

AIDS: Evolution of an Epidemic (2007)
Lecture Two—AIDS and the HIV Life Cycle
Bruce D. Walker, M.D.

1. Start of lecture 2 (00:00)

[music] *From the Howard Hughes Medical Institute, The 2007 Holiday Lectures on Science. This year's lectures, "AIDS: Evolution of an Epidemic" will be given by Dr. Bruce Walker, Howard Hughes Medical Institute investigator and director of the Center for AIDS Research at Harvard University. And Dr. Bisola Ojikutu, director of the Office of International Programs in the Division of AIDS at Harvard Medical School. The second lecture is titled "AIDS and the HIV Life Cycle." And now to introduce our program, the vice president for grants and special programs of the Howard Hughes Medical Institute, Dr. Peter Bruns.*

2. Introduction by HHMI President Dr. Thomas Cech (01:11)

Thanks for joining us again for these Holiday Lectures on the important topic of HIV and AIDS. These lectures actually are the centerpiece of a constellation of activities that are useful on this topic and I'd like to bring your attention to that. We have a separate web site called BioInteractive.org where all of this appears and on that web site you can find streaming videos of all the recent Holiday Lectures and animations from the lectures as well as interactive activities and interviews with scientists. I'd especially like to point out that now you can download those activities on your video iPod which should give you a whole new dimension to working out on your treadmill. Our next speaker is Dr. Bruce Walker. Bruce is an accomplished researcher and a caring physician. If you spend any time at all with Bruce you'll know that his knowledge, drive, and friendly demeanor make sense that he therefore is at the center of the hub of active people battling AIDS in both the lab and the clinic. As a physician Bruce's dedication to the health of patients has led him into the lab to study HIV not just in model systems but in people. In this lecture Bruce will focus on understanding how HIV infects the human body and how the body's immune system responds. Here's a short video to introduce Bruce.

3. Profile of Dr. Bruce Walker (02:55)

I'm an infectious disease physician. I see patients, but I also do research and what we have here is a model that is seen in many places in the United States, and other places in the world, where you have biomedical research right next to patient care as a way to really facilitate those interactions that are going to keep researchers focused on real-world problems of patients and keep the research really relevant. We can get blood specimens from patients over in our clinic and bring them right back to the laboratory here and it's that kind of proximity between research and clinical care I think that has really fueled advances in biomedical research. The Partners AIDS Research Center is actually a group of scientists that work together. I'm the administrative director of this. We have a number of other investigators, all principal investigators in their own right, that's part of what makes this so much fun is that we have people that are working on closely related areas like vaccines and transplantation biology and innate immunity. I hope what we can do with these lectures is get kids excited about science and get kids involved. This is a tremendous global problem that we're facing and that the next generation is going to continue to have to grapple with and there are ways for all those students out there to get involved in doing something about this and that's what I hope is that kids can see the excitement of science, the excitement of discovery and that kids will be motivated to get involved in doing something about it.

4. Introductory remarks by Dr. Walker (04:54)

Good morning and welcome to all of you. As you know from the video you just saw, I'm a physician. I went to medical school after college and as I was struggling to learn all the material that seemed a bit overwhelming, somebody told me that getting a medical education is like trying to take a drink from a fire hydrant. I really felt that that was true, it was just almost impossible to absorb all of the information that was being presented to us and yet when I finished and went to Mass General for my internship in 1980 I had never heard about HIV. I had never heard about retroviruses and what you know from what Bisola presented, that disease which came onto the scene here in 1980 has now killed more than 25 million people. So the big question here is how in the world did this virus that started out in a few gay men, first detected in a few gay men in California, how did that end up causing this spectrum of disease that it's caused?

5. How viruses cause disease (05:57)

And that's really what I would like to focus on in this lecture, is how do we explain really the manifestations that you've already heard about? Let's think about viruses to begin with. Viruses are essentially packages of genetic material. They're not able to replicate on their own but they carry all the information required for replication. What they need in order to replicate is a host cell and essentially what they do is they commandeer that host cell and force it to make viral proteins rather than host proteins and you can think of this sort of as viruses being commandos and taking over a machine tool factory and turning that factory into a bomb factory. So basically taking the components that are normally there and putting them to a different purpose that is the virus' purpose and as viruses are produced and go on to infect other cells, virus infection then spreads.

