Essentials in Immunology

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Lecture No. # 01

Introduction to the immune system

Hello. Welcome, my name is R Manjunath and I will be giving this course in essentials and immunology along with two other instructors, Doctor Anjali Karande and Doctor Depanker Nandi.

I am beginning this course to give you an introduction to this subject of immunology, subsequent to which the other, instruction, instructors will start their own portions for the course. So, this lecture 1, mainly consist of topics dealing with the history of immunology.

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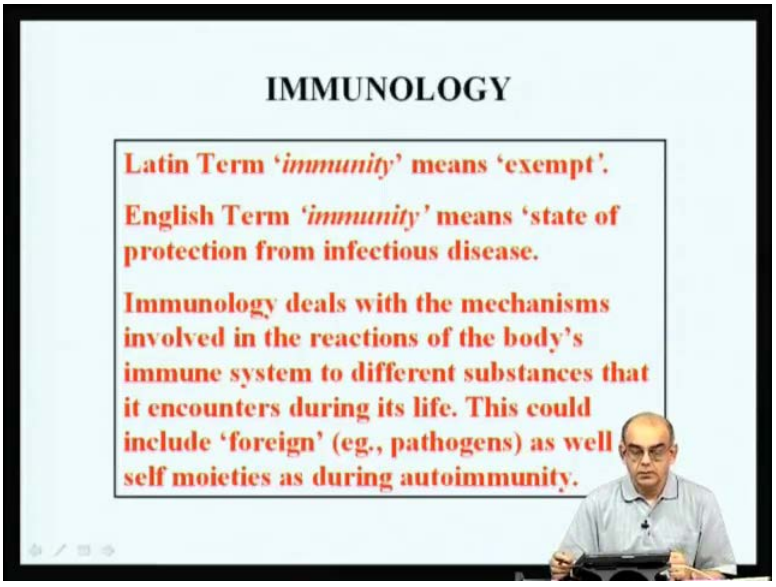

So, what is immunology? Now, immunology, immunity, the Latin term immunity actually mean, exempt and the English term immunity means, state of protection from infectious disease. And I am sure, that all of us, all of you also know that immunology

deals with the mechanisms that are involved in the reactions, the body's immune systems to different substances that it encounters during its life.

Now, these substances could include foreign, for example pathogens, like viruses, bacteria, which cause infection and disease, and not only that, it may also include self-moieties or self-protein, that can cause a reaction as what happens during autoimmunity. Basically, to kind of look at immunology, immunology is all about self and non-self-discrimination; that means, your body's immune selves distinguish between what is self to you and what is non-self to you. Also, there is an increasingly evident concept, that immunity involves actually perception of danger from non-danger.

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Now, if you were to look at the distinction between self and non-self, self and non-self actually is not so much peculiar only to higher organisms like mammals, it is also seen in very, very primitive animals, especially even in annelids; you will see here, that earthworms also exhibits this phenomena of distinction from self and non-self. If you take a piece of skin from earthworm from one locality and graft it onto another one from a different locality, here shown in red, you will see that the red earthworm actually rejects the black worm. However, if you have a skin graft that is taken from the same locality earthworm, then there is actually acceptance.

Now, this primitive distinction between self and non-self can also be seen in many other lower order specimens, like for example in corals. Now, all of you know, that coral have

a lot of polyps, now if you take these polyps, from one polyp and then graft it on to another polyp; the red one here is shown strangling the green one, which means there is a distinction between self and non-self. Actually, all these polyps are growing on the shell, which is inhabited by the hermit crab. Now, such sort of examples are evident in many other orders, orders of the immune system, like you will see in different phyla and so on and so forth. But the immune system has evolved into a very complex system in higher animals; much more about all these later on, during lecture in evolution of the immune system.

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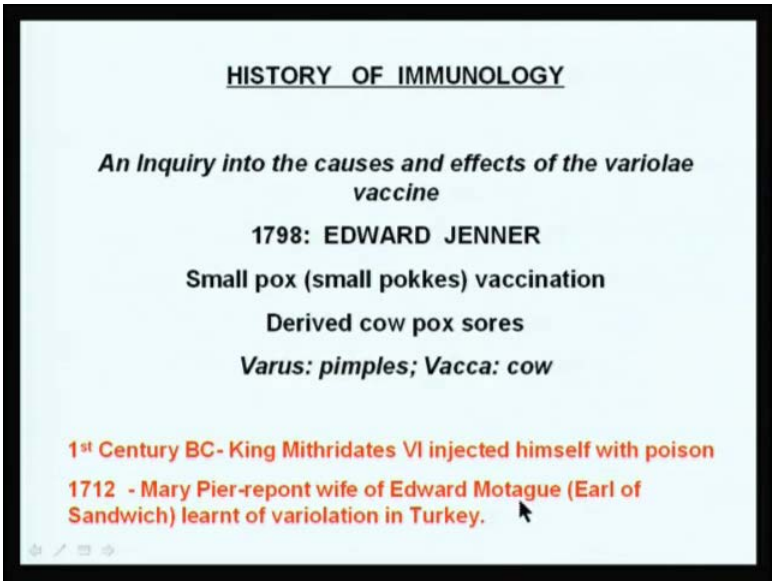

Now, to begin with the history of immunology; why history of immunology? Because if you look at history, you will actually come to appreciate some of the discoveries as they were made, and this discovery actually helps us to put together or put in place what we are learning in class today. Basically, this course immunology, I will be trying to make things clear and try to present them in a much more simple manner, the same thing, that is already available in many immunology text book, like Kuby immunology and several other text books, which we will give up, give a kind of reference to later on, during the classes.

So, to look at history of immunology, if you really look at it, probably began as man evolved; there is no beginning to this history of immunology. Now, if you look at various

historical texts, you will see the scourge the plague was causing during the ancient history and references have been made to this.

But a systematic entry of historical events actually, begins with what happened with Edward Jenner and you will see that Edward Jenner, in 1798 came up with a vaccination against the small pox. During those days, small pox was a scourge; it was killing a lot of people, disfiguring those who actually survived this disease.

Edward Jenner actually looked at the causes and effects of the variolae vaccine. So, varus means pimples and vacca means cow; so why this pimples and cow? Because during those days, it was a kind of a widely accepted observation, that these milk-maids or cow-maids, who used to milk cows, they used to come down with some disease called a cow pox, where they had source on their skin and these people rarely ever came down with small pox, and therefore, this association between pimples and cow and the word variolation. In fact, this small pox derives, or derives its meaning from small pokkes, pokkes means holes because these are the kind of poke marks that are a, that form on the face of individuals who come down with small pox.

Now, before this, actually, just to make some brief reference to the 1st, in 1st century BC, you find, that King Mithridates actually injected himself with increasing doses of poison to avoid being killed by murderer or who may poison him later, but the ironical part of this story is that when actually, he got depressed and he wanted to commit suicide by taking poison, the poison had no effect on him.

Now, another curious fact is this injection of poison, increases, increasing doses of poison is actually followed, when you make antisera against snake venom. So, how is antisera against snake venom made? They take very small concentrations of this snake venom, that will not kill horses and inject them to begin with, and then follow it up with slowly or increasing doses or increasing concentrations of this snake venom and get this antisera, which will neutralize the snake venom, which has to be given to people who have a snake bite and have had a chance to come to the hospital to receive it.

Apart from this, another historical fact, in, in 1712, Mary Pier Repont who was the wife of Edward Motague, she was a known beauty, but later on, after marriage, she was affected by small pox and therefore, her face became disfigured. Fortunately, she survived the attack and then, when her husband went to as an Earl of Sandwich, he was

an Earl of Sandwich and he went to, went to Turkey as an ambassador, she learnt of this practice of variolation, which was wide spread in Turkey, but was not known in other parts of the world. She took it upon herself to try and popularize this technique of variolation. And then, of course you know, in 1798, about the story of Edward Jenner, who injected the small boy with cow pox and then, actually challenged him with actual small pox material and saw, that the, that the boy was actually protected.

