2000 and Beyond: Confronting the Microbe Menace
Lecture One—Microbe Hunters: Tracking Infectious Agents
Donald E. Ganem, M.D.

1. Start of Lecture One (00:16)

From the Howard Hughes Medical Institute, the 1999 Holiday Lectures on Science. This year's lectures, "2000 and Beyond: Confronting the Microbe Menace," will be given by Dr. Donald Ganem, Howard Hughes Medical Institute Investigator, and Dr. Brett Finlay, Howard Hughes Medical Institute International Research Scholar. Dr. Ganem, who will discuss how infectious agents are detected and how epidemics of infectious diseases arise and spread, is a professor of medicine and of microbiology at the University of California, San Francisco. Dr. Finlay, who will discuss bacterial diseases, antibiotic resistance, and the role of molecular biology in providing potential solutions, is a professor of biochemistry, molecular biology, microbiology, and immunology at the University of British Columbia in Vancouver. The first lecture is titled "Microbe Hunters: Tracking Infectious Agents." And now, to introduce our program, the president of the Howard Hughes Medical Institute, Dr. Purnell Choppin.

2. Introduction by HHMI President Dr. Purnell Choppin (01:37)

Welcome to the 1999 Holiday Lectures on Science. As you can see, our auditorium is filled this morning with students from high schools around the Washington, D.C. area. Good morning. I also want to greet everyone who's gathered to watch this broadcast via satellite across the United States and Canada and on the Internet around the world. This year, we'll also be joined live by students brought together by programs that our institute supports at the University of Miami and Penn State University College of Medicine. No matter how you're watching, you're in for a treat. You're about to hear two superb scientists who will tell you about the discoveries that they and others have made about infectious disease. I know from my own career what an important topic this is. As a medical student and later as an intern and resident, I became fascinated by infectious diseases and the challenges that they represent. And this led me to a career in research on infectious agents and how they cause disease. I spent nearly 30 years in the laboratory studying viruses. When I started, we were just beginning to understand what viruses look like, and what proteins and nucleic acids they contained. Studies were begun at that time on what these proteins do, how they initiate infection and cause disease. And these studies are still proceeding. These experiments have paved the way for new vaccines and new approaches to treatment. It's been a wonderful time to be a scientist interested in infectious diseases. Unfortunately, scientists have not yet achieved total victory. Viruses and other infectious agents have come roaring back in recent years in new diseases such as AIDS and in the resistant forms of everything from tuberculosis to ear infections. So today, as before, it's a challenging time to be a researcher in this field. We need bright young people like you in this field to help develop the vaccines and the cures of the future, using the powerful new tools of molecular biology. We started these Holiday Lectures 7 years ago to give students the opportunity to hear firsthand from leading medical researchers. Most of the lecturers, like Don Ganem, who will speak shortly, have been investigators of the Howard Hughes Medical Institute. They carry out cutting-edge research at HHMI laboratories across the country. Our other lecturer this morning, Brett Finlay, who's from Canada, is the first to represent the HHMI International Research Scholars Program. HHMI also awards grants for a wide variety of science education activities across the United States. So now let's get started. Don Ganem is giving the first talk this morning. Like me, Don is an M.D. trained in clinical medicine who then became interested in medical research, specifically in viruses. He is an HHMI Investigator at our laboratories at the University of California, San Francisco. And he's very well known for his discoveries on hepatitis, HIV, and other diseases. Don will speak for about 40 minutes, then he'll take questions from all of you in the auditorium or watching on TV in Miami or at Penn State.After that, we'll take a short break, and then we'll hear from Brett Finlay. Don Ganem's talk is entitled "Microbe Hunters: Tracking Infectious Agents." We will now see a brief video to introduce him. Then, Don, it's all up to you.