6. Persistent viral infections have an acute initial infection phase (06:56)

It turns out that all of you in this room are infected with viruses right now. In fact if you had chicken pox as a child or infectious mono as a child you have that virus still alive in your body now. The reason that it's not causing disease is that you have an effective immune response that's helping to keep it in check. Now if any of you had those diseases you remember when you first became infected with chicken pox or mono, you feel really lousy, and part of that feeling of lousy is your immune system trying to respond and fight the invading pathogen and ultimately, even though that virus persists in your body you enter into a phase where you're asymptomatic and the virus is not causing any problem, again with an immune system keeping it in check. Well the question is, you heard from Bisola about these cases that we initially saw as AIDS cases then we realized that there was a spectrum before AIDS of HIV infection where people were asymptomatic. In order to understand what's going on with HIV we really need to understand the very earliest events and understand what happens when people initially get infected. So the question here is does HIV cause an acute illness like mono, like other viral infections and in order to address that

7. Video: Symptoms of acute HIV infection (08:14)

I'd like to show you a video of a patient of mine, Adam Barrett, and listen to what he has to say. What happened to me was in February of '85 I came down with symptoms of what I thought was the flu. What seemed to me to feel like a regular winter flu, which I seem to at that point have gotten every year of my life, went home, went to bed, little bit of a fever, malaise, nausea, headache, nothing too severe. Woke up at about 4:00 in the morning, very high fever, drenching sweats, body rash, progressed to stiff neck, severe headache, looking at any light at all really intensified the headache and what I knew at that point was that sounded like symptoms of meningitis which scared me. So I reported to the emergency room at Mass General for treatment.

8. Symptoms of HIV acute viral infection (09:13)

So imagine now that you're the physician that's seeing this patient in the emergency room. He comes in, the first thing you do is take a history to try and put his illness into some kind of context. He had fever, chills, shaking chills at times, headache, loss of appetite, joint pains, muscle pains, and malaise, which is basically feeling really lousy. Now probably just about everybody in this room has had those sorts of symptoms before. There was one additional critical piece of information that needed to be garnered from him, a question that needed to be asked, and does anybody know what that question is? The question was had he had any potential exposures to HIV because the question we're addressing here is does HIV potentially cause an acute viral syndrome. And it turned out that he had had unprotected sex 16 days before with another man, meaning that he had had a risk exposure for HIV infection. Well, the next thing that's done is the patient's examined, and on examination he had a skin rash that's quite characteristic of a lot of different viral infections. Viruses can cause these sorts of rashes, some of you may have had them in the past with other viral infections. The rest of his exam was notable for a couple of things. He had a fever, as he had reported in the history, temperature of 102 which is reasonably high. And in addition to his skin rash he also had swollen lymph nodes, so glands in his neck and under his arms that are areas where the immune system cells reside and these were swollen and in addition to that he had a stiff neck which can be an indication that the lining of the brain is inflamed and viruses can cause this, a number of different viruses can cause this. So given the history of what he had said, the physicians in the emergency room that day decided, well, this is probably just some viral syndrome. A lot of viruses that we haven't even identified yet cause this and people get better from these things and we go on without ever really knowing what the cause was, and he was sent home and told to return to the emergency room if he didn't start feeling better.

9. Why HIV tests show no infection during acute phase (11:27)

In addition, because of the history, a test was done to see if he had mono, and that was negative. He also had a test for HIV infection, and that test likewise was negative, an antibody test. So the question here for you is, is that antibody test definitive? Can we be certain that he doesn't have HIV infection? And in fact what happened was he went home and two weeks later he came back to the emergency room because he still didn't feel well and at that point his antibody test was actually positive. So he had developed the antibodies, and the question was why did we miss that early on when we first saw him with symptoms? Let me try and address that by thinking about what it is that we're really measuring with an antibody test. You're not measuring the virus, you're not measuring a pathogen with an antibody test, you're measuring the immune system's response to that invading organism.

10. Development of an antibody response (12:24)

In the situation with HIV infection, virus has to get across a skin barrier. It has to then be taken up by a specialized cell underneath, just below the skin called the dendritic cell. That dendritic cell then transports the virus to a lymph node and in the lymph node are specialized immune cells and that's really where the immune response to a pathogen like HIV begins. At that point B cells interact with the virus and those B cells, because of the ability to recognize HIV will be transformed into a cell called a plasma cell. That plasma cell now starts making antibodies. Those antibodies go out into the blood stream and one ends up then being able to do a blood test like you just saw up here in the first lecture and diagnose HIV infection. But this whole process takes a number of days, in fact it takes about three to four weeks for antibodies to develop. Remember Adam had... 16 days earlier had a sexual exposure that might have put him at risk.