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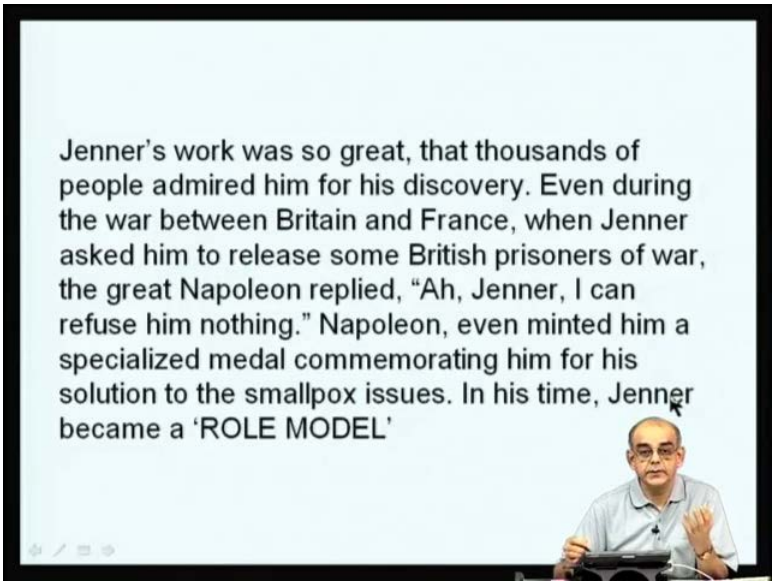

Now, Jenner was so, so great during that time, that thousands of people actually admired him for his discovery. He was so famous and so much admired, that during this war between Britain and France, this great person Napoleon whom all of you know about, actually when he was requested by Jenner to release some British prisoners of war, he said, Ah Jenner I can refuse him nothing. He even made or minted him a specialized medal, commemorating him for a solution to the smallpox issues. So, you can see that Jenner was actually a role model during his time.

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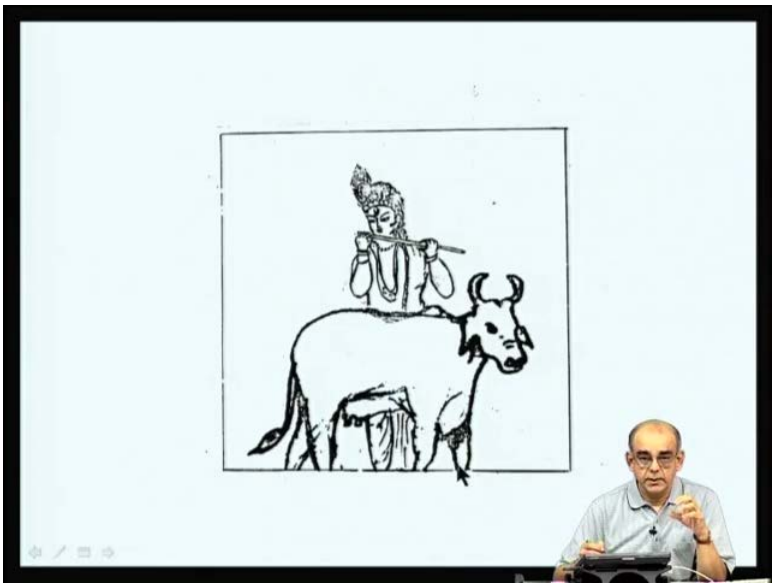

And then, strange co-incidents, that we were mentioning about vacca being cow, and here you have this supreme God head who is lord Krishna associated with the cow and much more interesting is a fact, that that these Gopicas, who are always going around with cows or cow maids were always lovers of lord Krishna and lord Krishna was loving them quite a bit.

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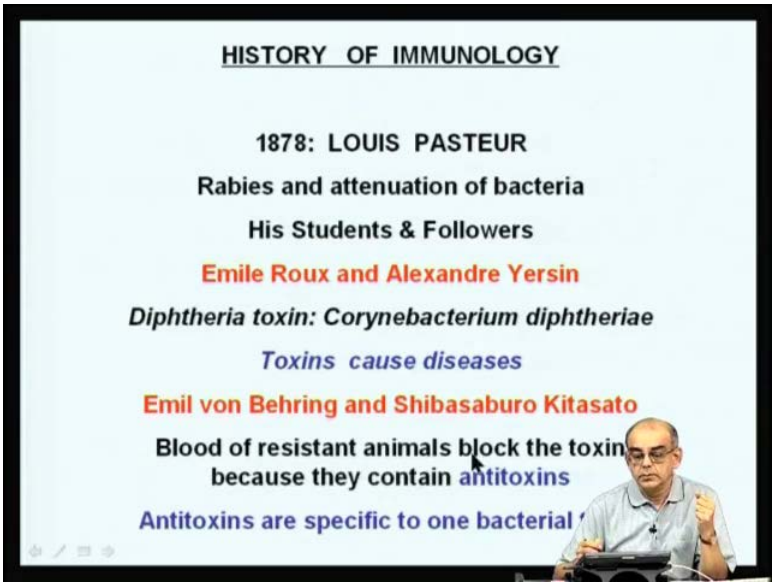

Now, going on, you see, in the history of immunology, in 1878, subsequent to Edward Jenner, it was Louis Pasteur who may, who is, who is a great name in your text books, he

is known for his rabies vaccine and of course, attenuation of bacteria. Now, his students and followers Emile Roux and Alexander Yersin also contributed a lot to, to the development of immunology.

Now, what they did was that they were working on corynebacterium diphtheriae. Now, this corynebacterium diphtheriae derives its term from diphtheria; diphtheria meaning throat. So, this particular organism grows in the throat of new born, yet this growth, growth in the throat actually kills the new born. So, how is it possible, that when the bacteria reside in the throat, it can kill the individual? So, they said, this particular organism is secreting a toxin and this toxin, they called it as diphtheria toxin.

So, they propounded this concept, that actually toxins cause diseases. Then, it was actually, Emil von Behring and Shibasaburo Kitasato who discovered, that when you inject these toxins because toxins were available by growing these bacteria in petri-plates, they have taken the culture filtrates of these bacteria and prepared these toxins. Then, one of the experimental models of those days was using chicken or using dogs or using rabbits or even guinea pigs.

So, they used to take this toxin and inject them into these larger animals and they took the blood of those animals, which were resistant to this toxin. In other words, they were not killed by this toxin and they found, that this blood of resistant animals had the ability to block the action of the toxin; in that, the toxic effect of the toxin when administered to an (( )) animal, it could neutralize the ability.

So, they said, this phenomena of neutralizing the effect of the toxin was actually because they had antitoxins. So, these antitoxins had the property of neutralizing the toxic nature of the toxin that was produced by these bacteria. Then, they found, that these antitoxins were actually very specific to each or that bacterial strain or bacteria. So, they found, that when you took these toxins that are secreted by one, one type of bacteria, they would be very specific to that bacteria and they would not neutralize the toxin, that is secreted by other types of bacteria.

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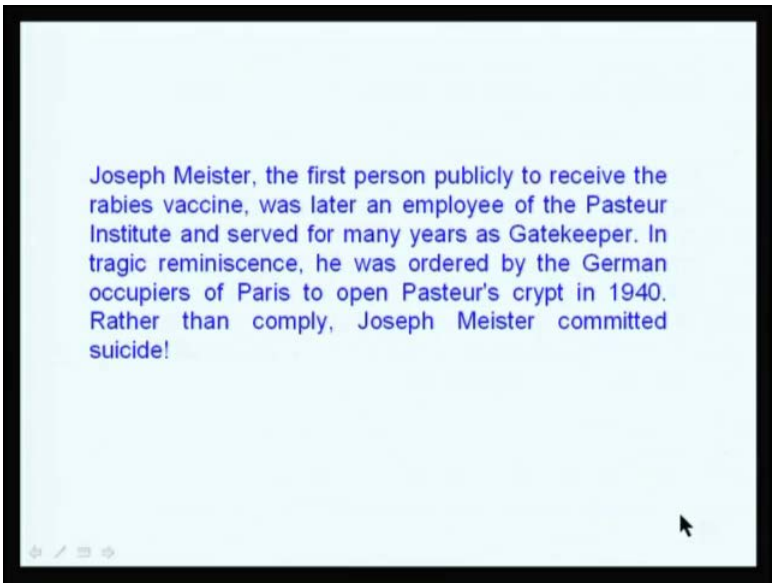

So, then, these students of this great person…, Actually, there is a kind of a tragic history, that is associated with this rabies vaccine because this person Joseph Meister, as a boy, as a 8 year old boy was a first person to receive this rabies vaccine. Then, after he became resistant to this rabies by this vaccination, he turned out to become an employee of the Pasteur institute, which was founded in France, all of you know about Pasteur institute, and he served in that institute as a gatekeeper for many years, for about 40 years. And the tragedy is that when the Germans invaded Paris during the war, they ordered him to open Pasteur's crypt in 1940 and he was so depressed, that he could not manage to do that, and therefore, he committed suicide.