3. Introductory interview with Dr. Donald Ganem (05:20)

So how did I get interested in science? Well, I think it all began in early high school. I read a couple of books that completely changed my mind about science. One of them was this old, old book called "Microbe Hunters" by Paul de Kruif which was about the late 19th-century bacteriologists and how they identified the causes of a lot of the major infectious diseases. I thought that was so cool. It was detective work. It was science, but it was science in the service of human illness, and I thought it was very, very cool. The other book that I loved was Jim Watson's book "The Double Helix," which was a completely different book. This was a book about the discovery of DNA. I was -- what I loved about this book was that it made the world of scientists seem very exciting. Those two things together got me interested in microbiology and molecular biology, and also, they gave me some insight into the fact that being a scientist was actually fun. I mean, this was interesting. This wasn't just a job. My lab is about studying viruses in particular and viruses that cause human disease. Our basic question is to try to figure out how virus replication works and how virus replication produces disease in a human being, how viruses are spread from one human being to another, and how that produces epidemics in the population. So in the case of KS, for example, Kaposi's sarcoma, we're trying to figure out how the herpesvirus that we know is linked to the disease actually causes it, not just in a general way, but specifically, what molecules are involved? On what cells are they acting? By what pathways do they act? Those are the sorts of questions that we think about. And we hope that over the years, things that we discover will wind up being the basis of a better understanding of the disease that will then one day wind up being the basis of a therapy. I would like for these lectures to do for at least one or two students in the audience what Paul de Kruif's book did for me -- get me interested in the subject, make me want to know more about it, but if I can get just a few people interested in it at the level that de Kruif's book and Watson's book got me interested in it, then I consider it a big success.

Good morning.

4. Bubonic Plague (07:41)

In 1348, a strange and terrifying new disease entered the shores of Europe. The disease was called, at the time, the Black Death. Now we know, of course, that this disease is bubonic plague, a bacterial infection carried by rats and transmitted to humans by the bite of infected fleas. The plague arrived in Europe with a ferocity that was difficult to comprehend. Let me read to you now a contemporary account written by a man named Angolo di Tura, who lived in the Italian city of Siena in 1348.

5. Historical account of bubonic plague (08:16)

"The mortality in Siena began in May. It was a cruel and horrible thing, and it is impossible for the human tongue to recount the awful truth.
The victims died almost immediately. They would swell beneath the armpits and in their groins, and fall over while talking. Father abandoned child, wife husband, one brother another, for the illness seemed to strike through breath and sight. And so they died. And none could be found to bury the dead for money or for friendship. Members of a household brought their dead to a ditch as best they could without priests, without divine offices. Nor did the death bell sound. And in many places in Siena, great pits were dug and piled deep with the multitude of the dead, and they died by the hundreds both day and night, and all were thrown into those ditches and covered with earth. And as soon as those ditches were filled, more were dug. And I, Angolo di Tura, buried my five children with my own hands. And so many died that all believed it was the end of the world."

6. Advances in biology in the 19th and 20th centuries (09:33)

Now, in the six centuries that have transpired since then, we have come to understand a lot more about infectious disease than people did at that time. In fact, the stunning advances of biology and medicine in the 19th and 20th centuries led to huge advances, some of which you will hear about from Brett in terms of vaccine development and antibiotics, advances in public health and sanitation that led to major triumphs over many infectious diseases and in fact inspired a complacency and a self-confidence that was misplaced. In the early sixties and seventies, leading scientists opined that the problem of epidemic infection was over, that we should turn our sights away from the problem of infectious disease and concentrate on chronic illnesses like cancer and heart disease.

