T cell activation, you would need to bring these down lymphocyte maturation repertoire selection in the thymus, for example, you have, you know thymic differentiation selection, and CTL, and cytotoxic T lymphocyte action, so these are important aspects that will be covered.

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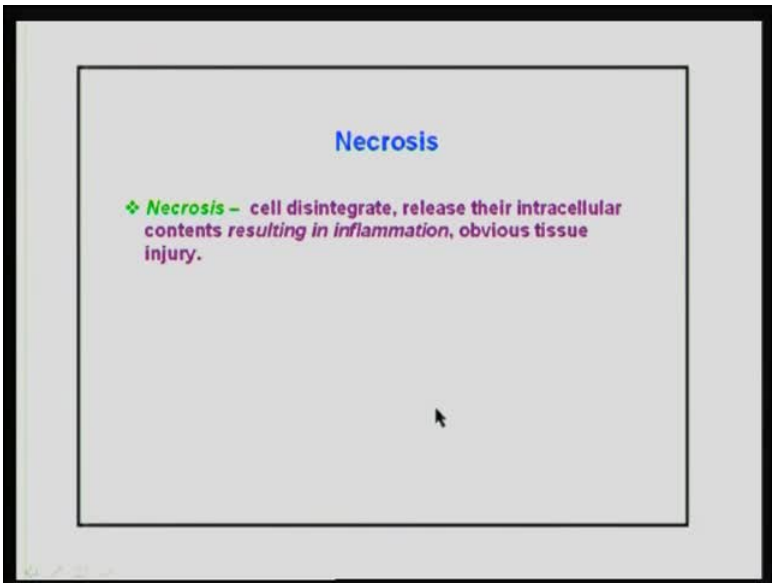

On the other hand, we discussed necrosis, where the cell disintegrates due to rupture of the plasma membrane, the release that inter cellular contents, and it results in inflammation. In other words, necrosis is somewhat of a messy situation, whereas apoptosis is a much more highly regulated method for cellular turnover.

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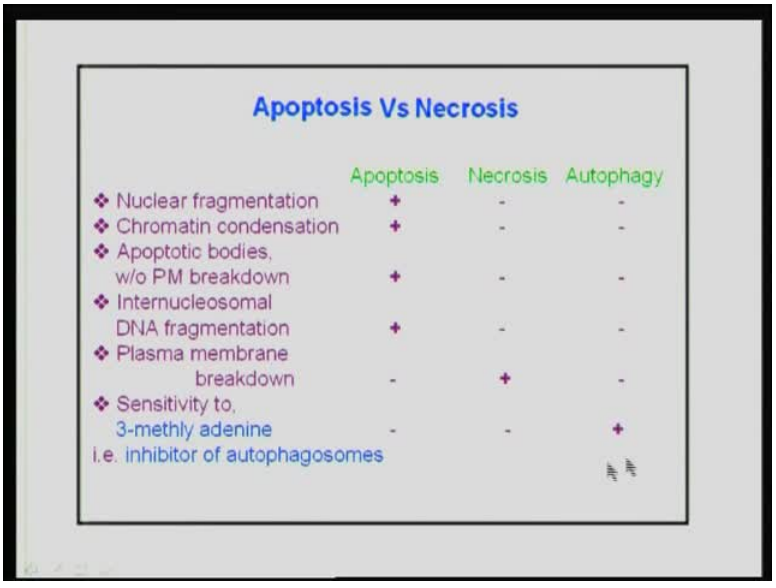

This slide it gives us little, we will look into some finer differences between apoptosis necrosis and autophagy. So, just a little bit about autophagy, what happens in autophagy is basically the cells sort of eat themselves up; so, autophagy that is what it means self-

eating, so that is what happens, and it is usually under conditions of starvation, and you have these giant vacuoles that develop, because that they are the basically the cellular contents are being eaten up inside these vacuoles.

Now, what are some of the hallmarks of apoptosis; now, you have nuclear fragmentation, in fact, this is important, and in fact, what you have is internucleosomal DNA fragmentation, so actually the ladders that one gets there is a particular ladder pattern that is got, and because that is telling you about the sites, where these internucleosomal cuts are generated. So, when you isolate DNA from apoptotic cells, there is a particular pattern that is got, and that is because it is the internucleosomal cuts, that are, that are seen.

So, as mentioned, you have chromatin condensation, you have apoptotic bodies without plasma membrane breakdown, that is the key, and inter nucleosomal DNA fragmentation so the plusses are ones, which I have listed to; so, that you can highlight, and then take a look at that.

Necrosis, you have the key, one is where you have plasma membrane breakdown, and that is the problem, and that is why the intercellular contents are ruptured, and it results in an inflammatory situation. Now, autophagy is characterized by the sensitivity to 3-methyl adenine, which is an inhibitor of autophagosomes, so which is shown over here; so, that is an important aspect. So, you can different these different processes by using different inhibitors, by looking at the morphology of cells, and by finally seeing, what the outcome is, so if you have an inflammatory like situation, then you know probably there is a lot of necrosis going on.

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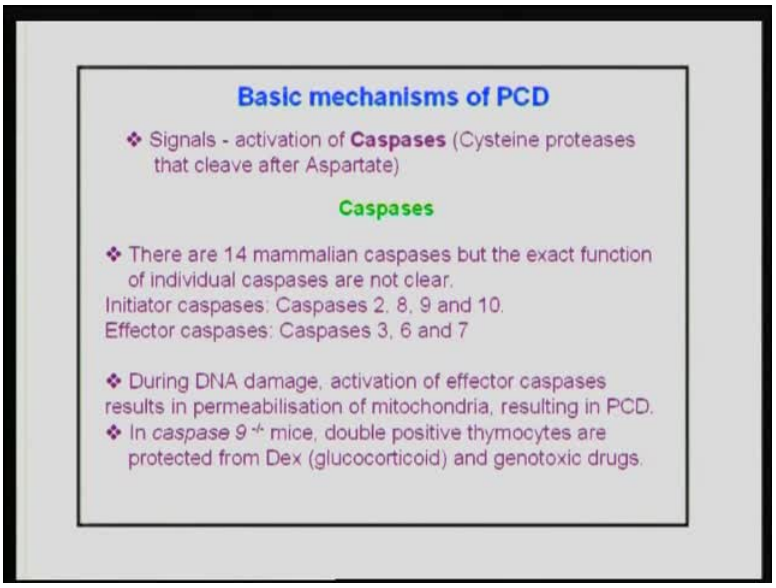

So, we will discuss some of the basic mechanisms of programmed cell death. The key enzymes that are involved in programmed cell death are activation of caspases; now, what are caspases? Caspases are nothing but these are cysteine proteases that cleave after aspartate, which is to say the active site amino acid is cysteine, and the substrate specificity is after aspartate; so, in terms of protein cleavage; so that is how they have got their name is depending on their active site enzyme, and what is their substrate preference or where do they preferentially cleave, because these are after all proteases.

Now, there are several mammalian caspases, and you perhaps have you know some of them are redundant, but by, and large you can categorize them into two types, one is the initiator caspases, and then you have effector caspases; now, initiator caspases are the key ones, that, that start it off, and out of which two are really important for the purpose for this talk, one is caspase 8 which is part of the extrinsic pathway, and other is caspase 9 which gets activated mainly through the intrinsic pathway; so, these the two pathways converge, and then activate effector pathways, which will do with the residual job. So, what this, means is that, they would initiate different pathways, where they would cleave different cellular contents, so that the cells undergo cell death, and one of the important functions of the roles of these caspase is to initiate ones, such that, the, you have permeabilization of mitochondria.

Now, mitochondria, this permeabilization of the outer membrane of the mitochondrial membrane is a very key step, it is almost a step by which, once that is done, you cannot go back, so that is a very important aspect, and there is a close link between the integrity of the mitochondria, and the cell death, it is something that, we will be seeing subsequently, just to give you an example, in the caspase 9 knockout mice here, the double positive thymocytes are protected from glucocorticoid, and geno toxic drugs, now what happens usually is once you give it glucocorticoids, the double positive thymocytes are highly sensitive to it, but however if they do not like caspase, if they do not, if these mice do not have caspase 9, then glucocorticoids cannot function, and that is what it shows that glucocorticoid function is mainly through the caspase 9 pathway in thymus sites.

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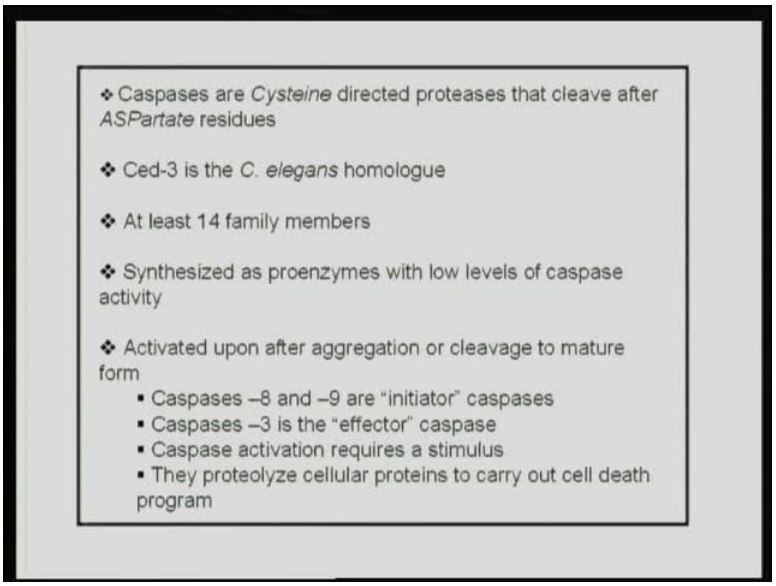

This is just a sort of a revision of something, that we had covered, and the, in the caspases, the ced-3 is the C elegans orthologue, and which is I had said that C elegans was the system, where this pathway was discovered, and one of the other important aspect is that, caspases are synthesized as proenzyme; so, as proenzymes they are ineffective, so it is only after cleavage, that their pro part is removed, that they become active, and hence the regulation of caspases or regulation of caspase activation is a very important aspect.