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So, such of the incidence in history, which is actually a great, you know, great incidents that actually make us remember so many things that have happened in the history of immunology. So, that was about Louis Pasteur and then his students.

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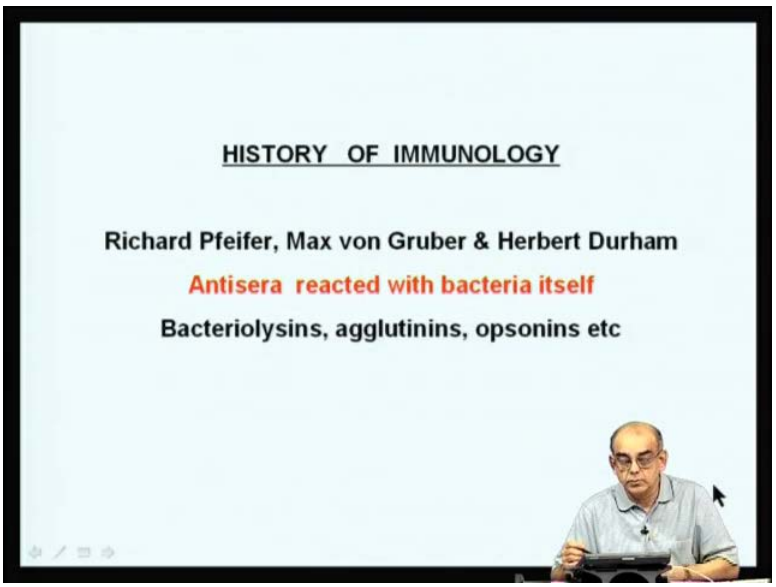

Then we come on to other kinds of observations, that were made by these scientists, Pfeiffer, von Gruber and Durham and they discovered, that when they injected these toxins to these animals, the blood or the blood or they call a body humor, which was known as, what it was known during those days? This is nothing but antisera. You just

take the blood and allow it to clot, and what you have when you remove the clot is called as the serum. And therefore, this antiserum had those antitoxin material and this antisera they found, not only it reacted to the toxin, it also reacted with the bacteria that secreted the toxin.

So, in other words, you had the body humor responding by making antibody or you inject this body, this body is nothing but the toxin. During dosage, which they refer to and this antibody would now recognize, not only the body that was injected, but also the bacteria, that was secreting, that was also injected into that, into that animal. And then, came the concept about how these, these antisera, there was lot of work, that was conducted on the subject of lysing bacteria with this body humor or antibody and including agglutination and opsonins.

Opsonins is nothing but a phenomena where you, you coat macrophages with these antibodies and the macrophage becomes activated to go and engulf those organisms. So, this, these phenomena was called as optionization.

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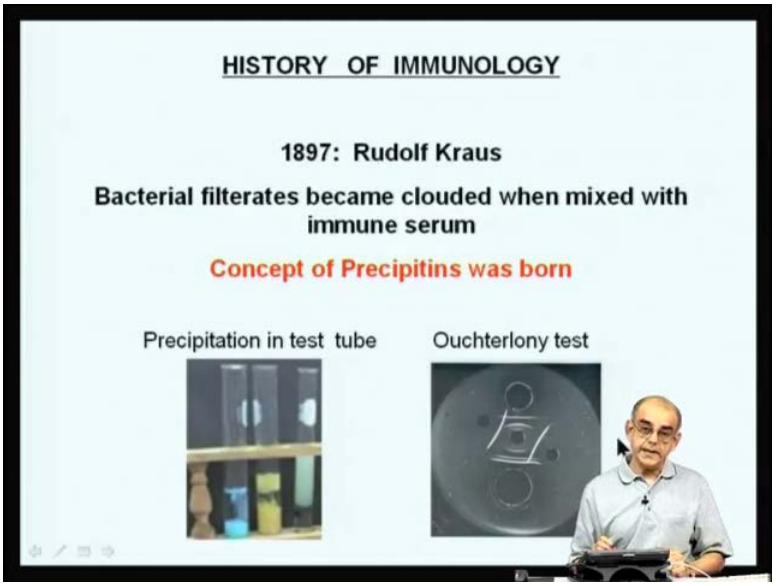

So, then, in 1897, a very important observation, which helps us to understand many of the simple things, that came later on, which we rarely look at it from that point of view when we study immunology text books in our BSc's and MSc's, and that is this observation in 1897 by Rudolf Kraus.

So what did he observe? He observed something, he was doing experiments, you know during those days people were experimenting, like in chemistry labs that we do in BSc, trying to identify compounds or different groups by mixing different chemical reagents in test tubes, like what you see here and then, if you had the presence of a precipitate you would conclude, that it is one group, when you did not have the precipitate, you would go on to test it for a different type of group.

So, this, actually these experiments that were done in the chemistry lab, is what was done by this Rudolf Kraus. And also, one more important point is that all of these experiments were done in macro volumes; you know, these days we talked, we talk about trying to do reactions with the small drop of blood, during those days it was more, in terms of 10 ml, 20 ml and 50 ml of blood.

So, they found, that when you took this immune serum or this serum that was prepared from these challenged animals or injected animals, they had the ability to cloud bacterial filtrates. In other words, you grow up bacteria and then you spring down and then remove the bacteria and take the filtrate and you mix them with this antiserum, they found, that there was a precipitate, like what is shown here in the test tube and in fact, this was what actually developed into some sort of a quantitation for the antigen-antibody complex.

Antigen being that, which was injected into the animal to produce this antibody, therefore it is called as an immunogen. Whatever substance that combines with the antibody in the test tube, that substance is called as the antigen. Therefore, the antigen combines with the antibody in the test tube to form a complex and at the right concentration actually precipitates out. The word immunogen was, is being applied or is, the term immunogen is used for or probably the same protein, but when injected into the animal.

So, if you look at later developments, in fact, the scientist Ouchterlony and all of you must have been exposed to this Ouchterlony test, where you have these white arcs forming between two wells, that have been punched in an agarose plate or a petri plate that is containing this agarose, which has been poured after being molten and then allowed to solidify. And therefore, you had antiserum in one well and you had antigen in other well, you allow it to diffuse and at the right concentration, which is also called as a

zone of equivalence, you had a precipitation here and in fact, this precipitation is actually nothing but this precipitate, that is forming in your Ouchterlony plate.

So, you can see, how these, this observation was later actually applied in order to look at similarity between different kinds of antigens and also to try and quantitate the amount of antigen, that is present either in the well or in other kinds of situations. In fact, this particular observation actually, developed later on by using radioactive tracers into a very, very, very sensitive technique called as radioimmunoassay, which was discovered by Yale.

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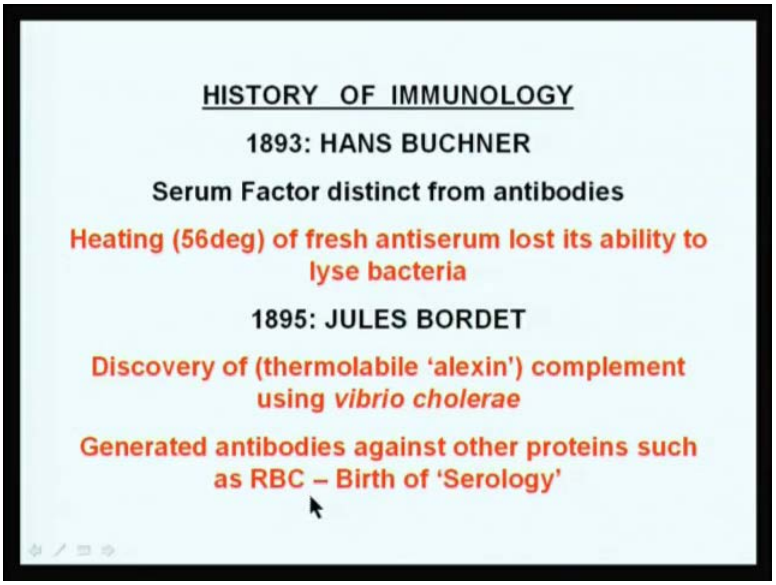

Now, going on, now the history of immunology. In 1893, Hans Buchner, he said, that there is something else, that is very distinct from the, that what was already discovered and what was widely discussed, as being kind of a something that was a great discovery, which it was during the time. He said that the serum has something else, which is distinct from antibodies, which had the ability to lyse bacteria; how did he find this out? He found this out by taking serum from 9 animals or those animals, which were not been exposed to that particular antigen or bacteria.