11. Measuring HIV RNA detects high HIV levels during acute infection (13:29)

So the question is, instead of measuring a reaction to the virus, can we measure the virus directly to see if in fact he has virus in his bloodstream? And it turns out that that actually can be done and this is one of the really big breakthroughs in understanding how HIV causes disease, was the development of a test that allowed us to directly quantitate the amount of virus in the bloodstream and that's done by a test called

polymerase chain reaction that gives you a precise quantitation of the number of viral particles. So what I'm showing you here are data that we've collected over the last couple of years from a sub-group of patients that have come into the emergency room at Mass General Hospital who have complained of symptoms exactly like or very similar to what you just heard about. Fever, sore throat, feeling lousy, many of these patients had already been seen by another doctor and had been told they had a viral syndrome and had been sent home and they came to us because they still didn't feel well. But they came in still thinking that they probably had mono. In fact what they had was acute HIV infection and you can see from these numbers that the level of virus, when you actually quantitate it is extraordinarily high in that very acute phase of infection. So what Adam had and what these people had was a viral syndrome, the problem was that viral syndrome was acute HIV infection and the symptoms that they were experiencing was probably because their body was trying to activate an immune system against that virus and in the process they were feeling ill. Now the important point here is that all of these people had symptoms and all of them thought that they had something else like mono. But all of them had negative antibody tests and the diagnosis would have been missed had the RNA test not been done.

12. HIV RNA can be detected before antibodies to HIV (15:18)

So what we know from these sorts of studies is that infection occurs and following infection there's this incredibly rapid ramp-up phase where virus is replicating enormously quickly, but during that time antibodies have still not been developed. The time of symptoms is usually a week or two after a person becomes exposed to HIV. At that peak level of viremia—viremia meaning virus in the bloodstream—people are usually symptomatic but not everybody is and then over time the viral load starts to come down and three to four weeks after infection, after exposure to the virus, antibodies develop and allow you to make a diagnosis using the test that we showed you before. Well, why is it important to try and understand when somebody becomes infected? It's obviously important for personal health reasons for a person to know that they're infected so that they can hopefully get into care and be followed by health care workers to make sure that they're getting the kind of attention that a disease like this requires. In addition it's important for public health reasons because the presence of virus in their blood stream means that, as we'll get into, that they will have virus for the rest of their lives at that point and it's very important for public health measures that people know that they're infected in an attempt to try and stem the transmission to other people by educating them as to what the problems are.

13. Does HIV infection progress the same way in all people? (16:54)

Well, one important question is, does HIV progress the same in every individual? I said that the people we'd seen in our emergency room were symptomatic when they came in. They felt a viral syndrome, but does everybody get that viral syndrome? And does everybody progress at the same rate? It's an average of 8 to 10 years to the development of AIDS from the time one becomes infected, but can we get a better sense as to whether there's variability from person to person. Well it turns out that if you look at people who've just become infected, even though they have a very high viral load initially, that viral load comes down to a relative equilibrium and that equilibrium is very different in different people. Within a year of infection there's quite a spectrum in terms of the level of virus in the blood stream. So one question that patients always ask is what's the prognosis? How soon am I going to get AIDS, how soon am I going to have problems from this infection during this asymptomatic phase? And so we can actually look at viral load one year after infection in people who've become acutely infected and then see if that predicts the subsequent course of disease by seeing what happens to those people over time and these were studies that were begun in the 1980s before any kind of drug therapy was available, so sadly we had nothing to do but watch patients get sick at that point.

14. HIV viral load as a predictor of disease progression (18:23)

So let's look at the people with the higher viral loads and try and determine how viral load impacts disease progression. Seventy-five thousand is getting high during this equilibrium phase. This is a Kaplan-Meier plot and what this shows is basically on the Y axis and at time zero, 100% of people are surviving without AIDS. What you see is that as time goes on there's a stair step phenomenon as sequential people develop AIDS and in fact if you look at this you can see that the first case of AIDS in this group of people occurred within about 6 months of becoming infected. But there's a lot of variability here. However, starting with a high viral load only about 10% of people were AIDS-free at 10 years. So high viral load, that's the level of progression that we're seeing. Let's now look at the other end of the spectrum of people that have viral loads of 10,000 copies or less. If you take a viral load, if you take that group of people and you do the same plot, here's what you find. After about four years is when you see the very first case of AIDS develop. So with a lower viral load the chances of progressing to AIDS, already you can see are much less, and look at 10 years out, it's actually about 60% of patients AIDS-free at 10 years. So what this slide shows you and what this study shows you is that viral load predicts disease progression and early on by just measuring somebody's viral load we can get an idea of how rapidly they're likely to progress which is critical information. It also gives us another piece of information in trying to put together the puzzle of how HIV is causing AIDS.

15. Viral load compared to helper T cell level (20:13)

And what you see here is a composite of kind of what we know at this point from what Bisola has told you and what I've told you. We now add in another phase in the course of disease, acute infection, with a very high viral load. It turns out that during that time people have a transient drop in T helper cell counts and then T helper cell levels decline slowly over time till the ultimate development of AIDS. But look at how viral load and T helper cell counts are related one to another. In fact it looks like they go in opposite directions at all these periods of time. So is there some direct relationship between virus and T helper cells that accounts for the fact that the more virus you have the lower your T helper cell counts seem to be? Let me take you to a video and let's look at what happens in the very earliest stages when a virus actually infects a cell.