7. Description of HIV and AIDS (10:19)

And just when people thought it was over, around the time that most of you were born, in 1981, 1982, a new disease arrived virtually simultaneously in America, in Europe, and throughout Africa -- a terrifying disease characterized by immune deficiency, opportunistic infection, and even tumors occurring in young men. That disease, I think you all know, is AIDS. And as of last week, the recorded toll is 33 million HIV-infected subjects across the face of the globe. Needless to say, the supervention of the AIDS pandemic put the lie to all of these optimistic predictions about how infectious disease was conquered and was no longer a problem. Now we know, of course, that that notion was foolish to begin with, that infectious disease, epidemic infection, is a part of the human condition. I'm going to show you that it's really a part of human evolution that we can never get away from infectious disease as a class. We can triumph over individual infectious diseases, but the concept that we're going to be free of infection as a species is a ridiculous one and one that nobody believes anymore. One of the other features about the -- living through the AIDS epidemic is that it has brought us all to a closer kinship with those pre-scientific societies that suffered earlier plagues. I think most of us now recognize that we are closer in our common humanity to those 14th-century victims than we perhaps might have thought, and this notion has reached even the popular imagination. This is not just a notion that scientists have. I want to show you a piece of artwork by a photographer named Joel-Peter Witkin that I happened on in the "Sunday Times" magazine section a few weeks ago in which the artist has juxtaposed the bones of a 14th-century plague victim with the body of a young man at risk for HIV. This subject is very much alive in the popular imagination. I think you'll agree.

8. What are epidemics? (12:11)

Now, in the two lectures that I have the opportunity to give you, I want to discuss our contemporary understanding of epidemic infection. You know, to laypeople and to members of pre-scientific societies, epidemics appear to be wholly mysterious. They came out of nowhere. As Angolo said,
they appeared to be spread through breath and sight. They had their own mysterious dynamic. They waxed and waned in a fashion that appeared to be wholly unpredictable and not comprehensible by the human mind. But in fact, of course, science shows us that epidemics, although they are hugely complicated, are understandable. We can understand the basic determinants of it, and what I'd like to do in this hour and the next is to discuss our contemporary understanding of infectious disease, of epidemic infectious disease, with particular regard to two questions: How do new epidemics arise? Where do they come from? How do they spread? And how do we identify, as scientists, the microorganisms that cause these epidemics, and how do we prove, once we have those organisms in hand,
that they are, in fact, the cause of the disease from which they are isolated? And I am going to concentrate in this lecture on the second question, and we will deal in the final lecture in the series with the question of where new epidemics come from.

9. Classes of microbes (13:24)

Now, let's make sure that we're all on the same page in terms of the cast of characters. There are many classes of microorganisms, bacteria, viruses, fungi, and parasites. Since, as Purnell mentioned to you, I'm a virologist, my lectures are going to concentrate on epidemics of viral disease. Brett will be concentrating on bacterial infection.

10. What are viruses? (13:41)

So let's touch base about what viruses actually are. Let me remind you of what I think most of you already know -- that viruses are complicated macromolecular aggregates that exist at the threshold of life. They have very simple genomes made of either DNA or RNA shown here wrapped up within a proteinaceous coat that is usually encoded by genes of the virus themselves. Now, for many viruses, that simple aggregate is the whole shooting match. These are the so-called non-envelope viruses like polio, for example. Other more complex viruses have an additional shell, or coat, made up of membranous lipids that are derived from the host cell membrane. These are the so-called envelope viruses, and these viral envelopes usually have within them additional proteins encoded by the viral genome called envelope proteins that play important roles in infection. Now, you all know that viruses are exceedingly small, and they are so rudimentary that, in fact, they are not capable of autonomous growth. They cannot live or replicate outside of the environment of a eukaryotic cell. In the parlance of the virologist, they are obligate intracellular parasites. They have to be within the environment of a cell in order to gain access to ribosomes for protein synthesis to other enzymes, for nucleic acid metabolism, et cetera. Now,

11. Demonstration: Size analogy of infectious agents (15:02)

you also know that viruses are submicroscopic. They are exceedingly small. Let me give you some sense of the scale that we're talking about here in more human terms. Let's imagine that I am the size of a single cell in your body. On that scale, if I am to represent a single cell, a bacterium of the type that Brett will be talking about would be about the size of this football. And let's see. Can you catch this? Nice try. OK, now you're infected. OK, so if a bacterium is about that size, a large virus like smallpox would be about the size of this double "A" battery. Don't panic. I'm not going to throw it. And a small virus like polio would be about the size of this aspirin tablet, OK? That's to give you some sense of the proportion here.