Now, when he mixed that serum along with the bacteria and allowed with the antibody or the resistant serum, he found, that this serum had the ability to increase the lyses of that bacteria in which it was mixed with.

Now, this increased capacity to lyse bacteria was actually, destroyed when you heated the serum to 56 degrees. So, he said, that this distinct serum factor was inactivated by heating to 56 degrees. In fact, that is what we still even today do for the inactivation of the serum factor, which was, which he called as alexin or nowadays called as complement. So, he was that, then in 1895, actually all these observations were, were actually concluded by Jules Bordet who discovered this particular complement by using vibrio cholerae.

So, you see, during those times, also called cholera, cholerae was abundant, and from where they got all these vibrio cholerae? Now, so, all these observations along with the discovery, that you could generate antibodies to many substances including other protein, other than toxins, other than bacteria, including things like RBC actually, gave rise to the birth of a subject called as serology, which is very much involved in immunology.

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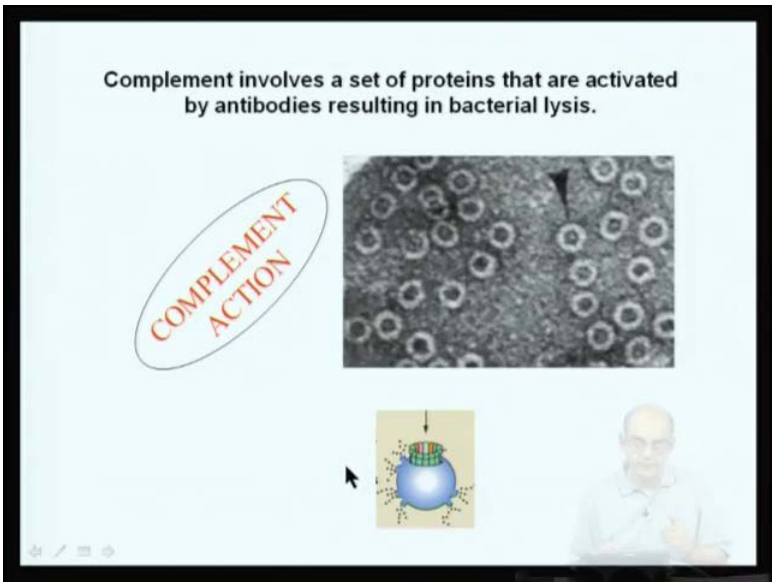

Now, what is complement? We were talking about complement, now complement actually involves a set of proteins, which you will be studying, studying about in later classes that are activated by antibodies. So, when antibodies bind to the bacteria, that they are specific to, these antibodies have the ability to activate the set of 9 fragments of complement, which actually come together like this over here to form pores into the cell to which the antibody has bound, and which has bound to complement. And these pores

have actually been visualized by electron micrography to see, that you can see this so clearly over there.

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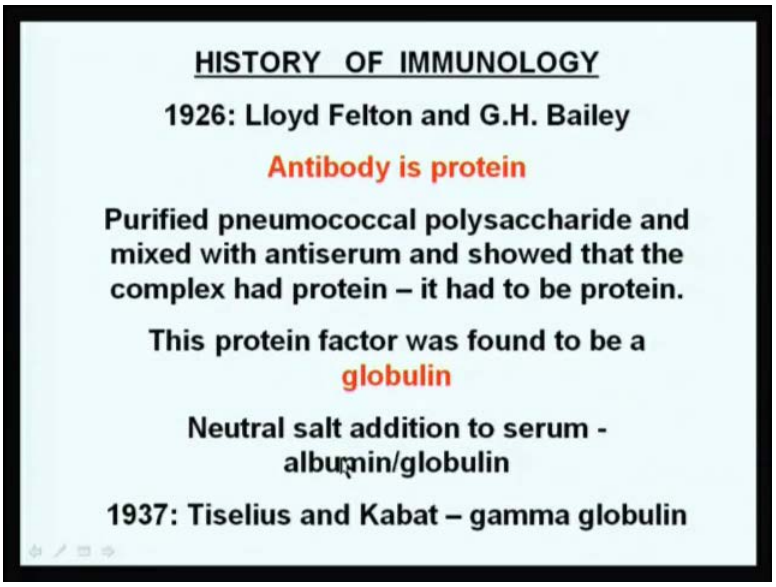

So, these pores, actually allow the contents of the cell to lyse out and therefore, you have bacterial lyses. Now, going on with the history; you see, 1926, Lloyd and Felton and Bailey came up with the landmark discovery, that antibody is actually protein in nature and it was not any other kind of material, how did they do that? You know, what they do, they, it is a simple concept, but during that time, it was a really, it led to a great discovery.

Now, what they saw was, they took purified pneumococcal polysaccharide. Now, all of you know, what are polysaccharides? And polysaccharide, suffice it to say, that it is not a protein. Now, they took this polysaccharide and they mixed it with antiserum that they had developed by injecting this pneumococcal polysaccharide into animals, like rabbits. So, they took this antiserum that they had blood from rabbits and they mixed it with this polysaccharide. Now, as I told you earlier, there was a cloudy and there was a precipitate. So, they could take that precipitate by spinning it down and they found, that this complex, that the precipitated complex had protein, by testing for protein.

So how, how is it possible, that when you had a pure polysaccharide, when you mix it with antiserum, you had protein in it? And therefore, that had to be the antibody that had bound to the polysaccharide and therefore, it had to be protein in nature. Then, came the

discovery that this protein factor was actually, globulin. Now, what is globulin and what is albumin? How was this discovery made? This discovery was actually, made in 1937 by Tiselius and Kabat who discovered, that these antibodies were actually gamma globulin.

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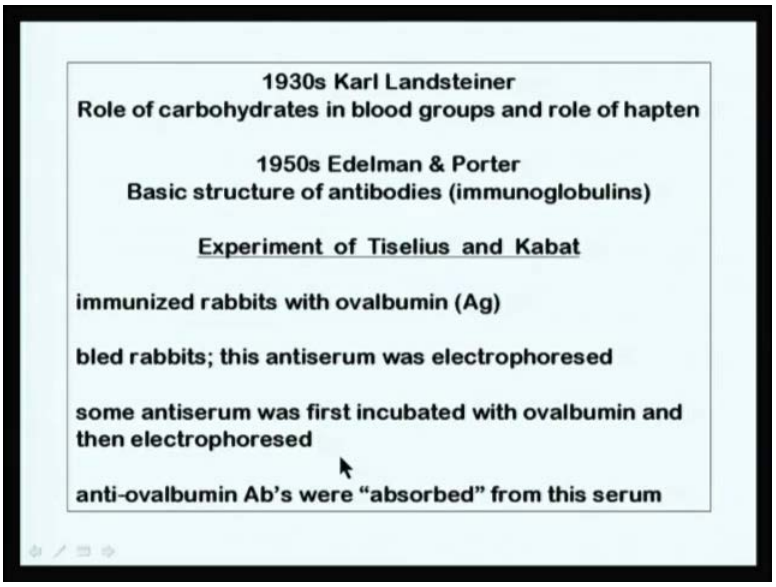

Now, just before, before or during this time, when Tiselius was discovering electrophoresis, which we, which we take for granted these days in any lab... During the 1930s it was Karl Landsteiner, a very great scientist who actually looked at the role of carbohydrates in blood groups and all these A B O blood grouping that we know about, that we have to have our blood, blood group tested for getting blood in case of accidents and so on and so forth, was actually discovered by, in 1930, by Karl Landsteiner. And he also discovered the role of hapten, which is a small chemical, also can make an antibody, but it cannot induce antibodies in animals unless it is coupled to proteins; more about this in your antibody class.