16. Animation: HIV life cycle (part 1) (21:08)

So this is HIV, it's a typical retrovirus, meaning that it has an outer envelope and in the center it has two copies of RNA as well as an enzyme here in blue that's reverse transcriptase which will ultimately turn that RNA into DNA. The virus itself with this outer envelope protein actually directly infects T helper cells. The way that it does this is that as it comes up to the cell surface, it uses receptors that are on T helper cells and exclusive to T helper cells, which are CD4 molecule, which really defines T helper cells, it's a surface receptor that binds to the envelope protein. That causes a conformational change and allows a second receptor to grab hold of the envelope. This is the chemokine coreceptor, it's also called CCR5 and we'll talk about that more. What happens now is that the stalk of the envelope protein pierces through from the virus into the host cell and starts to draw the cell membrane and the viral membrane together and what ultimately happens is fusion of those two membranes and the viral genetic material is injected essentially into the cell and the envelope protein is left at the cell surface.

17. Helper T cells orchestrate the immune response (22:34)

So in fact the problem with HIV infection, the very central problem with HIV infection is that on the right we have T helper cells. T helper cells have a T cell receptor and CD4 on their cell surface. They recognize antigen presenting cells that are basically the initiators of an immune response. The antigen presenting cells as Bisola told you will have taken up a foreign pathogen, they'll present an epitope, a small amount of protein from that pathogen at the cell surface. This will lead to an interaction between these two cells and the T helper cell will secrete substances called cytokines which are basically chemical messengers that give messages to other cells to help orchestrate an effective immune response and this leads to activation and proliferation of B cells and T cells. The problem is that these same helper cells that are supposed to be

orchestrating an effective immune response, also happen to have the receptors that HIV uses to get into cells. And so HIV is an infection of T helper cells, it does that because of CD4 and chemokine receptor on the cell surface and so you can see already that there's a linkage here between what we're seeing clinically in immune deficiency and what we've discovered, what scientists have discovered in terms of how HIV actually infects cells.

18. Animation: HIV life cycle (part 2) (24:03)

Well let's go on to another animation to look at what happens after the virus gets into a cell to get a better sense of everything else going on. The virus has a matrix and a capsid protein shown here in green and red that essentially are digested when it enters into the cell. That releases viral enzymes and the viral RNA. Here we have reverse transcriptase which take the viral RNA and using host nucleotides converts that viral RNA into a single strand of DNA. While it does that it makes some random errors which is characteristic of reverse transcriptase, it has very poor proofreading activity. That single-stranded DNA now is again reverse transcribed into a double-stranded DNA. At that point another enzyme that has come in with the virus in the beginning called integrase essentially grabs hold of that double-stranded DNA and carries it through a nuclear pore into the nucleus of the cell. Within the nucleus of the cell it finds the host chromosome and it basically, the integrase enzyme makes a nick in the host DNA and allows for HIV to insert itself into the host chromosome and that right there is what establishes lifelong infection. Now RNA polymerase comes along and makes messenger RNA. Those messenger RNAs encode for different viral proteins, they end up associating with ribosomes at the surface of the rough endoplasmic reticulum and here's a piece of mRNA that's making envelope protein which is directly produced into the endoplasmic reticulum and it's shuttled then through the endoplasmic reticulum and taken to the cell surface where at the cell surface it become embedded in the cellular membrane and at this point coalescing with other envelope proteins that have been produced, you have this cluster of envelope proteins now on the surface of this infected cell. At the same time there are other messenger RNAs that are being produced that allow for translation of other viral proteins. So here are additional viral proteins being made which are going to be used to make up the key components that the virus ultimately is going to need. These are transported again to the cell surface to the area where these envelope proteins are and a strand of RNA as well as some of the enzymes are part of that complex. This then buds off at the cell surface at this point but it's still not a mature virion because the polyprotein chain needs to still be digested into its component parts. That's done by an enzyme called protease. Protease breaks up those polyprotein chains and ultimately allows for them to coalesce and form the mature structures that make up the final virion and now you have a mature infectious virion that can go on now to infect other cells. Once that happens, now the cell can produce tons of viruses and this is really what then keeps the whole process going.

19. Summary (27:37)

Well let me at this point make some simple conclusions and then let's take a couple of questions. HIV is predominantly an infection of the immune system's helper T cells that have the CD4 receptor. Viral load predicts disease progression and gives us a way to tell patients what to expect but also gives us insights into pathogenesis. There's a window period following acute infection when antibody levels are negative and yet viral load is very high and finally HIV integrates into the host chromosome and thereby establishes lifelong infection and actually I want to just call your attention to this. Does anybody get *The New Yorker*? Anybody read *The New Yorker* this week? There's an article in here about retroviruses and summarizing the fact that a large fraction, a significant fraction of the host genome is actually made up of viral DNA that's been added into the chromosome over time and in fact about 8% of our genome originally derives from viruses so these get integrated into the host chromosome and then get passed on to children from parents.