12. How does a virus infect a cell? (15:48)

Now, let's look at what happens when a cell gets infected by a virus, a process that's easy to model in this demo. What's going to happen once that virus enters the individual cell? I have prepared a short animation that I'd like the staff to roll now,

13. Animation: Viral infection (16:09)

and before we start it in motion, let me just remind you that here is the virus particle with its envelope and proteins and genome, and you're going to see this represented as being within a pipette that's going to be dropped onto a petri dish containing human cells that are going to be susceptible to infection. And microscopically now -- let's stop the video here -- and this is a human cell. Here's another and another with the cytoplasm, the nucleus, and the cell membrane. And what you are going to see are virus particles coming in contact with the cell surface and binding to specific proteins on the cell surface called receptors, and they will do that by virtue of their envelope proteins. So let's roll the tape, and you will see that happening now. Here is a blowup of a single cell. Keep your eye here. These are the cell surface receptors. Here comes the virus particle that's going to bind. Now let's stop the tape, and I'll tell you what's coming. Next the virus has to deliver its internal contents -- the DNA or RNA -- into the cell, and it

does that by creating a fusion event between the plasma membrane and the viral envelope. That's going to deliver the internal contents of the virus, including the genome, into the cell, and the next thing that you'll see is that the incoming virus particle is going to be disassembled. That's imperative so that the cell machinery gets access to the genome to begin replication. Then you will see the genome be replicated, but not yet, please. You will see the genome be replicated, and simultaneous with that, new viral proteins will be expressed. OK, let's roll the tape. Here comes the disassembly stage. The genomes will, in this case, enter the nucleus -- not all viruses have to do that -- and now you see replication. Let's stop the tape now, and the next step in viral replication is going to be making these -- wrapping these progeny viral genomes back into particles by taking newly synthesized viral proteins and assembling them around this central core. That will give rise to daughter virus particles that can then bud out of the cell. So let's watch those steps. Roll the tape, please. Here comes the assembly reaction, and these cells are now -- the capsids are going to now go to the cell surface and bud. OK, stop the tape again. So what we've just witnessed is a single cycle of viral infection. A single particle infects a single cell and gives rise to many progeny -- on average, 100 or so progeny per cell -- some viruses up to 1,000 or even more. Now what's going to happen? These budded viruses can now emerge and infect the other cells in the culture. What we're going to see in the next part of the tape is the spread of virus from the initially infected cell to surrounding cells in progressive waves, each cell infected by a single particle, giving rise to 100 or even 1,000 progeny and so on and so on and propagating waves of infection until the entire dish of cultured cells is destroyed. So let's roll the tape now to completion. Here you can see that that single infected cell is going to be able to infect many surrounding cells. Each one of those cells is going to, in turn, be able to release hundreds or thousands of particles and infect many of the surrounding cells. And you see propagating waves of infection that will one day soon, in the matter of a couple of days or even less, spread across the entire dish -- an extremely robust replication cycle that's capable of generating many, many hundreds or thousands of infected cells in a short period of time.

14. Why do viruses replicate the way they do? (19:33)

Now, why do viruses have to engage in this extremely robust form of replication? Well, if we think back to our earlier analogy of size, it turns out -- it's easy to understand that viruses that want to infect a multicellular organism like a human being have a gigantic task before them. These submicroscopic particles have a very big job. How big is that job? Let me give you a feel for it. On the scale that we just talked about where I would represent a single cell, in a tiny drop of human blood, there would be as many cells as there are people in the entire San Francisco Bay area, OK? And I remind you that, in a person my size, there would be six liters of blood in the circulation. We talked about the population of one tiny drop. And then, of course, all the cells of the blood represent only a tiny fraction of all the cells that are in a human being. So viruses have a gigantic task before them if they are going to take over such an organism, and that's the principal reason they have developed this highly robust replicative cycle. OK,

15. How do you know if something is an epidemic? (20:34)

now, that's all I'm going to say about fundamental virology, but if you understand that much, you are ready now to start thinking about epidemics, OK? Now, let's begin our discussion of epidemics with a little thought experiment. I want you to imagine that, at your high school, there has been a picnic, 100 students attend, and then one or two days later, you discover that 35 of these students have developed an illness. They have a fever, a low-grade fever, nausea, and vomiting. I want to put to you the following question: is this an infectious disease? Let's see a show of hands. How many of you think that this little outbreak of fever and nausea and vomiting must be due to infection? Now, you've got to vote. Let's see a show of hands. Raise them really where we can see them. Great. OK, let's look at the converse. How many of you think that there might be an explanation for what happened here that doesn't involve an infectious agent? OK, why don't you tell me why you think so? Can you invent a scenario whereby you could have a simultaneous outbreak like this and not have it be due to a pathogen?