But in 19, 1950's, it was Edelman and Porter who actually came up with the basic structure of immunoglobulin molecules and that, this immunoglobulin molecule was having two chains, and that is, that it had a heavy chain and it had a smaller, smaller, smaller chain and it is written as a y, which has got disulphide bridges; more of that later on.

Now, if you look at these incidences, how, what was it that told, told Tiselius and Kabat that actually, this antibody was gamma globulin? What is the experiment that they did? They immunized rabbits with ovalbumin. You know, during those days, all these experiments with blood grouping and the fact, that blood was easily available in a slaughterhouse because they would kill, they would kill the sheep and so there was a lot of blood available, so they could have excess to red blood cells. They could take this blood, spin out the blood cells, RBC's and experiment with it.

So, they took this as an antigen, which was easily available and they started injecting into various animals. So, in fact, sheep red blood cell is a very, very convenient antigen, which is highly immunogenic; immunogenic, meaning that it has the ability to be strongly induced the formation of antibodies in different animals, like rabbits. So, if you took sheep blood cell and injected them into, into rabbits, you would get anti-sheep red blood cell antibodies and of course, all these injection has to be in an animal different from the one, that the RBC was taken from and that is because of the phenomena called as tolerance, which will come to study later on.

So, they injected these rabbits with ovalbumin, so sheep red blood cell was a convenient antigen, ovalbumin was another convenient antigen, which you could get from eggs and another famous antigen is called as KLH, called as keyhole limpet haemocyanin; limpet is a bivalve and haemocyanin is the blood pigment, that is available in these bivalve and keyhole is a place, it is a beach and therefore, they would take all these bivalves, which washed up on the beach and extract this blood pigment and use it as antigen.

So, after injecting this ovalbumin, they bled these rabbits after sometime, so they had antiserum that was available. This antiserum, they electrophoresed, so they subjected it to electric current and they separated various components. Now, along with doing this experiment with this immunized serum, they also did another kind of a treatment. Some of this antiserum they took and incubated it with ovalbumin, which was the antigen that was injected in the first place and then, so the principle expected was that the anti-ovalbumin would combine with the ovalbumin antibodies and therefore, try and see what would happen when these were electrophoresed.

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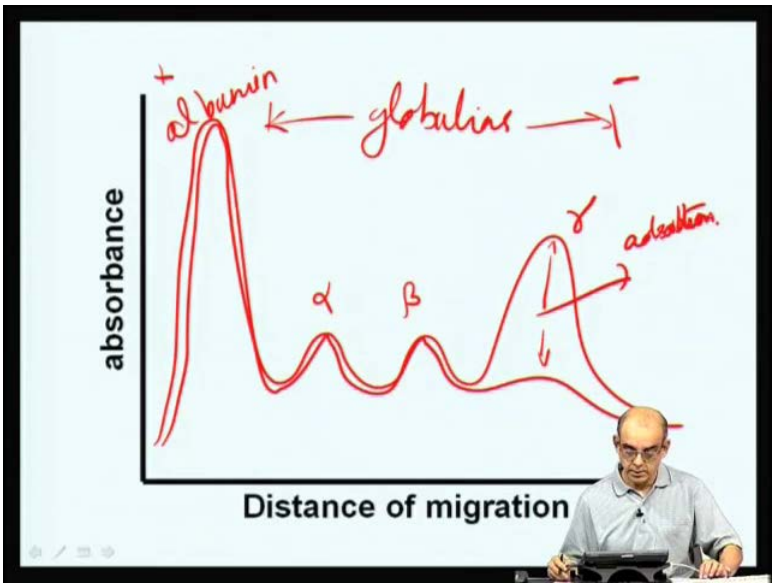

So, they did this electrophoresis and they, they found, that you had several peaks that was separating. So, if you were to mark this particular separation plus and minus, and then, you looked at the absorbent. During those days, they were actually electrophoresing serum in kind of, large volumes in different material, like kind of a round walled glass tubes, and they would look at the absorbance of the various bands, that were separating and they found, that actually when they looked at the absorbance, there was one peak and then they had three other peaks, like that. So, they called, this one was turned out to be albumin, which a lot was known, about which lot was known because there was lot of egg albumin and albumin would always be used for cooking or preparing various kinds of things in the kitchen.

So, apart from albumin, they had three other peaks, called as, they called it as globulins. So, anything coming later and later then albumin was called as globulins and they called it as alpha, beta and gamma. So, your alpha globulin, you had beta globulin and you had gamma globulin. So, they took this material now, which was combined with the ovalbumin. So, the immune serum that had combined with the ovalbumin and they electrophoresed and this is the profile that they got. They got this peak very much as it was, they got the alpha, they got the beta also, but the gamma was severely depleted and this depletion, basically, becoming because of the absorption, because of the absorption of the antibodies.

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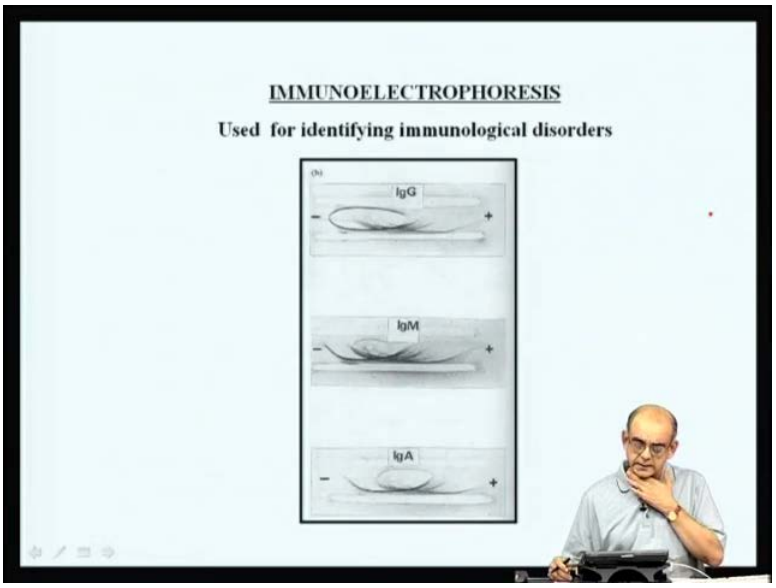

So, this was the experiment that Tiselius and Kabat did to discover these were actually, gamma globulins. So, you will see, that this kind of experimentation and the use of these kinds of experimental techniques, including the precipitation are actually used even during these modern day immunodiagnostics, to look at immunological disorders. So, this is a technique called as immunoelectrophoresis, basically it is an electrophoresis combined with the octal unitype of double diffusion, where you have the formation of arcs.

So, in this immunoelectrophoresis, those patients, which do not make IgA or IgA insufficient or do not make IgM can be diagnosed by the disappearance or the non-appearance of a particular or the absence of a particular precipitate, that forms when you incubate these serum with anti-IgM, anti-IgA or anti-IgG. So, you see, that this kind of precipitation technique, how, how well it was used even for immunodiagnostics.

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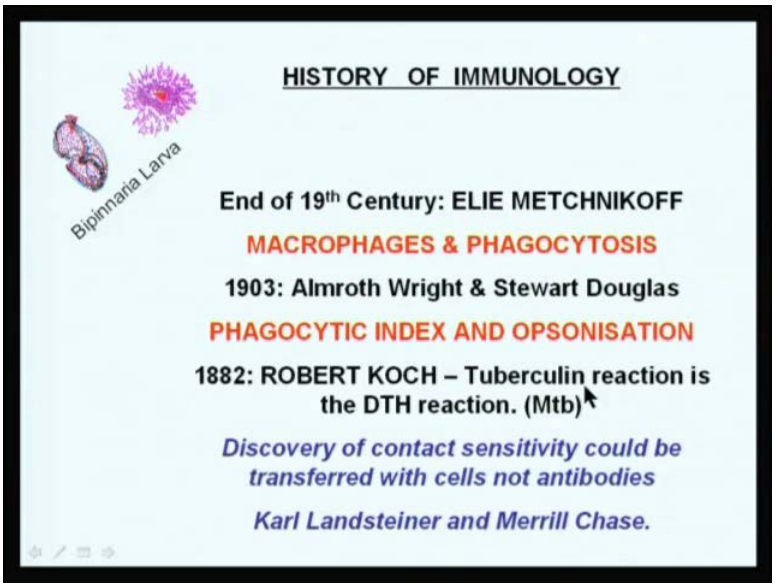

So, while all those things where happening, there was other kinds of discoveries being made, which was made at the end of 19 century. All of you have heard of Elie Metchnikoff and Elie Metchnikoff of course, you always put him together with the discovery of macrophages and phagocytosis.