So, these are activated caspases are activated upon aggregation of cleavage to form the mature form, so it is a mature form that is going to be active, and once again examples of the initiator caspases are caspase 8 and 9 which is something, that we will be seeing fairly soon, and you have caspase 3 as the effector, this is an example over here as the effector caspases,

Now, caspase activation requires stimuli, and this is something that we will discuss. So, the stimuli can be essentially of two different forms, one is a receptor activation and that is true through the extrinsic pathway, where you have signals from the outside; usually, you know binding of the Fas-FasL, the Fas receptor to the FasL ligand or activation of the TNF alpha receptor, the tumor necrosis factor receptor is the, that is part of the extrinsic pathway, and once you have this, you have activation of caspase, especially caspase 8, and then you have the activation of the downstream once.

The other way is to activate through the intrinsic pathway, by which you have usually cellular stress or the responses which generate cell stress, and because of the problems in cell stress, this is translated down and this results in increase in ros, increase in activation of caspases, especially caspase 9, which ultimately feeds down into the mitochondria and then results in cellular death.

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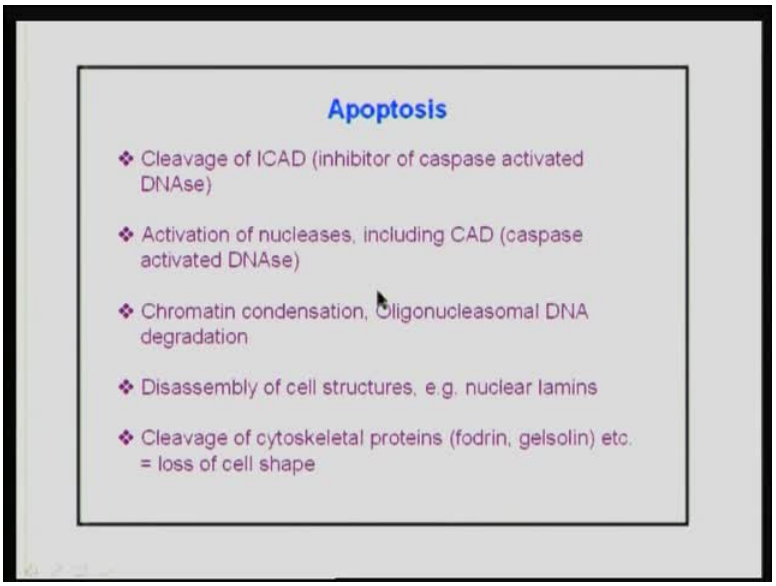

So, apoptosis now in once you have the mitochondrial pathway, and you have this entire cellular bit going down, you have different processes occurring, and so what this slide is

tells us is about the different processes that occur; so, how does a actually a cell die, you activate the caspases, and then you have cell death, so how is it the activation of caspases finally leads to cell death, because there are so many events occurring, so here are some of the events, that I have listed that you ought to be familiar with. The first part is that cleavage of ICAD, now ICAD is inhibitor of caspase activated DNAs; now, usually what happens is this inhibitor it prevents the action of this DNAs, so once you have cleavage of ICAD, so now what happens is that, DNAs is now free to function, and it results in cleavage of DNA.

Now, activation of nucleases including cad or caspase activated DNAs, so again they will cause mixing to DNA, cause DNA damage, and it will essentially kill the cell chromatin condensation which is something that was mentioned oligonucleosomal DNA degradation, disassembly of cell structures nuclear lamins; so, the nuclear envelope part is composed of proteins known as lamins, a large number of them, and so those get those are some of the substrates of these caspases, as a result of which the nuclear envelope is no longer, as an integrated whole body holds as an organ, it starts leaking out; so, things are ultimately, that is part of the process, that occurs during apoptosis, you have cleavage of cytoskeletal proteins, fodrin, gelsolin and it results in loss in cell shape.

So, in you can see there are different processes occurring, first is DNA is damaged, the genomic DNA is damaged because of activation of these DNAs alright. The second way is that, and this affects chromatin condensation, apart from the mitochondrial problems, where you have outer membrane permeabilization, you have then the nuclear lamins are affected, and as a result of which the nuclear integrity is lost, and also you have problems with cytoskeletal proteins which affects cell shape.

So, you see, once you initiate the pathway, you have lot of other processes occurring which will certainly affect the way, the cell function and the cell shape, and finally it would sort of get neutralized into apoptotic bodies, which are then removed by different mechanisms, and that is again something, that we will be studying at the last part of this lecture.

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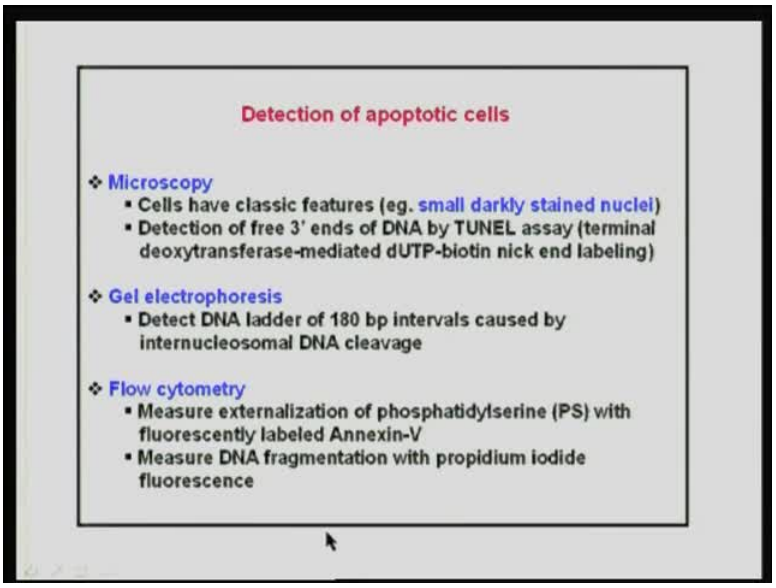

So, how does one study apoptotic cells; so, there are different ways of studying apoptotic cells, the first one is microscopy, and I have said that, because of this condensation of the nucleus, these cells have classic features, they are darkly stained nuclei, especially using hex staining, and if you are very good at looking at apoptotic cells, this is certainly a way for you to sort of tell that.

You have the other common ways of detection is by doing what is known as a Tunel assay, and that is something that we will see, now what happens over here, there are nicks made as was shown in the previous slides, there are nick made in the DNA, and because of these nicks, you have some the free a three prime ends are there, and what is done is you label the free 3 prime ends in the different nicks present in DNA with the DUTP, and the DUTP is labeled within a particular way, so you can detect it, so but DUTP gets incorporate, and do this nicks only in the presence of a particular enzyme known as terminal deoxy transferase TDT, and so you can then look at the incorporation of DTT into these nicks and that is known as a Tunel assay.

The other ways that are, that you could look at, you could do gel electrophoresis and look at DNA ladder, in which is, what I said that you can look at this 180 base pair intervals, and that is caused due to the internucleosomal DNA cleavage, then you have flow cytometry, you have you can look at the externalization of phosphatidylserine; now,

in this case what happens is phosphatidylserine is usually present inside the plasma membrane.

Now, however epitudic cells, they flip it in epitudic cells phosphatidylserine is flipped on the outside, now why is it flipped, because epitudic cells once they are dying, they show some signs, and these signs are known as eckmiflags; so, these eckmiflags are assigned for other cells to sort of ingest this apoptotic cells, and because of the flip, age of phosphatidylserine, there is a particular molecule known as annexin 5, which binds to this; so, you can have a fluorescently labeled molecule annexin five for example, and it will bind to it.

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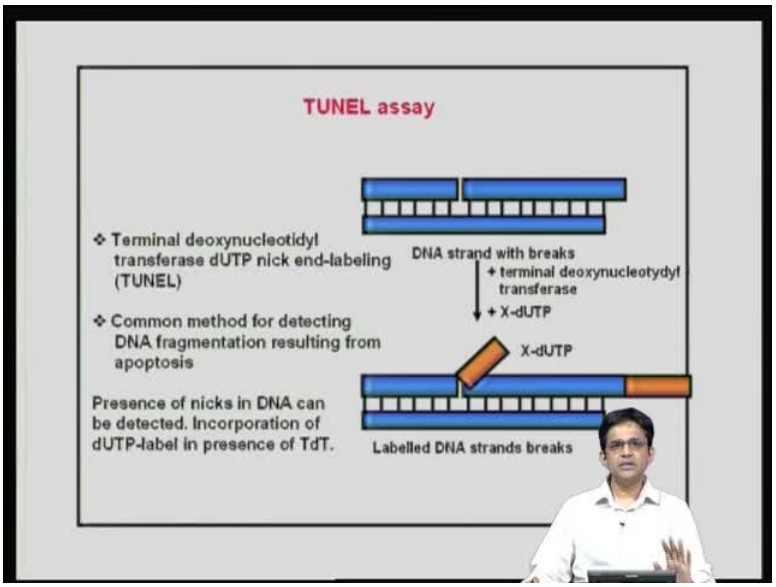

Now, what you can do is, you can distinguish your healthy cells from your apoptotic cells, you can get some good numbers on it using flow cytometry. The other mechanism is to look at DNA fragmentation using propidium iodide; now, in this case what happens is that, once you have because of the nicks the genomic DNA integrity is lost, and you have hypodiploidy, so in a cellular population, you can look at the number of cells with hypodiploidy, and as there is increased apoptosis or cell death, you have increase in hypodiploidy.

So, there are different ways of a detecting epitudic cells, I think it is important for a students to be little familiar with the techniques to detect apoptosis, and that is what was shown; there are different methods, we will discuss a little bit one in a slightly greater

detail which is the Tunel assay, which is what shown over here, you have these DNA strands with breaks that are generated, and in these breaks, you can incorporate DUTP, and X is X stands for a labeled molecule, and so for example, DUTP labeled to biotin, and you come in with the antibiotin and antibody against biotin conjugated to fiitjee, so you can see, you know these cells undergoing cell there, and so what happens is DUTP gets incorporated here in the presence of terminal deoxytidyl transferase TDT, and so Tunel stands for terminal deoxynucleotidyl transferase DUTP nick end labeling, because what is happening is only the 3 prime end gets incorporated with, so if you have a lot of mix over here, only these the label will be incorporated in certain parts, and will show up in a cell, and that is read out, that the cell is undergoing death, and what is shown over here the presence of nicks in DNA can be detected by the incorporation of this; so, just a small one example of how apoptotic cells can be detected.