I thought it could possibly have been a result of something at the picnic instead of everyone getting it from maybe one person.

Mm-hmm. When you say "something at the picnic," what kind of a something might that be?

A food or --

I think you're barking up the right tree. Fundamentally, what you're talking about is that, yes, clearly these 35 people acquired something at a picnic. The question is, was it a microorganism, or was it something other than a microorganism? And you're correct in suspecting that it might be something other than a microorganism because, for example, why couldn't it have been a poison or a toxin or some chemical substance that contaminated the food? Or even a poison that might have been deliberately put there? Any one of those things could have given rise to a simultaneous outbreak involving the 35 people. So, this might be due to an infection that was present in the food -- infectious organism present in the food -- or it might have been due to some sort of chemical substance, OK?

16. How would you know if a microbe caused a disease? (22:30)

Now, I want you to think about the following: can you imagine designing a study that doesn't involve laboratory science but just involves looking at the nature of the outbreak that would tell the difference between what was acquired at the picnic being an infectious organism or some kind of chemical substance? Anybody have an idea how you might tell the difference -- not by laboratory work, but just by looking at infected subjects or diseased subjects? Yes.

Maybe if it was able to transmit itself later --

Super, absolutely right. The comment was, if there was evidence of spread of that disease from the group of people who were at the picnic to other people that they contacted who were not at the picnic, that would be evidence of what epidemiologists call secondary transmission, or person-to-person spread. And no chemical substance could do that because chemicals don't replicate, right? They get -- when they make it into the human body, they get diluted, whereas microorganisms, as I showed you on the tape, can replicate and spread from one person to another. So even without laboratory science, the demonstration that there is person-to-person spread of an agent -- very astute, by the way -- is indicative of the presence of a probable infectious agent. Now, let me give you a real-life example of this -- oh, I should say that the science of the study of patterns of disease occurrence and spread in the population is called epidemiology. And epidemiology usually plays a key role in the initial investigations of an outbreak and establishes the case for or against an infectious agent being present and often provides additional powerful clues, as you'll see shortly.

17. HIV epidemiology (24:06)

Let me give you a specific example of how this played out during the AIDS epidemic. In the early phase of the AIDS epidemic when it was noted that this immune deficiency was strongly linked to male homosexuality -- to groups of gay men in San Francisco, Los Angeles, and New York -- some initial ideas about where this outbreak came from revolved around the notion that they might have been exposed to toxins or certain recreational drugs or even that it was a reaction to proteins that might be present in human sperm. Now, of course, all along we suspected that there might be a pathogen, an organism that caused this disease, but the decisive piece of evidence that there was such an organism in AIDS was epidemiologic studies that showed that there was transmission by blood products, by shared needles and intravenous drug abuse, and even spread from infected moms to their newborn babies. These examples of

person-to-person transmission clearly established that AIDS must be due to some transmissible pathogen and set us on the pathway to look for and ultimately discover the virus HIV.

18. Job of an epidemiologist (25:11)

So, at this point in the workup of an epidemic, the job of the epidemiologist is largely complete, and now the ball is in the court of the laboratory scientists. Given that there is evidence for a microorganism in a disease process, how do we go about getting our hands on that microorganism? It's not a simple job, but this is something that microbiologists have been doing for over 100 years. There are classical