So, it is said, that if you look at the books, you actually read, that Metchnikoff actually, on, discovered this particular, or thought of this idea during a Christmas eve because he had this Tannenbaum in his home on which all these gifts where hung up for those kids during Christmas eve, they would get these the next day as a Christmas present. So, he found, that when he took a thorn from the tree and then, because his house was near the beach he had a lot of access to star fishes, you know star fishes come up on the beach quite often, and these star fish actually have a larva, called as a bipinnaria larva.

Now, the unique thing about bipinnaria larva is that they are transparent. So, what is happening within the larva, if there are cells within the larva and in fact, there are cells in, in this larva, you can see these cells moving around. Now, that is an advantage with these larva, in fact, modern day biology also has its equivalent, like having transparent systems, model systems to study in, that is, the, the, the, the, you know, the, once the zebra fish hatch out from the eggs, they are transparent and you, there is a lot of work that is being done on how these cells combat mycobacterium tuberculosis by looking, looking at how these cells move within those fishes.

So, here, you see that when he poked the thorn, he came back after a couple of hours and he found, that this thorn was actually surrounded by these mobile cells, which were motile within the larva. So, therefore, he actually got the idea, that these, these cells where surrounding the thorn in order to engulf the thorn. Now, what we study phagocytosis, you can imagine, that all these things were derived from this observation, that Elie Metchnikoff made.

Then, of course, in 1903, Almroth Wright and Stewart Douglas, they came up with a system for measuring this phagocytosis, called as phagocytotic or phagocytic index. And then, you had the phenomena of opsonisation, discovery of opsonisation, as I have already told you earlier.

Then, of course, another epoch discovery was made by Robert Koch and this epoch discovery was the discovery of tuberculin reaction. You know, this tuberculin reaction is a reaction that is being still done nowadays, to look at your exposure to micro-bacteria. So, this tuberculin reaction was actually discovered, that it was, what you called as a delayed type hypersensitivity reaction and of course, this was then later on discovered, that this discovery of delayed type DTH reactions could actually be transferred only with cells and not with antibodies. So, if you had a sensitized individual, it is something similar to your reaction to poison ivy.

So, if you transferred, you took cells from a sensitized animal and injected into a non-sensitized animal and then, exposed it to this particular tuberculin, you had a, kind of, something redness and making of kind of a wheel on the, on your skin, on the animal skin later on, after certain, certain amount of time. So, all these actually, actual discoveries were actually, alluding to the fact, in addition to antibodies something was being contributed by the cells because macrophages are cells, they are cells, that are motile within this larva and this DTH reaction could be transferred, not by serum or antibodies, but only by cells when they were given to the other animal.

So, therefore, all these observations were actually pointing out, that in addition to what is called as humeral ability, body humor containing these antibodies, there was something else, that that contributed to a full blown immunity, and that, that, that the contribution was coming from something other than antibodies and that had to be cells.

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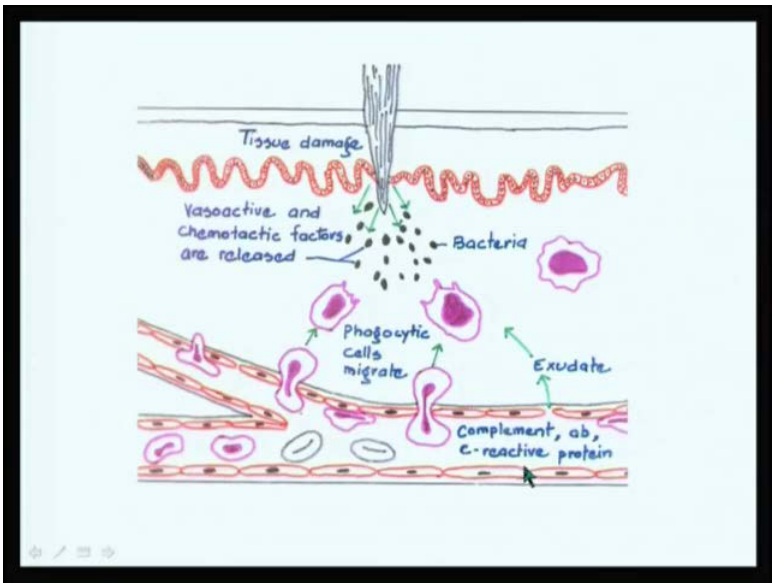

So, therefore, if you look at, if you look at the entire scenario, you see, that all of the discovery of macrophages, in fact, these neutrophils actually coming out from your capillary when a thorn is poked into your skin, rather than the bipinnaria larva, you had, you know, the remarkable thing about this is that the bacteria in the thorn themselves, actually serves as an attractant to these neutrophils on macrophages.

Now, these are all components of what you call as innate immunity, which I will be describing to sometime later, which involves the migration of macrophages. Not only do they have to migrate to the place where you have poked the thorn or the thorn was poked into your or into your skin by accident, you find, that those cells have to move out exactly in that location and not anywhere else.

So, these neutrophils have to move out from your blood vessel or blood capillaries and come out and then migrate towards the thorn and then phagocytose the bacteria. In fact, as I told you, bacteria have certain molecules, which are chemotactic to these cells and these molecules are present in the cell wall of bacteria. So, these, these cell walls actually, you heard of formylmethionine F-mate ryu fi methionine amyl leucyle phenylalanine.

So, this tripeptide, which was formylated because formaylation is something, that happened in the bacteria and not in higher animals, so you see, this formyl peptide could actually be chemotactic to the neutrophils. So, that was how the macrophages actually

still, I mean, which was discovered so long ago, play such a great role even today in your bodies.

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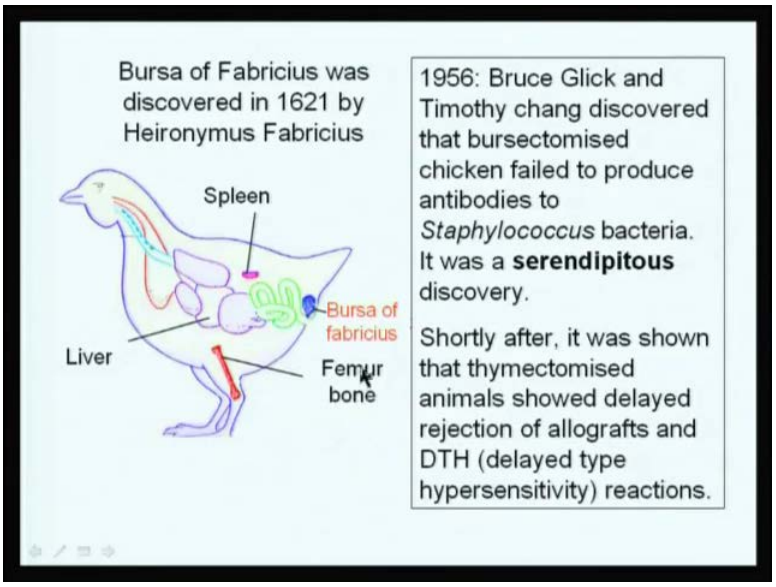

So, then the cellular immunity was actually strengthened by the fact, the discovery of this function for the Bursa of fabricius. So, there was a lot of experiments being done, looking at this clouding or precipitation of antibodies with, that is specific to a particular antigen and then, of course, all they were trying to quantitate the formation of this complex. So, if you had injected an animal, let us say, with ovalbumin, they would take the blood and try to see by this immune-precipitation reaction to see, how much antibody was being made in the blood of those rabbits or sheep or whatever.