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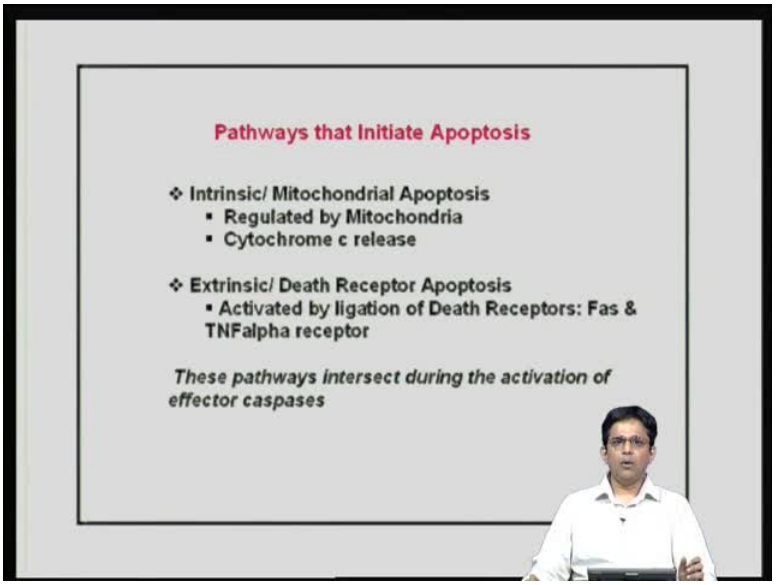

Now, as I mentioned there are two main pathways for the initiation of apoptosis, the first one is the intrinsic or mitochondrial way in which is regulated by the mitochondria and over here, in this case, you have release of cytochrome c.

The other pathway and then you have formation of the, so one is the mitochondrial and that is because of once a cells are under stress, and they feel, it is now time for us to die, and then you have activation of this intrinsic pathway.

The other pathway that is seen extrinsic or the death receptor pathway, and this is usually done upon activation by ligation of the death receptors Fas and TNF alpha or and or TNF alpha receptor family.

Now, the two pathways interact at the during the activation of the effector caspases, and now as mentioned a previously caspases are present in poor form, they need to be activated, and so the signals for activation come from these different, two different pathways which is the intrinsic and the extrinsic, extrinsic because it needs the signal from the outside to trigger, and intrinsic because it cell stress generated, so something you know maybe it is a e r stressed or high amounts of reactive oxygen species or nitrogen species, so on, so forth.

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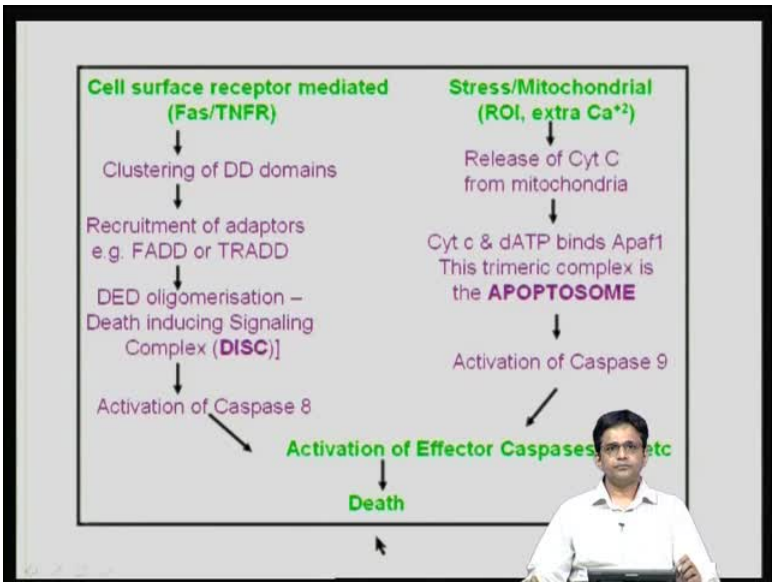

So, this is a pathway, and we will go over it somewhat slowly, so this is the cell surface receptor mediated pathway, and this goes through the Fas or the TNF receptor, and over here what happens is, you have clustering once the receptors come together Fas is the receptor, and FasL is induced under some conditions, and the when once you have binding of FasL with Fas, you have this activation of this particular pathway, and what is shown over here is you have clustering of the death domains; so, you have the receptors coming together, as a result of which a parts of the intracellular domains contain the death domain, and you have clustering of the death domains, now what this will do is it will recruit adapter molecules, and these adapters are for example the Fas associated

death domain containing proteins or the TNF receptors associated death or domain containing proteins.

Ultimately, what this results in is you have oligomerisation of death effected domains that is important part; now, these oligomerisation of this death effected domains constitutes a complex known as the death inducing signaling complex known or disc; so, once you have this, the what this does is it results in activation of caspase 8, remember pro caspase 8 is now converted into the active or mature caspase 8. Once, you have this, you have activation of the effected caspases which will ultimately finally lead to death.

Now, there are different pathways that are involved in it, and we are just discussing the initial parts, so want to be make sure that, you have you understand this particular pathway or the cells of the intrinsic pathway in very clear terms, its mainly goes through the Fas TNF receptor, and you have the clustering of the domains, and then which will recruit adapters, so and ultimately, you have formation of the death inducing signaling complex which activates caspase 8, that is why I said caspase 8, caspase 9 are the important.

The other ones, the other a pathway is the stress pathway over here, because of endogenous stress or any other forms of stress, the cell and high amounts of let us say calcium or reactive oxygen intermediates, you have release of cytochromes C from mitochondria. What happens over here, cytochrome C the dATP, now binds to APA form, and this trimeric complex is known as the apoptosome; now, apoptosome results in activation of caspase 9, and once you have this, you have activation again of the effector caspases resulting in cell death, so you have two pathways which ultimately the final outcome is cell death, but there are two different pathways that are involved in this.

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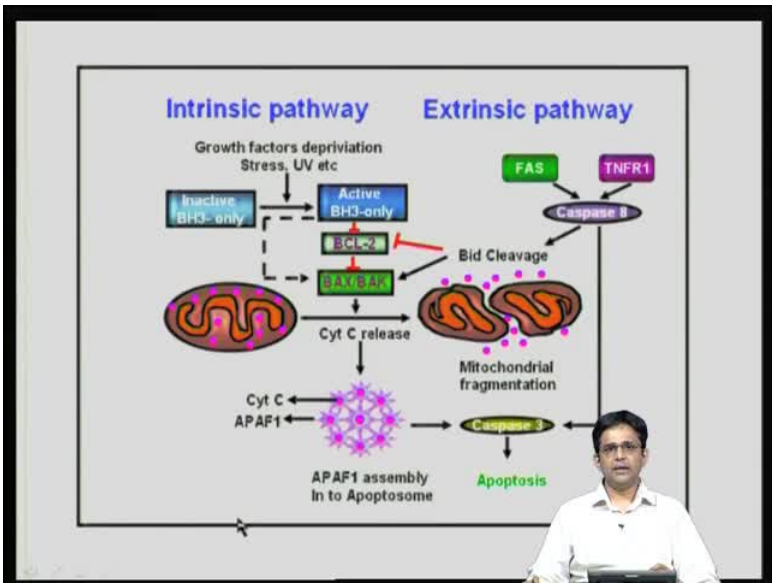

And this is shown in a little bit of a different slide over here, which is showing the Fas in the TNF receptor pathway, which results in activation of caspase 8, and its shown over here, it goes on over here, and this is the intrinsic pathway, where you have dipriviation of growth factors activation of this pro apoptotic BCL family proteins, it is something that we will discuss; ultimately, what happens is you have cytochrome C release, and then your formation of what is shown over here as apoptosome, and then activation of the effector a pathways, and you can see over here, that you have mitochondrial fragmentation.

What is also shown in this slide, and I would like you to pay attention to it, we will deal with that little bit later is caspase 8 apart from activating the effector caspase 3 which is shown over here, also results in activation of this molecule known as bid, what bid does it goes on activates bax and bak from here, and I which also result in the release of cytochrome C from the mitochondria.

So, bid is an important molecule, in the sense that, it links the two pathways, so once you activate Fas or TNF receptor you have activation of caspase 8, and that has a pathway, the, which will target the effector caspase is directly, but it also has a mechanism to of activating other proteins, and therefore, example bid which will go on, and turn on the other pathway, which results in excess secretion of cytochrome C from the mitochondria,

and over here, you can see mitochondrial fragmentation, and which will certainly contribute to cell death.

So, you have the two pathways, and one example was also shown of bid which sort of links the two pathways, and of course, as mentioned previously caspase 8 is important for the extrinsic one and the intrinsic one is caspase are 9.

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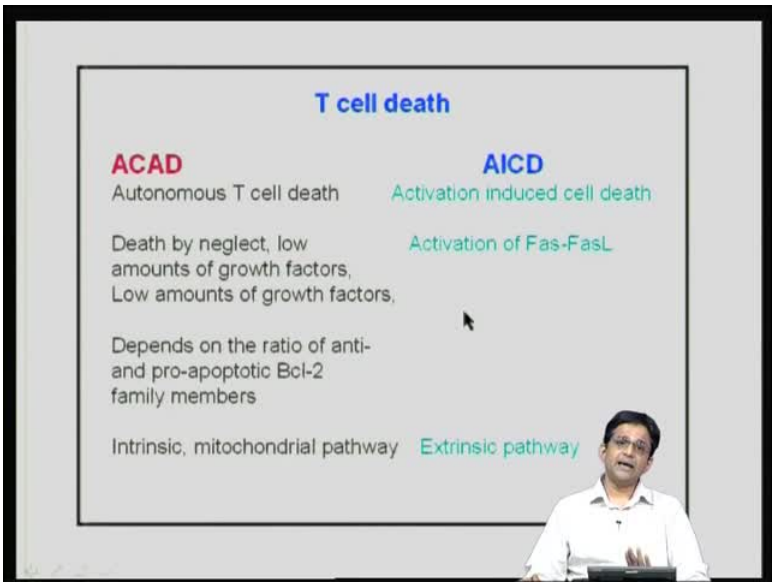

So, in terms of T cell death, there are two main forms, and again students need to be little bit familiar with it; the first is a is autonomous T cell death are known as ACAD, and what happens over here ACAD is a form, where T cells undergo death, because you have to growth factor dipriviation, so for example, they are been activated, they produced IL2 but with time IL2 amounts have dropped and once these IL2 amounts drop, then they undergo death, because it is sort of naturally takes care of the fact, that T cells have activated, they are no longer getting activated perhaps, because the antigen is no longer present, and now it is time to die, and so because of lack of for growth factors, these undergo death.