20. Q&A: What can be done after accidental HIV exposure? (28:56)

So let me stop there and I'll be happy to take some questions and see how my arm is for throwing t-shirts. I see one there. Let's say somebody's exposed and they know they are, and let's say a paramedic goes to respond to someone who's HIV positive and they find out later that they were exposed. What steps can they take to avoid infection? We will get into that a little bit more in the next lecture, but let me just briefly say that there is a period of time when the use of potent anti-viral drugs and this is really within the first few hours of exposure, when that can potentially prevent the initial round of infection. But if you wait for 8, 12, or 24 hours or more to initiate those drugs, the likelihood that you're going to stem infection is really gone. So there is a way to potentially do that and what you're trying to do is basically prevent even that first round of integration of the virus into the host chromosome. So I was ambitious here in picking somebody so far back, but let me see. All right. [applause] There you go Bisola. Bisola play basketball, not baseball.

21. Q&A: What conditions can HIV survive in? (30:13)

Yes? What conditions can the virus exist in outside the body? So how does HIV exist outside the body? Fortunately it doesn't exist very well outside the body but if you go back to those early days of the epidemic in New York there was a lot of fear that it might be transmitted by utensils, that by sharing a toothbrush, etcetera that people might be transmitting infection. The virus dies relatively quickly when it's left on its own in the ambient air. So it's not a hearty virus that sticks around. It's also something that is not that easily transmissible through any given contact and we'll get into that a little bit more in some of the subsequent lectures in terms of what the chances are for transmission with an individual exposure. Here we go. I missed that time.

22. Q&A: If the virus integrates, how does T cell count decline (31:18)

Yes in the blue sweater. If the virus just stays in the host cell genome and doesn't destroy the cell, how does T cell count decline? Well the cells actually die over time so viruses will be made for awhile, but those cells actually will decline in number because an infected cell ultimately will die. I think we're going to stop with questions now but we'll have more time after the break to continue. Let's go on and let me tell you a little bit more about pathogenesis.

23. Is the immune system trying to keep HIV in check? (31:51)

So we talked in the last hour about the fact that T helper cells decline over time. Partly that's because those cells become infected. These are a central orchestrator of the immune response and so that helps to make a link to the fact that people have... that HIV and AIDS are immunodeficiency diseases. The question is, does the body try and fight back? When we were talking earlier about chicken pox and Epstein-Barr virus, I mentioned to you that your body is holding those viruses in check. So the question is does that happen in HIV infection? Well let's look at the other piece of information that we've learned from the beginning part of this lecture which is that virus levels are really high in acute infection and then those levels start to drop down. So the question is if you look at this from the perspective of an immunologist, the question is, well, actually, is that dropping because an immune response is trying to hold the virus in check, it's just not ultimately doing the job that it needs to do and not clearing the virus, but is it actually working partially for some time. Well let's look at the different parts of the immune system to try and answer that question.

24. Humoral immunity and antigen binding (33:07)

One major arm of the immune response you've already heard about from Bisola and that's the generation of antibodies by B cells. That part of the immune response is called humoral immunity and B cells secrete antibodies to directly neutralize free virus. Now it turns out these antibodies have a characteristic structure, but they're endowed with a lot of specificity and in fact that specificity comes because of sequence differences in the antigen-binding sites so that the antigen-binding site is the part of the antibody that

actually attaches to the foreign protein. Basically it's shape recognition so antibodies recognize a shape, a foreign shape in the body and basically then can directly try and neutralize whatever that is.

25. Antibodies neutralize HIV by binding to its surface proteins (34:03)

So let's look at antibodies in HIV infection and just ask the question about whether these things exist and in fact when HIV first came on the scene we knew that there were antibodies because we were detecting them using the blood test. The question is are those antibodies actually able to do something to not just bind to the virus but also to neutralize its ability to infect other cells. So these... on the top is a viral membrane and basically the crystallographic structure of envelope. So this is all drawn in proper dimension. You have CD4 and the chemokine co-receptor CCR5 on the target cell membrane down at the bottom and basically what antibodies do is they bind to the envelope spike and that prevents an interaction then with the receptors and allows for the virus to be prevented from entering a cell. So clearly we see these things generated in HIV-infected individuals and in fact, when tested in tissue culture, scientists were able to show that they could remove the infectiousness of HIV by binding to HIV.