So, when you look at all these different kinds of experiments, there was an experiment done on the Bursa of Fabricius. Now, this Bursa was actually discovered way back in 1621 by a person called a Hieronymus Fabricius, and therefore, it is called as Bursa of Fabricius. Now, during that time, when there was a lot many experiments were done to see what is the function of this Bursa and birds, so one of the ways, you look at organ function is to try and remove that organ and then, try to see organ functions are, or what is absent in the person or the animal, that the organ has been removed form. So, you have heard of various kinds of terms, like bursectomy, thymectomy, hypophysectomy to look at the functions of all these various organs.

So, they were doing lot of experiments involving bursectomy, so they would remove the Bursa and then try to take this chicken and then, try to see what, what type, what type of function would be missing in these bursectomized birds, but to their surprise they had never come up with any abnormality of these birds.

So, they were at a loss to explain the function of the Bursa. Now, the function of this Bursa was actually found out by accident, so in other words, it was a serendipitous discovery by two graduate students in 1956. So, look at the discovering in 1641 and the function being attributed in 1956. So, 2 graduate students, called as Bruce Glick and Timothy Chang, they actually discovered the function of this Bursa and as I told you, it was a chance discovery. The chance, the happening was, that several students went to, went to Timothy Chang to try and learn, how to evaluate the formation of these immune complexes or the precipitation with the antibodies?

So, Timothy Chang did not have any chicken in his lab or birds in his lab, so he borrowed these birds from a different lab, from Bruce Glick's lab and then he took these birds and injected these birds with staphylococcus bacteria, to show the students, that after sometime you will get these antibodies, that could precipitate this staphylococcus and to his surprise and to the chagrin of all the students who had come to learn this technique, they found, that the whole experiment failed because there was no immune-precipitation, not there was no precipitate being formed when they mixed the serum with the staphylococcus.

So, then he was very much perturbed, then when he was discussing with, the whole matter with the Bruce Glick, he found that, that he had maintained some record. So, they went back to all the records and there found these birds had the Bursa of fabricius removed, so they were in fact, bursectomized. So, then came the discovery, that actually, Bursa was involved in the formation of antibodies and this Bursa was making cells. It was not that the Bursa was related to the secretion of antibodies, but cells in the Bursa of fabricius, was what was responsible for the secretion of antibodies later on, when they were exposed to a particular immunogen.

So, this, this was the discovery of the function of Bursa of fabricius and therefore, all these cells were named after the Bursa, will start with a B and therefore, it was called as a B cell. Now, you know how the B cell derives its name from? Now, simultaneously,

there were lots of experiments, as I told you, to look at the function of the thymus. So, they would take out the thymus and please note, that all these bursectomy, removal of the Bursa or the thymus, has to be done at very early stage.

So, this bursectomy has to be done soon after hatching and thymectomy has to be done soon after, soon after the, you have the birth of the (( )). Therefore, you see, it is called as neonatal thymectomy; so, the thymus actually regresses in adult life. So, if you remove the thymus form an adult, there is no effect on the immune response; only if you remove the thymus from a neonate, you will have its effect on the various cells of the body. During those times, they actually found, that removal of the thymus also led to a decrease in the number of cells that were circulating in the blood.

So, there was a lot of immunology, immunochemical kind of techniques, immunohistological techniques or immunochemical techniques, that were being done during those days, one of the test were to look at what different types of blood cells were available in your blood. In fact, that is something that is done even in modern day diagnostics to look at different kinds of, different blood types in the blood, that is drawn from a patient.

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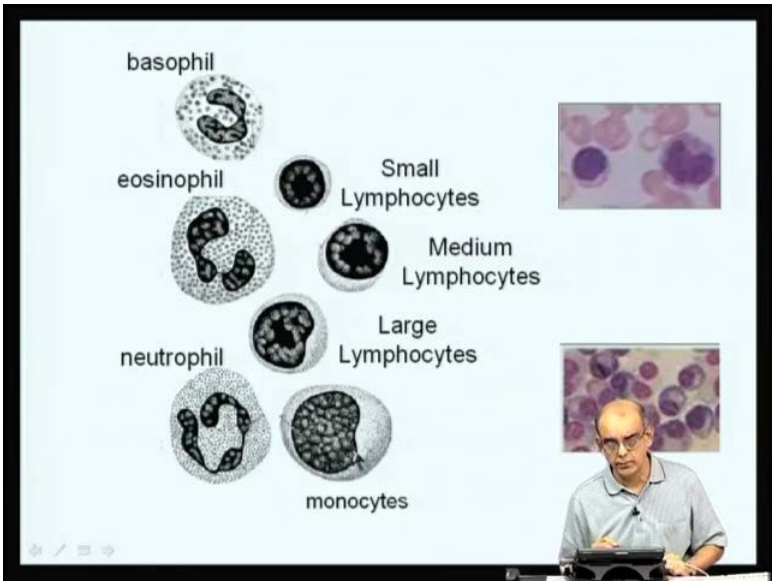

So, when you look at this, you see, that when you stay in with either with haematoxylin, eosin or do what is called as (( )) test, you find, there are different kind of blood cells and they differ by morphological criteria and there are, there are cells with large nuclei and

there are cells with a segmented nuclei and there are cells with kind of a biload nuclei. So, there were small cells also, smaller compared to all these other cells, called as basophil, eosinophil and neutrophils and these small cells they are called as lymphocytes.

So, during this bursectomy and thymectomy, they found, that the number of these small types of lymphocytes and they would stay in differently also, outside of the nuclei, the cytoplasm will stay in differently and the other cells will stay differently as you can see in these 2 pictures. So, they had some sort of inkling, that the cells also played a major role when you removed the thymus of the Bursa and Bursa of course was implicated in the formation of antibodies.

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So, came about the concept, that that the immunity actually derives from 2 different arms or 2 different types of immunity in your body, called as humoral immunity, which is actually resulting from the B cells and therefore, they make antibodies circulate in your blood, they can combine with the antigen in a test tube and these were called as humoral immunity because it is in your body humor or blood as opposed to cellular immunity, which involve cells and these included a lot of cells, different kinds of cells, B cells, T cells, which are involved in killing and so forth.

So, now we realize, of course, that to get a full blown optimal immune response to a pathogen, you need the participation and the cooperation between humoral immunity and cellular immunity. So, looking at all the different kinds of aspects and looking at all the

different kinds of cells, let us see, what are the different, different kinds of cells of the immune system?

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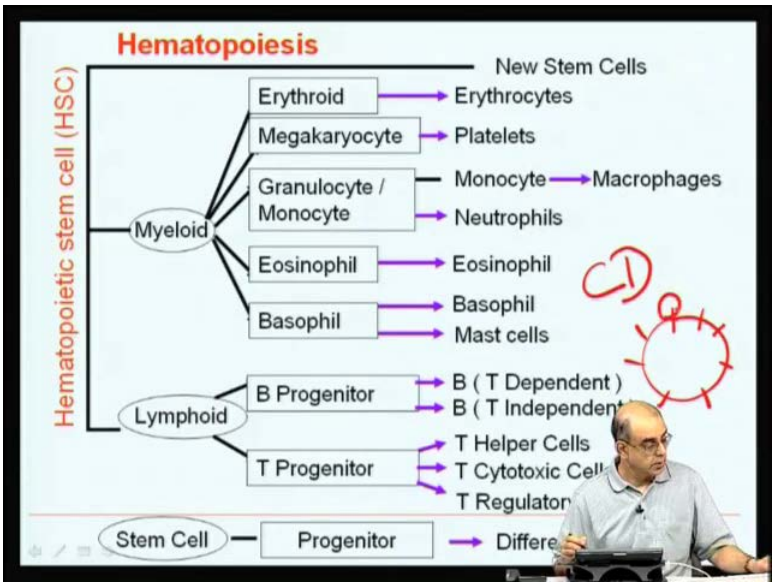

And if you look at the different cells of the immune system, you look at any immunology text book, you have here, what I have represented here is that how all these differentiated cells, which you have come to hear about now, like the monocyte, neutrophils, eosinophil, basophils and other types of cells, that are listed in this slide, actually differentiate from precursors.