Now, in the autonomous T cell death pathway, the ratio of the anti and the pro apoptotic Bcl2 family members is most important; so, the ratio is important, because it determines whether the cells will undergo cell, whether it will the ratio of the pro verses apoptotic, whether we will determine, whether the cell finally undergo cell death or survives, so

that is an important pathway, and it is the intrinsic or the mitochondrial pathway, which plays an important role in the autonomous T cell part.

In the activation induce cell death pathway, the key pathway over here is activation of Fas-FasL cell, and it is the extrinsic pathway which is playing an important role over here.

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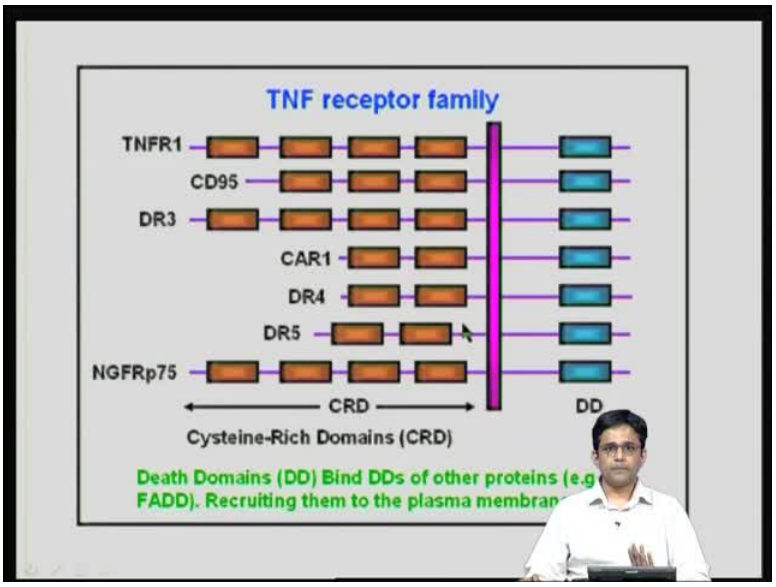

And this slide shows you about the different the TNF receptor family protein cell CD95 is the Fas, and you have different family members, you can see similar similarities in organization of the which domains, and the DD domains or the death domains, which are important in recruiting the other proteins and forming this complex or the disc.

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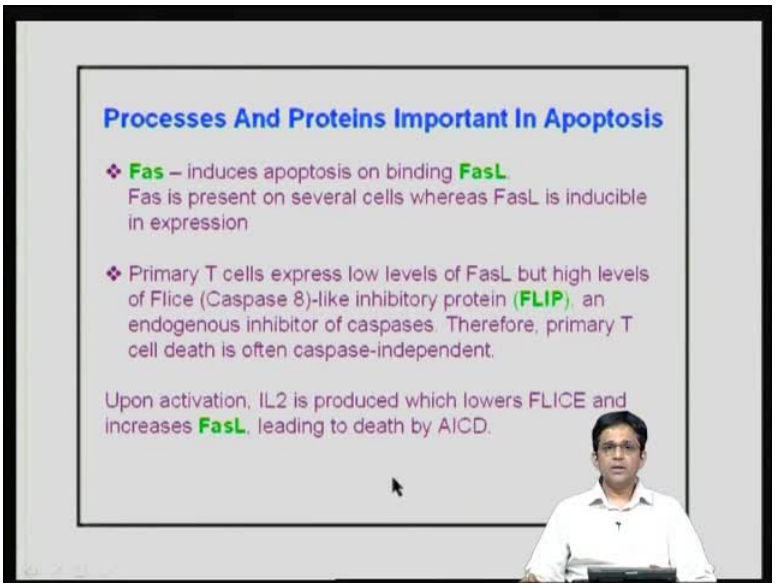

So, as was mentioned the important proteins over here are Fas and FasL; now, Fas is the receptor, FasL is induced usually upon activation, and the binding of this two result in cell death, it is also important that primary T cells express low levels of caspase 8, like inhibitory protein known as flip, and therefore, primary T cells often do not die by caspase dependent mechanism often their death is caspase independent, and upon activation what happens IL2 is produced, and this is after 4 to 5 days of being activated in the presence of IL2, and subsequently only at the latest stage, you have lowering of flice levels, you have increased in FasL, and which then will now undergo death by AICD.

So, the initial primary T cells usually die in caspase independent manner, and whereas it is only the activated cells are the latest stage, the, they die in by AICD, and that is because it takes time for the induction of FasL, and so you have now the interaction of Fas-FasL which results in this, and this is again something that is important.

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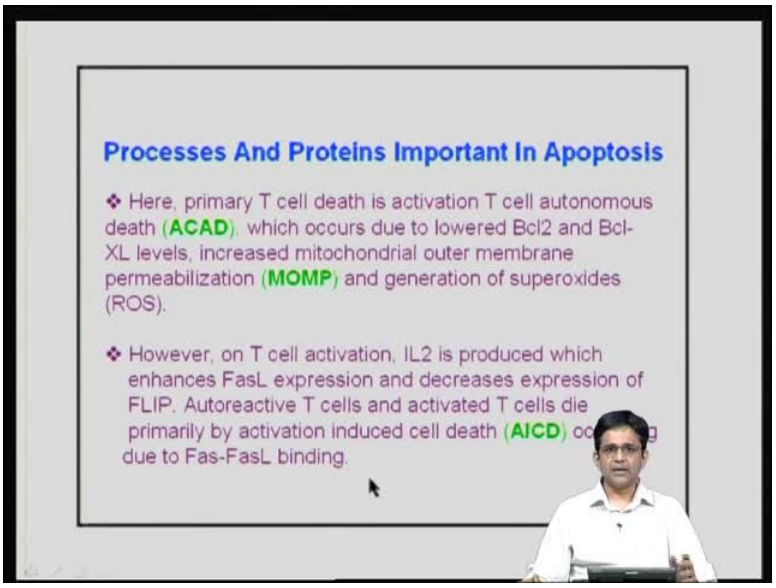

Now, as was mentioned over here, in the autonomous T cell pathway its due to lowering of Bcl2, Bcl-XL, and these are the two main entire apoptotic proteins Bcl2 Bcl-XL, and you have increased mitochondrial, outer membrane permeabilization generation of mitochondrial a ros, and which is an important aspect.

Now, IL2 enhances FasL expression, and decreases the expression of flip, and this is again important, because again this this part of subsequent T cell activation, probably mainly on the AICD, but the initial T cell activation death pathways are probably going through the autonomous T cell pathway, so one needs to understand these differences again.

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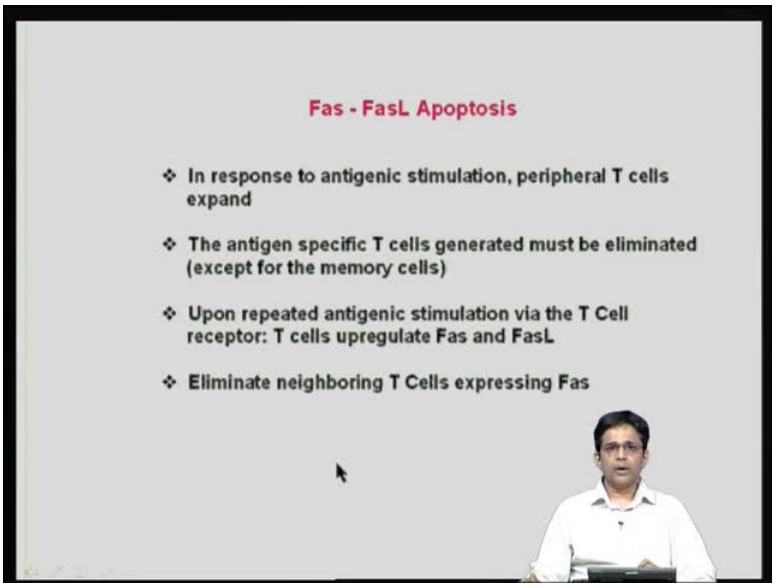

So, little bit more about Fas-FasL apoptosis in response to antigenic stimulation peripheral T cells will expand, now majority of these T cells need to be eliminated except for a small pool of memory T cells; now, upon repeated antigenic stimulation, and upon you know expression of a high amounts of IL2, IL2 induces the expression of FasL, and so then, you have the AICD pathway which is turned on, and then you eliminate these cells.

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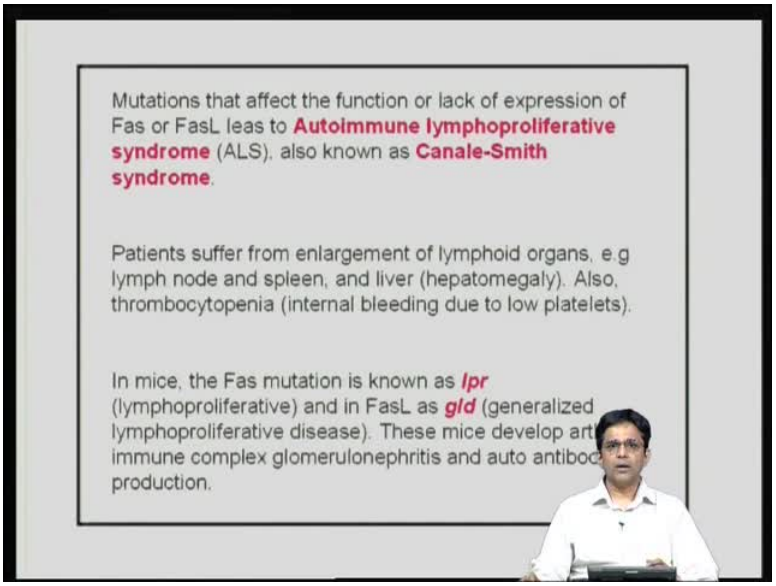

So, now the question is what happens, if you do not have a Fas or FasL or you have mutation which effect the oligomerisation or activation of these receptors; so, that you cannot activate the caspase 8 or the intrinsic or the extrinsic pathway, so what was shown is that, this leads two disease known as autoimmune lymphoproliferative syndrome, and originally, it was known as the Canale-Smith syndrome.