26. Can neutralizing antibodies prevent initial infection? (35:19)

Well the question is, having made that observation that people do make neutralizing antibodies, one can hypothesize then that those neutralizing antibodies might actually be so good that they could maybe prevent infection. And the idea here really takes us towards the idea of a vaccine, towards thinking about, you know, is this something that you could use to prevent infection from occurring. So let me explain to you an experiment that was done. In this experiment scientists took antibodies that were able in the laboratory to neutralize HIV and they infused them in a monkey model of AIDS virus infection. So they gave monkeys high amounts of these antibodies. Then what they did was they tried to replicate what we thought was happening in terms of transmission by placing the AIDS virus in the vagina to see if these antibodies would prevent infection from occurring and I'll show you the data in terms of what happened with this experiment. On the left hand side you see, on the Y axis, plasma RNA concentration, so that's the amount of virus, and what you can see in the control animals on the left, these are two animals that didn't get any of the HIV-specific antibody but just got a control antibody, you can see that there's a high level of viremia that occurred. But in the animals that got the neutralizing antibody, they're basically flat in that they never got infected. So in fact these neutralizing antibodies can be quite good and yet even though they've been pulled out of somebody who's HIV infected, they don't seem to be doing that job in infected individuals, even though in an experimental model we can see a lot of efficacy. We're going to come back to that point and it really is part of the central point as to why HIV is not ultimately cleared by the immune system.

27. Mechanism of cytotoxic T lymphocytes (CTLs) (37:16)

Well there are other arms to the immune system. There's humoral immunity and then there are two parts of cellular immunity. This is in terms of adaptive immunity. Helper T cells that secrete cytokines, we've already heard about that, those are the central orchestrators of the immune system and then cytotoxic T lymphocytes that are actually able to kill virus infected cells. Let me show you what cytotoxic T cells do. Cytotoxic T cells recognize viral epitopes that are expressed on the surface of an infected cell through... which are being presented by something called an MHC Class I molecule. Cytotoxic T cells come in and by virtue of a T cell receptor and a molecule called CD8, they're able in kind of a lock-and-key fashion to make contact and deliver a lethal hit and kill that infected cell. Let's go back to the machine tool factory analogy. The commandos come in, they take over the factory, and they start making bombs. Think of the MHC Class I molecule, that thing in blue there, as a factory worker and the viral peptide as a piece of the developing bomb. The factory worker hangs out one of the windows waving the piece of the bomb to alert the community that something bad is going on inside that cell, and basically to say, eliminate this factory for the

greater good of the community, and that's essentially what cytotoxic T cells are engaged to do. Let's go to a video to try and look at how this happens within a cell.

28. Animation: Antigen presentation and CTL (38:51)

You remember messenger RNA and new viral proteins being made essentially to assemble new virions, but something else can happen to those viral proteins. There are host proteins called ubiquitin that can tag these proteins. Once ubiquitin has tagged one of those proteins it's carried to the proteasome and the peptide chain is fed into the proteasome where it's partially digested into shorter peptide fragments. Those shorter fragments now are acted upon by additional peptidase enzymes that are present in the cytoplasm and broken up into smaller peptides. Those peptides now go to the endoplasmic reticulum, gain entry through a molecule called TAP, and once inside the endoplasmic reticulum, if they're the right conformation they will bind into the binding groove of a developing MHC Class I molecule. That's the factory worker grabbing a piece of the developing bomb. They're then carried to the cell surface from the endoplasmic reticulum where they then get embedded into the surface of the cell and at this point now they are giving an alert to cytotoxic T cells that something bad is going on inside that cell, it contains foreign protein. Cytotoxic T cell comes along and if it recognizes foreign viral protein in an MHC Class I molecule, the T cell receptor on a cytotoxic T cell will be able to directly engage through a conformational recognition, along with the CD8 molecule and that leads to the release of granzymes and perforin that actually kill the virus-infected cell. Now ideally, you'd kill the cell before any progeny virions are produced and in fact what happens in real life may be quite different. Here you see viruses budding from the cell surface. If cytotoxic T cells have not gotten there in time, then the cell will not be killed and in fact progeny virions can be produced. But if the CTL gets there soon enough it can kill the cell. If it gets there late and lots of progeny have already been produced and that cell's going to die anyway, it may be a lot of effort for not much effect by killing a cell after it's already had this explosion of new viruses that go on to infect other cells.

29. Video: CTL killing a target cell (41:26)

Well, let me show you this now in a video that Mike Oldstone at Scripps Institute in San Diego put together. Let's run this. This is a cytotoxic T cell that you see on the top and on the bottom is an infected cell. What you see is a cytoplasmic bridge that occurs between the cytotoxic T cell and the infected cell. Serine esterases, these are things like granzymes are released then and enter into the infected cell. At this point what you'll notice is that that infected cell is going to start to contract a little bit as these enzymes start to punch holes in the surface membrane of this infected cell. At this point as those punctures are made you'll see those cytoplasmic contents balloon out from this cell, the infected cell as the cell is dying. That cytotoxic T cell then can go on and find another cell and take care of it. Now in all of your bodies right now this stuff is going on. You have cytotoxic T cells that are helping to clear pathogens that you've been exposed to and this is normal part of the immune response.