So, in this slide, I have put together in a way, that shows you by an arrow, an arrow mark standing for the final differentiating here, differentiated cell and then they are coming from one, that is put in this square, which is called as the progenitor cell and this progenitor cell actually, coming or developing from what is called as a stem cell.

Now, this tends to the concept of a stem cell is very much evident these days. It is becoming important for, for biology and both, in terms of scientific and legal implications because you can have a stem cell regenerating different types of cell in your body. And looking at the differentiation of these different kinds of blood cells, you realize, that all these different kinds of blood cells with its different morphological characteristics and also different types of contents within them, actually derive from different kinds of precursors. So, you have actually, what is called as a hematopoietic stem cell, as opposed to a flurry potency stem cell; flurry potency stem cell is one, which

has the capacity to differentiate into different types of cells, as opposed to hematopoietic stem cell, which is a stem cell that gives rise to only to blood cells

So, a flurry potency stem cell is what gives rise to a hematopoietic stem cell and this hematopoietic stem cell then starts to develop into different kinds of stem cell. So, in this case, you will have a myeloid stem cell and you have a lymphoid stem cell. Now, all these stem cells nowadays are distinguished by the presence of markers. Markers, meaning kind of proteins, they are also called as cluster of differentiation (CD); they are labeled as CD because they stand for clusters of differentiation. So, these are found on the cell surface, so you can actually look at the presence of these by looking at specific reagent that bind to these cell surface proteins.

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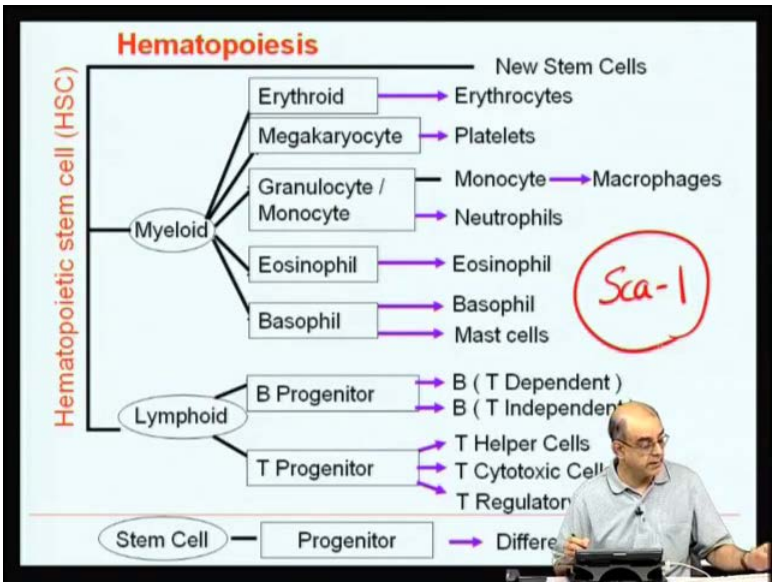

So, these are, these are specific to certain kinds of cells and those which are common, different kinds of cells, so these are called as markers. So, you have a marker called as stem cell antigen-1; so, this stem cell antigen-1 is actually a marker for this flurry potency stem cell. So, using this presence of these marker nowadays, they can isolate these stem cells and look at the presence of stem cell antigen-1, and make sure, that is, that it is in fact, and purify them, that I am, make sure that it is in fact, a stem cell.

So, you can have these different kinds of stem cells and you see, that this develops into 2 different kinds of, like you have the lymphoid stem cell and the myeloid stem cell, and when you look at the myeloid stem cell, you see, that it gives rise to erythroid progenitor,

meaning, that this is a precursor through the erythrocytes. In addition to this, of course, you have this HSC, having the ability to multiply, not differentiate, making more of its own kind, called stem cells that means, all these stem cells have the ability to differentiate into different kinds of cells in the blood.

So, apart from this erythroid lineage, you have the megakaryocyte lineage or the megakaryocyte progenitor, which gives rise to platelets. Now, platelets are vasoactive in your blood and they are very much important in clot formation, they are the ones, which actually help in making your blood clot and not bleed to death.

So, then, of course you have granulocyte-monocyte lineage, which gives rise separately to a monocyte progenitor, which finally differentiate into, what are called as, tissue macrophages and these tissue macrophages are called by different names in depending upon, which skin or under which skin, that they are found in your body.

Now, this same progenitor give rise to, what are called as, neutrophils, which is also responsible not only for chemotaxis, but it is also responsible for making different kinds of reactive substances, like oxygen radicals and so on and so forth. Then, you have the eosinophil progenitor, which gives rise to eosinophil; basophil progenitor, which gives rise to the basophils; and also, it gives rise to mast cells. So, all these take part in allergic reactions. In addition to this, of course, giving, coming down to lymphoid lineage, you have the B progenitor, which gives rise to different kinds of B cells, nowadays being distinguished by the presence of specific cells of this markers.

But in this slide, I have just shown you 2 types, called as T dependent, T independent because these B cells, some of them, which are T dependent or dependent on T cells to get cytokines from them and then, so they are dependent on help derived from T cell. Whereas, there are other kinds of antigen, that activate B cells in a T independent manner. Now, of course the T progenitor, which gives rise to different kinds of T cells, called as the T helper cells because they help B cells to make their antibodies; T cytotoxic cells, which kill infected body's infective cell; and of course, regulatory cells, which have the ability to have a feedback mechanism to regulate different kinds of cells.

Now, all these different kinds of cell, that you are seeing here in this, in this, in this particular table, you find all these are determined by the availability of growth factors, for example, GMCSF played very, growth, great role in the differentiation of granulocyte

and monocytes. Similarly, IL-8, IL-7, IL-4 and IL-5 are very important for B cells. So, like this, we will come to in the lymphokine class, as to different kinds of, different kinds of lymphokines, that help all these different kinds of precursor cells to differentiate into all these different kinds of mature cells.

So, the basically, all this time-table of development is actually driven by the presence of lymphokines, lymphokines that are available during the stem cell, stem cell development or during, during the body's immune reactions, where these particular progenitor are present.

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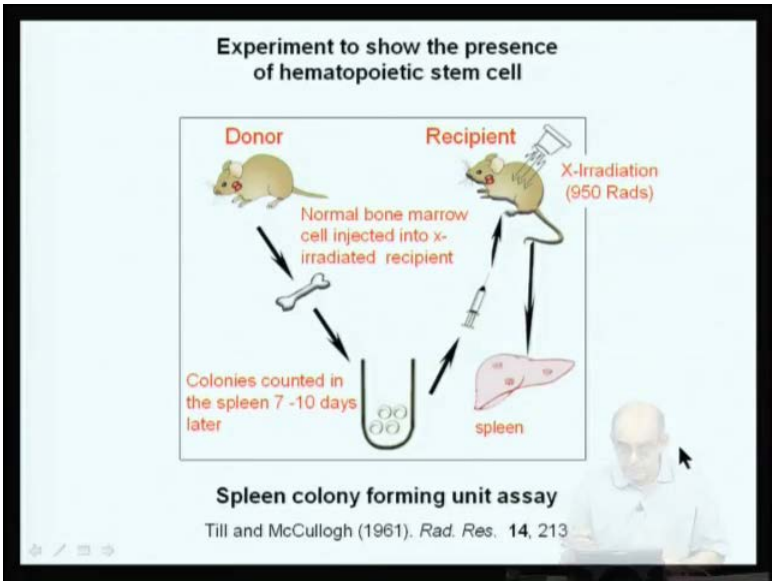

So, then, of course I will have to describe to you an experiment, that shows the presence of the hematopoietic stem cell where they showed, that in fact, there is one type of cell, called as a stem cell, that gives rise to all the different kinds of cells in the body and therefore, you are actually reorganizing this body immune system, by, by, from this particular stem cell; more of this in the next class, because I am out of time in this for this lecture number 1. And therefore, I will conclude this lecture and then, when we come back to lecture number 2, will look at this experiment and then go around to look at how B cells and T cells were actually discovered and all these different other kinds of experiments.

So, to end this particular lecture, you have this lecture number 1 showing you the different events in the history, immunology, including the discovery of the Bursa of

Fabricius, and then, in the next class we go on to look at, how stem cells, are, can repopulate immune system or make up a mature immune system and how, how it was shown and the various kinds of cells, that derived from it and experiment that were done.