So, what happens over here is that, you have enlargement of lymphoid organs of especially lymph, and spleen, and you have and liver, so hepatomegaly, so that the cells that usually die are not dying through this pathway, as a result of which they are accumulating, because they are accumulating the sizes of these lymphoid organs, another organs increase, because you have accumulation of these lymphoid cells, and they cause what is known as autoimmune like syndrome, in patients also it causes thrombocytopenia, which means that, there is internal bleeding due to low number of platelets; usually, the platelets are usually able to take care of internal bleeding, but however if there is excess of internal bleeding though the number of platelets are already taking care of it; so, in the circulation, there are very low numbers or platelets which can be life threatening.

Now, in mice mutations in Fas are known as lpr and lpr stands for lymphoproliferative and Fas for mutation in FasL is known as gld or generalized lymphoproliferative disease, so and these mice, in fact, develop arthritis immune complexes, and they have immune complexes nephritis, which means, there is a problem with the kidney function, that is because you have accumulation of these antigen antibody complexes which do not get filtered easily, it clogs up the kidneys and it effects the filtration, and which is what results in inflammation, and problems in kidney function, you also have autoimmune auto antibody production. So, you can see mutations in Fas-FasL result in autoimmune disease, and these are key examples of this, it is a very important aspects, because one needs to ask if a particular protein or set of proteins is playing an important role what happens in terms of in vivo function, what happens in terms of disease, so this aspect is key.

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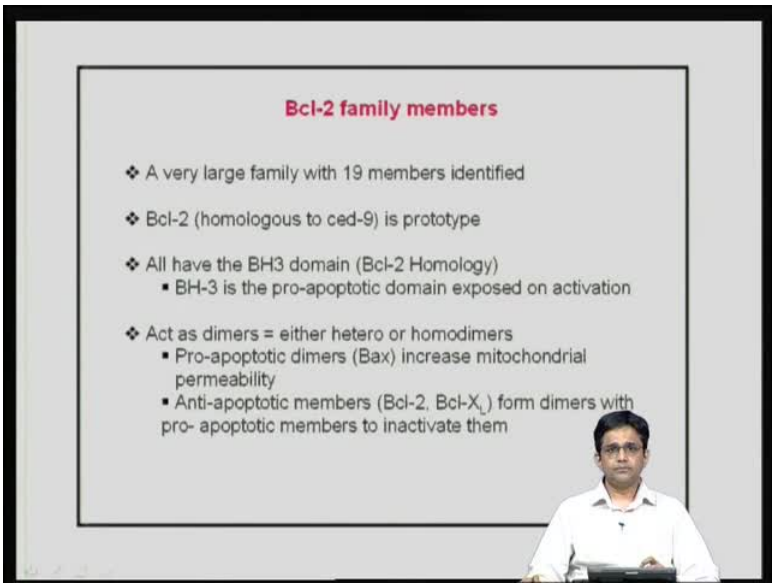

So, a little bit about, now that we talked about the surface receptor and ligands, we are to look in a little bit into the Bcl-2 family proteins, because I said the ratios of the pro apoptotic, and the anti-apoptotic Bcl family members play important roles, so one must be a little bit familiar with this; so, these are large member of Bcl family proteins and Bcl stands for B cell leukemia, because they were discovered from that source, and now there are several family members that are known, and the Bcl2 is homologous to said 9 which is present in C elegance.

Now, what is interesting is all have the BH3 domain or the Bcl-2 homology domain, and the BH-3 is the pro apoptotic domain exposed on activation; now, they act as a dimers of hetero or homodimers pro apoptotic dimmers, for example, bax increase mitochondrial permeability, the anti-apoptotic members Bcl2 Bcl-XL from dimers with pro apoptotic and you know, and try and sort been inactivate them, so it is important to understand the way the Bcl2 members function.

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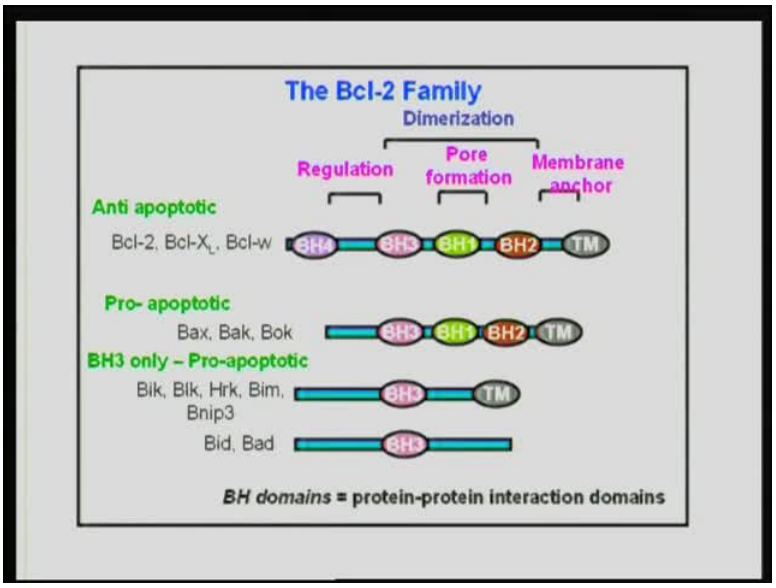

Now, if you just look at the organization, you have memory and anchor, you have pore domains that are important in pore formation and in regulation, but you can see the BH3 domains are conserved in all these different family members, and the main anti apoptotic ones are Bcl-2 Bcl-X L, I would just like to remind you, that one of the mechanisms during the T cell activation, that we study was the by which CD28 excess the costimulated of T cell activation.

Now, not only the CD28 enhance cytokines IL2 production, another cytokines, it also increases the Bcl-2 and Bcl-XL, so it acts at two ways, one is it enhances T cell activation, it also enhances T cell survival, and so the key members of the pro survival family members are Bcl-2 Bcl-XL.

On the other hand, the pro apoptotic members are shown over here, you have bax, bak, bok, so on, bid and bim, and these are all important family members, which have different functions, because there are so many of them have redundant functions, and some of them are specific for certain situations, and knockouts in mice, I have shown they roles in different situations.

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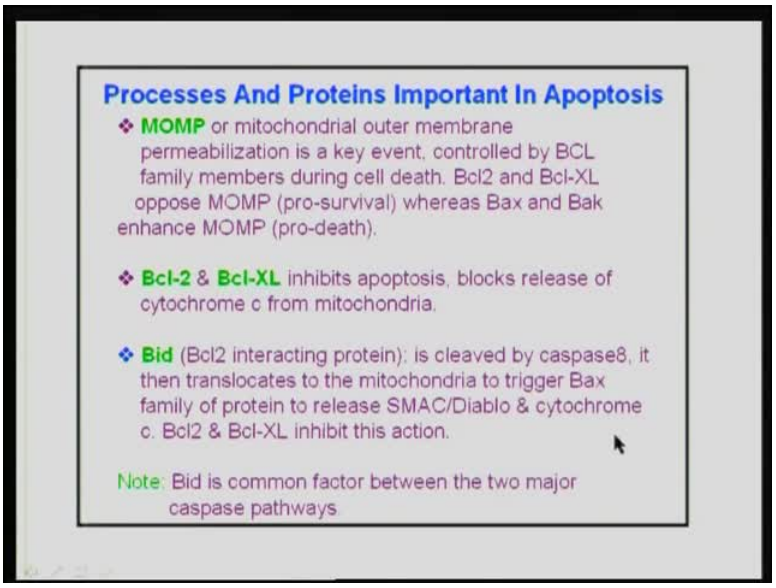

So, some of the important aspects over here, I had said that the mitochondria is clearly a very important player, and you have the mitochondrial outer membrane permeabilization, it is an very important, and this permeabilization is controlled by Bcl family members, so for example, Bcl2 Bcl-XL, they oppose the permeabilization of the mitochondria, and therefore are pro survival, so they help in survival, and whereas in bax and bak enhance a cell death.

So, if you over express Bcl2 Bcl-XL in cells, usually they tend to survive better, they are more, they are even to resist test better, and unfortunately, because of this, they also play a role in cancers, because in cells where you have higher expression of Bcl-2 or Bcl-XL, they would tend to survive more and it might sort of help in tumor cell survival; so, you have this aspect also that one needs to understand.

On the other hand, you have Bcl2, so Bcl2 and Bcl-XL, they inhibit apoptosis, they blocks or release the cytochrome c from the mitochondria, you have bid which is cleaved by caspase 8, and it translocates to as was shown in that diagram, it translocates to the mitochondria to trigger the cytochrome c release from the mitochondria, and I had said that bid is the common factor between the intrinsic and the extrinsic pathways. Now, bax and bak, these are again pro apoptotic, and whereas bim is another pro apoptotic member, and in fact, knockout studies have shown that, it bim plays an important role in the survival of granulocytes.

Now, NF-kappa B is playing an important role in the process in two NF-kappa B activation has been shown to increase T cell survival, and therefore, if you inhibit NF-kappa B, there is increase in ros, there is increase in bim, and you have increase in T cell death.