30. DO CTLs help limit HIV infection? (42:42)

Well now the question is, you know, we're still dealing with the fact that HIV infection is a progressive infection. It never gets completely cleared but I showed you that cytotoxic T cells, at least in theory should be able to recognize an infected cell. Well let's ask the question then, can we prove by doing an experiment that cytotoxic T cells are generated in somebody who's HIV infected and can those cytotoxic T cells limit viral replication? So the experiment we're going to do is we're going to take CD4 cells--T helper cells--and we're going to infect them with HIV and add in cytotoxic T cells to see if we can prevent progeny viruses from being produced and we'll measure in the cell's supernatant whether there is any production of viral proteins. So what we're measuring on the Y axis is HIV p24 antigen, that's part of the Gag protein of the virus. And what we have on the X axis are the days after infection. In yellow you see what happens when you add HIV to CD4 cells. Virus replication occurs and actually goes to quite high levels in this experiment

in the laboratory. If you add cytotoxic T cells obtained from a patient who ultimately went on to develop disease, what you can show is that those things are so potent that they can completely eliminate virus replication in that culture. So we have this challenge now to understand how is it that we're having progressive infection in somebody who's HIV infected and yet we have neutralizing antibodies and cytotoxic T cells that are being generated and I think for this we have to go back to really looking at again the central orchestrator of an effective immune response. What cell is that? It's the T helper cell.

31. How helper T cells orchestrate an immune response (44:34)

And let's just visit this again. Remember you get a lot of opportunistic infections with HIV infection. Antigen-presenting cells present pathogen epitopes to the immune system and they initiate an immune response by activating these CD4 cells which then go on and release cytokines and that cytokine release leads to activation and proliferation of B cells and T cells including cytotoxic T cells.

32. By eliminating helper T cells, HIV disables the immune response (45:07)

The issue in HIV infection is that these pathogen-specific T helper cells are exactly... as they become activated, because they express CD4 and because they express the chemokine co-receptor, they're also precisely those cells that get infected by HIV. So you have a function of these helper cells, which is to orchestrate an effective immune response, and sadly you have a receptor on their cell surface along with CD4 that allows virus to get into those very cells. Now think about the situation that we were talking about before, you have somebody who, you know as T helper cell levels decline, you start to get opportunistic infections. Well why is that? It's because those T helper cells as they're getting activated to try and fight CMV or other diseases, they're slowly getting whittled away by infection from virus because virus is not being controlled completely in these individuals. Now this is one aspect then and if we look at this, basically the problem is that you're losing these pathogen-specific T helper cells, you're losing the chemical messengers from them that are intended to orchestrate an effective immune response and finally you're losing the activation and proliferation and, you know, activity of the immune system that's supposed to be then doing the job to control whatever that initial infection was. So as a result you have a progressive breakdown as you're trying to defend yourself against various pathogens, you're actually offering up those central conductor of the orchestra of the immune system, those are basically being knocked off, and so as a result you have an ineffective immune response. Tomorrow we're going to talk some more about other mechanisms that also contribute to immune failure, but this is really a central problem in HIV infection and it's probably the most important thing to understand from this lecture is that basically... that HIV is an infection of the immune system.

33. Summary (47:23)

So let me make a few conclusions and then we'll throw some more t-shirts. The immune system responds in an attempt to control HIV infection and actually is associated, the induction of antibodies and cytotoxic T cells is actually associated with this decline in viremia. HIV-specific neutralizing antibodies are generated and actually can be shown to be effective in the laboratory but they clearly are not doing the full job in infected individuals. HIV-specific cytotoxic T cells are also generated, but they also are not doing the full job and at least part of the explanation for that is that both of these effector arms of the immune response, both B cells and T cells are dependent upon T helper cells in order to orchestrate an effective immune response and it's the loss of those T helper cells that is contributing to the inability to ultimately control all these different opportunistic infections. In fact, most people who die of AIDS die of opportunistic infections by just no longer being able to combat those things. I have to say in the beginning when I started out as a physician and we were seeing these patients, we had never seen anything like this because people had multiple different opportunistic infections at the same time. A complete collapse of the immune system and it was just a total mystery at that point. It's really remarkable to see what we've come to understand in terms

of how HIV actually causes disease. For years it just seemed like a black box. But I think we understand now much more precisely what's going on. We still have a ton to learn and hopefully you all will help us to learn that. So I'll stop there and be happy to answer some questions. I think you had a hand up last hour so I'll start with you.

34. Q&A: What causes a person to have a high or low viral load? (49:23)

You said like that the different loads of the virus can predict like the progression, but what causes a person to have more than another? That's a really excellent question. So why would one person have a high viral load and another person have a low viral load? And why would people who have a similar viral load have some differences in terms of the rapidity of their disease? Does anybody know? I told you already that some cases of AIDS developed within six months. What's the longest that somebody's been infected with HIV without developing disease? Probably 30 years now and counting. Some people actually seem to tolerate it and that will be a big part of the lecture that we'll talk about tomorrow and it has a lot to do with a different genetic makeup in individuals, and with the immune system and how it actually reacts to the virus. So that's a great question and you're only in the second row, so let me see. Yup. Great. In the middle here.