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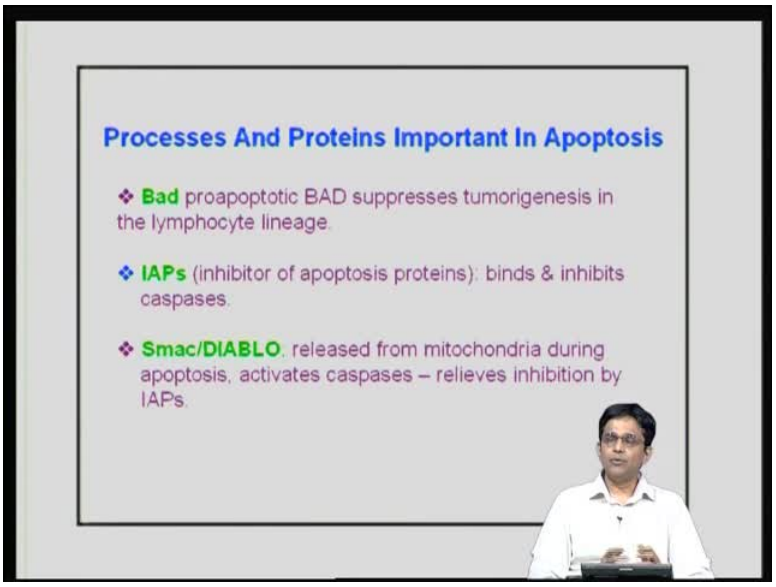

There are some other molecules that are important, so for example, you have a bad which is also pro apoptotic, then you have the IAPs or inhibitors of apoptotic proteins, these inhibitors, they actually bind and inhibit caspases, so they are inhibitors of caspases, then you have Smac and DIABLO which are released from the mitochondria during apoptosis these activate caspases, and it relieves the inhibition by the inhibitors of apoptosis.

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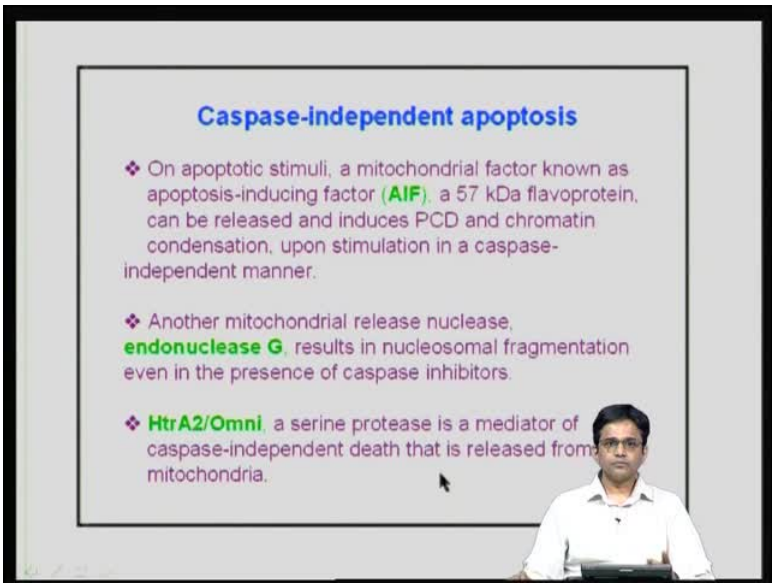

So, by enlarge as was mentioned that you have this pathway is or the apoptotic pathway is caspase dependent, however under some circumstances, you have caspase independent apoptosis to, and some of the molecules that are important during caspase independent apoptosis had been identified, one of which is apoptosis inducing factor which is a 57 kDa protein, which induces program cell death chromatin condensation in a caspase independent manner.

The other one is endonuclease G which results in nucleosomal fragmentation, even in the presence of caspase inhibitors, then you have HtrA2 Omni as a serine protease, as a mediator of caspase, independent death that is released from the mitochondria, so you had, you have these different molecules which result in caspase independent apoptosis.

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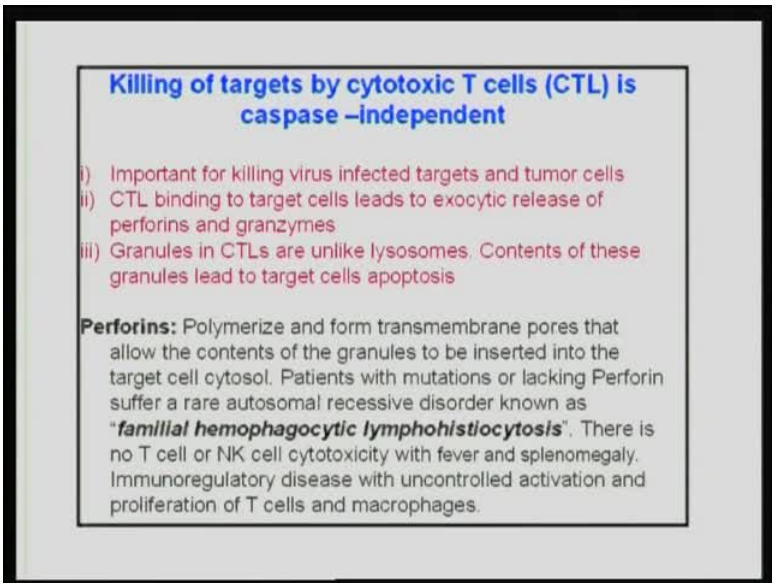

Now, the killing of CTL targets is important, is important for T cell function, and is independent of caspases, and why is it independent of caspases primarily, because of two particular proteins, one is perforins, the other is granzymes, we had briefly touched upon it in some of our previous classes, and I think I will just go a little bit in slightly greater detail over here, because it directly relates to T cell function, and since we are in this area of death, I think it is important to know what are the mechanisms associated with how CTLs actually kill target cells.

So, why is this is this important, because it is important for killing of virally infected targets, and tumor cells, CTL binding on target cells leads to exocitic release of perforins and granzymes, and what are perforins, paerforins, are these pore these form pores, once you have interaction of between the CTL, and the target, then you have pores being formed, so that now these contents from these granules can be released from the CTLs into the target cell, what is important is the granules in CTLs are unlike lysosomes, and these contents lead to target cell apoptosis; so, it s important to understand what are the intracellular contents of these granules, but before that we will look into perforins, and these polymerize, and form transmembrane pores, that allow the contents of the granules to be inserted, now what happens to patients, if they lack perforin, and in fact, they there is a particular disease, that is known as familial hemophagocytic, lymphohistiocytosis, so in these patients that there is no T cell or NK cell cytotoxicity, and these patients have fever splenomegaly, splenomegaly means inflammation of this spleen, and there is

proliferation of immune cells again, because the immune regulatory mechanism is somewhat compromised.

So, there is a major problem in patients, again that have mutations in perforin, which affect the function of perforin, and or they lack perforin. So, the other important aspect, that one needs to understand is granzymes, and perforins are important not only for NK cell for a for CTL, but the same mechanisms hold true for natural NK cell kali; so, basically, if you do not have a perforins what happens is your two arms are compromised which is your CTL arm is compromised and your NK cell arm is compromised.

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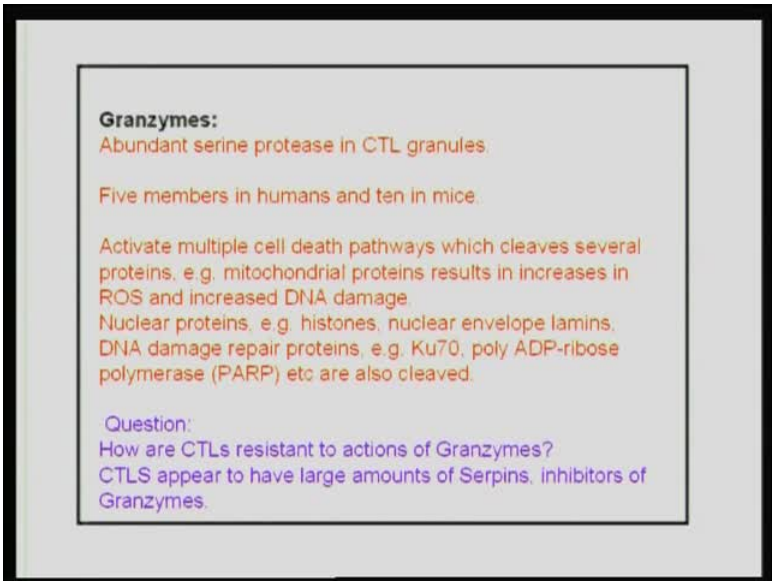

So, next one we will go on to the granzymes, and the granzymes are constitute a larger chunk of these contents in cells, and they are abundant, they are serine proteases, and there are different, five different members in humans, ten and mice. They activate multiple cell death pathways. So, again, they are, these are, they are doing it in a caspase independent manner, because they themselves are the are proteases which can do this, and so, they can cleave a mitochondrial proteins, they can again cleave nucleoproteins, they cleave a DNA damage repair proteins, especially one important one which is parp which is often used as a marker, again for cell death to know the parp cleavage is associated with cell death, and that is often studied, so granzymes are able to do all these.

So, one of the questions that I had is, you have the CTLs, that are having all these vacuoles, these granules which contain all this toxic material, what makes them, how is it

that the CTLs are able to resist the killing themselves, because suddenly the contents of the granules kill target cells, but what about the CTLs themselves, now it turns out that CTLs have a large amounts of inhibitors of these granzymes known as serpins, which will which inhibit the function of granzymes; so, basically what happens is CTLs themselves are somewhat resistant to the action of these granzymes, and therefore the target cells do not have high amounts of serpins, and therefore, once these granzymes get into the target cell cytosol, it causes great amount of damage leading to death.

An important aspect that we need to consider is, you have, let us say in the thymus, you have large amounts of apoptosis going on, large amounts of cell do not are not able to survive selection, and they need to be killed; so, how is that they are cleared, what are the mechanisms, by which they are cleared, and that is an important aspect; so, it turns out, that you have both professional APCs which is macrophages, and dendritic cells as well as non professional cells that are involved in this clearing mechanism.

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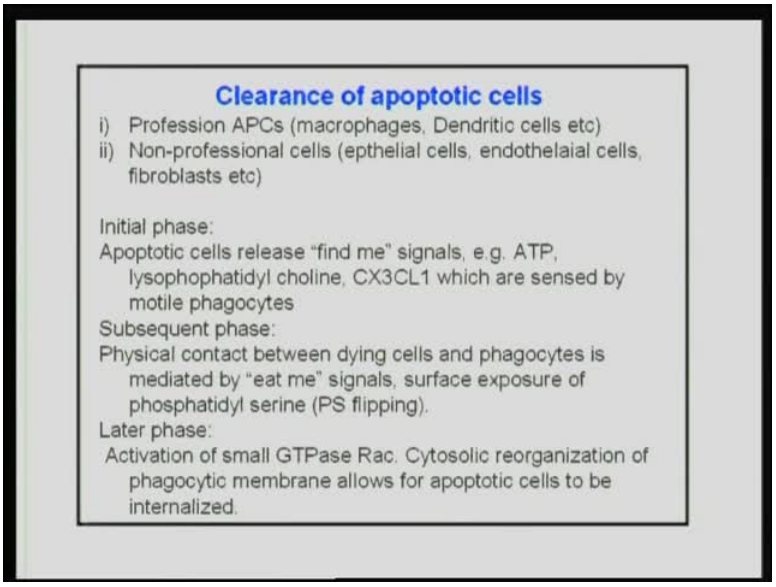

And there are different processes that are involved here, and I will briefly go over them. The first is apoptotic cells released what is known as find me signals, which is ATP lysophophatidyl choline, a chemokine known as a CX3 CL, which are sensed by phagocytes by, and these phagocytes are motile; so, they can go once this is released by concentration gradient, they are able to sense it, and they go into and try, and find these phagocytic cells, what happens then is, there is a subsequent contact between the dying

cells and the phagocytes, and this is mediated by what is known as eat me signals; so, I had eat me signals or eat me flag, and we had discussed one such flag, which is known as phosphatidylserine, and it was one of the basis for detection of apoptotic cells.

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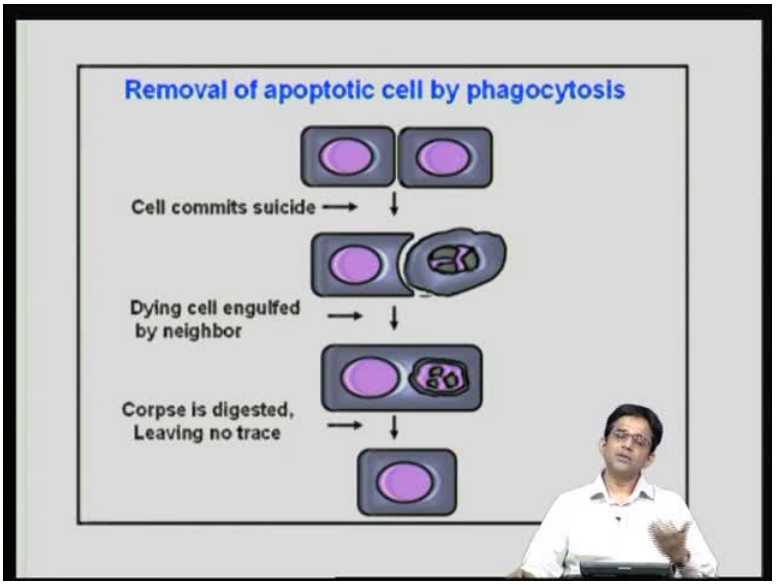

Subsequently you have activation of small GTPase known as Rac, you have cytosolic reorganization, and it allows for the for the apoptotic cells to be internalized; so, there are different processes involved in clearance of apoptotic cells, and this is I think an important aspect, that again one needs to look into. This is sort of a pictorial representation of this, so you have this particular cell that is undergoing death, and this is engulfed by the neighboring cell, and this, and it the corpse is digested, and it leaves no trace, so there is no evidence, that, that you have all these processes that are occurring.

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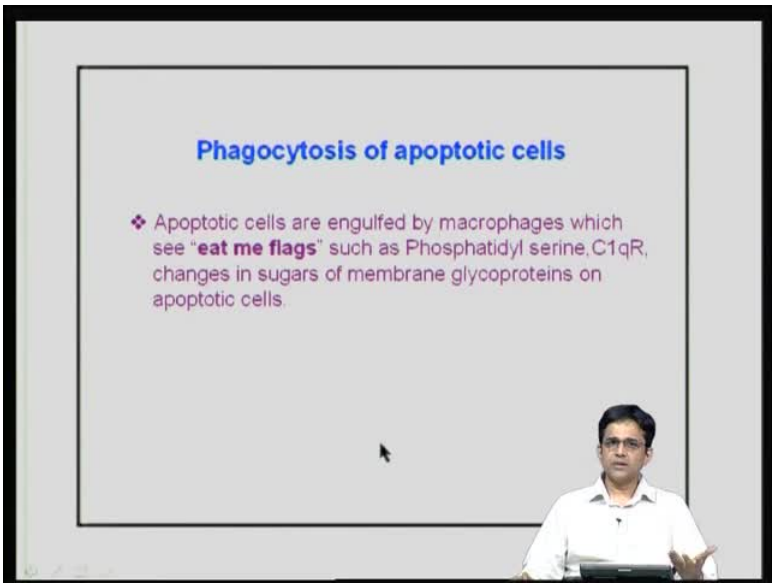

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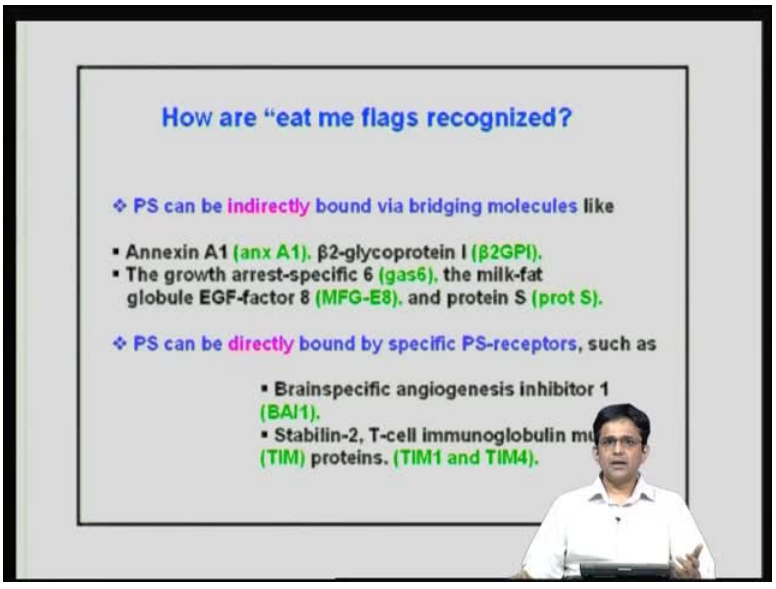

And this was mentioned that you have these apoptotic cells which express eat me flag such as phosphatidylserine you have other ones also, that is expressed. Now, how are these recognized, there are two main ways by which they can be recognized phosphatidylserine, for example can be bound by bridging molecules, and one of which, there are several, and one of which is the milk fat globulin EGF factor 8 molecule.

So, what happens is they bridge, so they bind to phosphatidylserine, they bridge, and then, now, this, they attached to some other molecules which sort of recognize it, and can

take it out the other is phosphatidylserine can be specifically bound by particular for receptors that recognize it, and there are several again over here, you have brainspecific angiotensin or bal 1, you have stabilin 2 and, you have the TIM proteins TIM 1 and TIM 4 that will recognize phosphatidylserine and they will ingest it.

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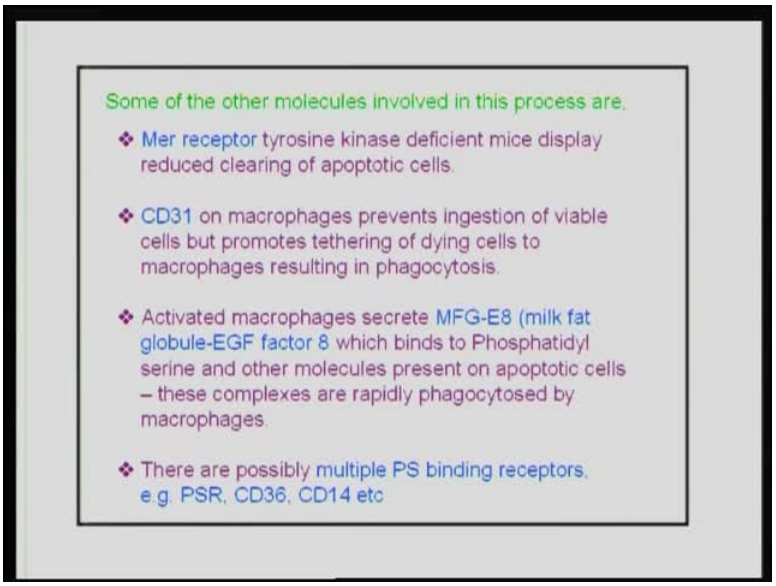

Now, moving on to the other molecules that are important in the clearance of this, you have the mer a receptor, a mer is tyrosine kinase, and this is important in the clearance of a apoptotic cells, you have different molecules that is involved over here, I am sort of running through them down you have CD31 on macrophages, which results in increased phagocytosis, I had mentioned the milk fat globulin EGF factor 8 which binds to phosphatidylserine, but and, these are complexes are phagocytes by macrophages; there are multiple phosphatidyl binding receptors CD14, CD36; CD14 is something that again we had looked up during our initial classes on innate immunity.

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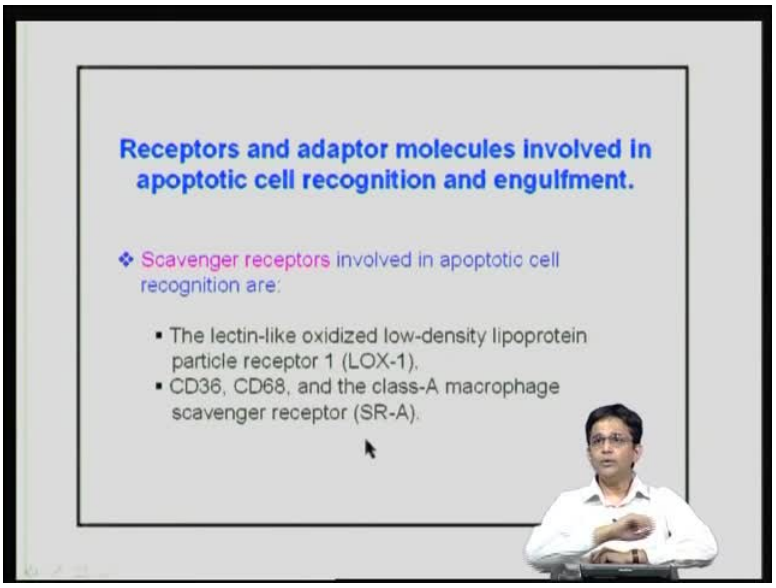

Now, then you have scavenger receptors, now scavenger receptors are ones that sort of bind to oxidized LDL proteins, and these you have different types of scavenger receptors, again CD36, these are macrophage scavenging receptors, they bind to all different types of molecules, and their job is to basically scavenge, and these are also important, and scavenger receptors are also part of the mechanisms by which apoptotic cells are recognized and they are sort of cleared out.