35. Q&A: Do mutations cause changes in the HIV envelope proteins? (50:26)

Would mutations to the HIV virus cause any kind of difference to the membrane proteins that form around it? As far as the disease and how it replicates? Yeah, it brings up a whole issue about the virus and mutations in the virus. In fact HIV is not a single virus, but HIV is a number of closely related viruses because remember I talked about the HIV reverse transcriptase, that it makes errors as it's replicating the RNA into DNA, has poor proofreading mechanism. And so even within a single individual the virus starts to evolve very rapidly and some of those mutations can affect the way that the virus enters cells, the way that it replicates within cells. Most of that information is yet still to be fully discovered. We can see the end result of a difference in replication, but understanding all of the molecular events that are occurring and how particular mutations are affecting the ability of the virus to grow are really challenging questions that have real implications because of the possibility... if you can figure out what makes it harder for a virus to grow maybe that gives you then an opportunity to be able to develop some kind of therapeutic intervention to try and replicate that. Now I have to throw this between two television cameras. See how we do. Oh. Okay. Good catch. Oh yeah, we had one here.

36. Q&A: Do some people not have the receptors that HIV uses? (52:06)

You said that the reason as to why AIDS gets through the cell is because of the receptors, so are there some people who don't have like receptors, and if there are, can they get AIDS and stuff? That is a great question. And it actually prefaces something that we'll still talk about tomorrow. So the question was, does everybody have these receptors such that is everybody susceptible to HIV infection and as you'll subsequently learn, in fact, there are genetic mutations that are present within the human population in the co-receptor for HIV entry that make it very unlikely for those particular people to become HIV infected. That's one thing that we've discovered. There are in addition some individuals that have been really heavily exposed to HIV and yet have never become infected and my own personal feeling is that most of that reason we still don't understand but there are mechanisms that are keeping people from becoming infected. These are for example, some people that have been sex workers with tons of exposures who've never become infected and the question is, why is that? And this is an area really of intense investigation as well. Again trying to understand from patients the manifestations of disease in those patients, what's going on and trying to use that information then to develop interventions to make a difference. So here's another t-shirt. Good. Way back up in the corner there?

37. Q&A: Why can't we use antibodies to HIV as a vaccine? (53:45)

If the monkey experiment was so effective, why don't we try introducing antibodies as kind of a preventative vaccine? Well that's also a very good question. There has been a big attempt to try and induce antibodies, to have them present ahead of time to protect people from becoming infected and the problem is this issue of HIV diversity that we'll get into a lot more subsequently. Antibodies have tremendous specificity through those antigen binding sites. Each of you has about a million different antibodies in your body right now, but you have the ability to make about a trillion different antibodies depending upon what pathogen gets into your body. And the problem with HIV is that HIV varies and the ability of the antibody recognizing one virus to recognize another virus that evolves in vivo is not that great and that's part of the problem. In the blue sweater up there? Yup.

38. Q&A: Could you make drugs to attack HIV's protease or integrase? (54:48)

Would there be a way to like create a drug that could attack protease or integrase to prevent replication that way? That's a great question also and you guys are really understanding the basic concepts of this lecture. In fact it's understanding the viral lifecycle that led to the development of drugs, so once we knew that protease was one of the enzymes that was critical to making mature viruses, that led to the development of protease inhibitors which you'll hear about in the first lecture tomorrow. But that's exactly the key where this basic science discovery that tells you the individual steps in the life cycle ultimately translates into a pill that you hand to a patient. And I have to say when I was... again, when I was taking care of patients early on in my career who had HIV infection, we as physicians could do essentially nothing for them in the late stages of illness, and when drugs became available to treat patients it was just extraordinary what a difference that made. Patients that we had given up for, we were sure they were going to die, and they were sure they were going to die, actually returned to fully functional lives from those medicines and I think is a real tribute to what biomedical advances can mean for real live people. I think we have to stop. Oh, I threw out my arm! Thank you very much. [applause]

39. Closing remarks by HHMI Vice President Dr. Peter Bruns (56:22)

Well thank you, Bruce, that was a terrific lecture. It's amazing how sophisticated the life cycle of these viruses is and how the body responds to that. Now there were plenty of other questions here waiting which is frustrating for people here and for those of you who are watching this lecture on the DVD and how do you ask questions? We figured that out. We have a site on our web site called Ask a Scientist. Put in a question, our volunteers will email it back and we'll even post good questions and answers for other people as well. So come back tomorrow for more of these wonderful lectures and Bruce and Bisola will again speak on AIDS and HIV and talk about how science and the health care system together can work to prevent and treat AIDS. Thank you. [music]