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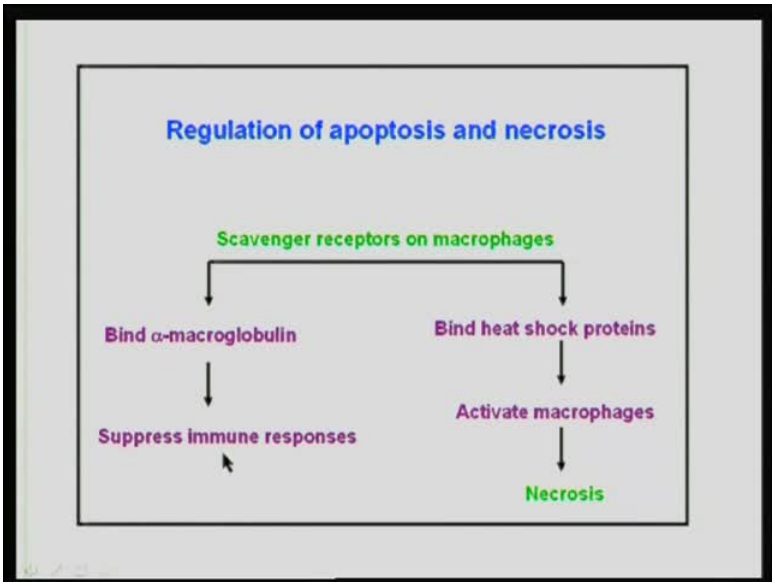

Now, I will show a particular cartoon over here to sort of tell about why this is important so, of what is shown over here is, you have scavenger receptors on macrophages, now, and these bind to alpha macroglobulin in one case, now in one case, if they bind to alpha globulin which is secrete by apoptotic cells and it suppress the immune response.

On the other hand, if the cell burst open, and you have intracellular contents being released, and for example, they, these scavenger receptor is bind heat shock proteins, then they activate the macrophages and it results in necrosis; so, this is, where you have a single receptor, that can bind to a particular, one let us say alpha macroglobulin the suppress immune responses or they bind to heat shock proteins, they activate macrophages and resulting in necrosis; so, you what is shown over here is depending on what these molecules mind bind to, you can get different responses, and this is important, because it is also important in terms of regulation of apoptosis versus a necrosis.

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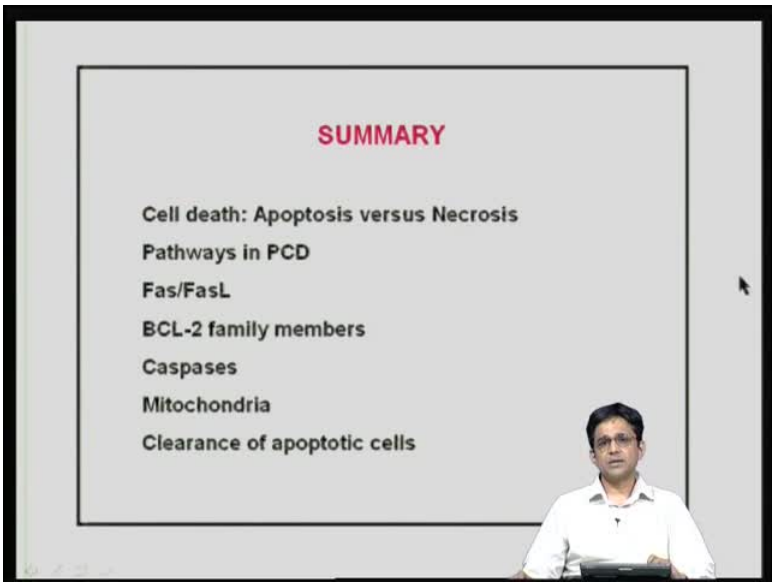

So, I will briefly, I will try and summarize this classes an important class on T cell survival and death, and so we will just the go over some of the important aspects; so, the first thing is we talked about the importance of cell death, there are different types of a cell death you have necrosis, and necrosis results in inflammation that is most important thing, and you have rupture of the plasma membrane, as a result of which intracellular contents are released; so, that is a, that is an important thing, whereas in apoptosis what happens is, you have outer membrane permeabilization, but there is no rupture of the

plasma membrane; so, the apoptotic bodies are sort of ingested as apoptotic proteins without release of the intracellular contents; so, it is a very efficient process by which this occurs.

Then, you have autophagy, autophagy is sort of a cell feeding, you have these joint vacuoles that sort of develop during starvation, and the sort of the cells eats them cells up, but they do not release their intracellular contents cell; so, these the apoptosis and autophagy are the two important players or pathways involved in program cell death.

Now, we also talked about the pathways in program cell death, there are two main ones, one is the extrinsic pathway, which relies on the Fas-FasL, and the TNF receptor, and the other is the intrinsic pathway, which gets turned on when the cells are stressed, and activation of these pathways result in activation of different types of caspases in terms of the intrinsic is the caspase 9, the extrinsic is the caspase 8; remember the caspase are made as a pro form, and they need to be cleaved in the mature form, and you have different processes that are involved over here, they finally it results in activation of the effected caspases, and then subsequently you have the outer memebrane permeabilistion of mitochondria, and once these caspase is effector, caspase are released, you have other processes occurring too, so cytoskeletal proteins are in are cleaved, you have nuclear lamins being cleaved, so the nuclear envelope is damaged, and you have DNAs is being activated as a result of which the genomic integrity is lost; so, you have all these different processes that are occurring and that one needs to be a little bit aware of.

Now, we, it is also important to understand roles of Fas-FasL,and the best way to look at, it is to look at mutations or people or mice, where the, it is a non-mutation or you have some other mutations, by which these molecules cannot associate, and form active complex, and so in case of Fas-FasL, people have splenomegaly, they are hepatomegaly, they have increased in numbers of in the size of their immune organs, because you have large number of these immune cells which cannot die, and which they are around which results in autoimmune in autoimmune disease.

We also discussed the importance of the BCL family or members BCL family members; now, there are two main types of BCL family members, you have the pro apoptotic and the anti apoptotic. The two major ones that pay important role in resisting apoptosis is Bcl2 and Bcl-XL, and they are the main ones, and the pro apoptotic, there are several of

them, bim, bax, bak, and some of these result in permeabilization of the outer membrane in the mitochondria; so, you have cytochrome c coming up out, and then formation cytochrome c gets together with d ATP and APF 1 to form the apoptosome which will activate the caspase 9 pathway, and we talked about the role of bid which sort of links these two the intrinsic and the extrinsic pathways.

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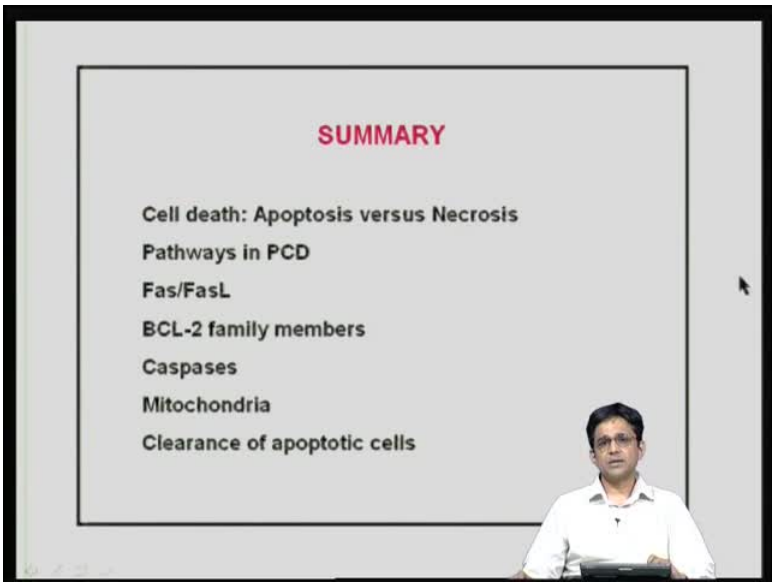

We also talked about caspases, you have the two main types the initiator and the effector caspases, I talked about a little bit about the functions of these caspase 8 is for the intrinsic pathway, primarily involved in the extrinsic pathway, sorry, and the caspase 9 is in the intrinsic pathway, you have the effect of caspases, and we talked about the different roles a apart from mitochondria, they have the activated nucleases the degrade cytoskeletal proteins, the degrade nuclear lamins.

Now, the mitochondria is really the main player doubt here, the outer membrane permeabilization of the mitochondria is almost as signal, ones that happens, it cannot, the cell cannot go back, the cell is destine for apoptosis, and therefore, the integrity of the of the mitochondria is really important, and the integrity of the mitochondria the main players, that are, there are actually the BCL family of proteins which play important role in either maintaining or enhancing death, and the way they do it, they are different mechanisms by which they do it, and they are clearly the really the important players in this process.

Then, once you have so much death, you have quite lot of death going on, what are the mechanisms, by which they are cleared, and we talked about the different mechanisms, you have signals being sent out like ATP lysophosphatidyl choline, and these are sort of sensed by phagocytes by, and so they come towards these apoptotic cells, and then you have binding of this between, which you have these signals, again send off by apoptotic cells phosphatidylserine is a good example of it, and then you have activation of the Rac, GTPases, and the neo cytokine reorganization, and then ultimately these are phagocytose.

So, we also discussed the importance of mutations in two of thethe genes Fas-FasL, it results in Canale-Smith syndrome, and we talked about the autoimmune disease, that it results in we also talked about perforin; now, mutations in perforin which is important for CTL cytotoxicity, because it forms pores, it results in inhibition of CTL as well as NK cell function, and this greatly compromises the host as, and there are immune regulatory problems as a result of which you have autoimmune, because it seems to be activated, we also talked about granzymes, and I think I will end over here.

Thank you.