19. Classical methods of finding a disease agent (25:36)

methods here that you've all probably learned about in school and involve the direct microscopic exam of the tissue. Now, of course, for viruses, which are submicroscopic, they are not visible in the light microscope. They can be seen in the electron microscope, but that's not a very sensitive way to look for viruses in a disease because you need many, many viral particles in order to see them in microscopy. The other way to try to attempt isolation of a pathogen is to do animal inoculation or to inoculate cells in a petri dish, as I showed you on the animation. That's a very powerful method. Both of those are powerful methods, but they do not always succeed. For example, suppose the cells in the culture that you have don't happen to have the receptor for the virus as you saw in the animation. If they lack that, infection is impossible. Or suppose they have receptors but they are different than the ones employed by the virus in the human body. A lot of human viruses can't be transmitted to animals because the animal analogues of the receptors don't work for virus binding. The point is that there are lots of limitations to these classical methods.

20. Genomic method of finding a disease agent (26:42)

Fortunately for us, the march of science has produced an entirely new way to look at searching for causative microorganisms which is wholly different from traditional microbiology. Rather than search for the causative organism itself and try to get it to grow as a living thing in an animal or a test tube, the goal of the new methods, which I refer to as genomic methods, is to clone the DNA or RNA of the pathogen. And one of the powerful features of this kind of science is that it is relatively independent of the nature of the organism. You don't have to know the category of virus that you're looking for or even whether it's a virus or a parasite in order to undertake this method. It makes a very minimal number of assumptions. Now, I want to emphasize that the subject of genomic methods or approaches to new pathogen discovery is an exceedingly complicated and highly technical one. And there is no way in the confines of 40 minutes that I can tell you all about how this is done. There are many methods that are involved. They're getting more technically complicated and more sophisticated all the time. What I want to do is

21. What is molecular subtraction? (27:44)

tell you about one generic type of approach that is fundamental to the intellectual enterprise of new pathogen discovery. And it's a class of techniques called molecular subtraction, and I warn you ahead of time, it's a little complicated. I'm going to show you a very primitive version of molecular subtraction just so you get the idea of what the technology is all about. The goal here is to identify a new pathogen that's present in diseased cells and not present in normal cells. So if we can get specimens of diseased tissue and normal tissue -- and the normal tissue can either be from the same patient or another patient who's not infected. We're assuming now that the diseased tissue has some infection in it. One makes RNA from both preparations, and to the diseased preparation of RNA, one makes a copy of that. One copies that RNA into DNA with the enzyme reverse transcriptase -- that does that for a living, copies RNA into DNA -- and we destroy the original RNA, and we are left with a copy of DNA that is complementary to all the

RNAs that were in that original diseased tissue. This is so-called cDNA, complementary DNA. Now, this is a very complicated gamish of DNA sequences. It represents all of the messages that are normally found in those cells, plus any messages or RNAs that are unique to the infected tissue. For example, those that might derive from a pathogen. So the pathogen RNAs represent a needle in a haystack here. And the question is, can we somehow figure a way to reach into that haystack, blind, and pick out the needle, OK? How do we do that? The way we do that is to subtract away all the hay. OK, and how do we do that? We do that by taking advantage of the fact that the RNA from the normal cells is going to be able to base-pair with the normal RNAs in the diseased tissue. So everything in this tissue that is identical or complementary to a normal RNA can make hybrids when you mix these two specimens together and allow hybrids to form by base-pairing by Watson-Crick base-pairing. So all the normal messages in such a tube can be made duplex or double-stranded by hybridizing to excess amounts of the normal RNA. Then there are many biochemical tricks for getting rid of all the double-stranded material. What you're left with is single-stranded material that is single-stranded because it could find no complementary sequences in the normal tissue. That means that in this final pool of very small amounts of material are materials that are unique to the infected tissue. Included in that will be the potential genomes of pathogens. It's a clever technique called subtraction. It exists in many guises technically. It's been souped up and jazzed up in recent times by a lot of technical bells and whistles that I won't trouble you with, but the fundamental concept here is you're trying to identify a pathogen by the difference between an infected tissue and an uninfected tissue. Does everybody follow? OK. If you understand that much, it's downhill from here. OK.

22. HIV in Kaposi's sarcoma: A molecular subtraction example (30:46)

Let me give you an example now, a specific example, of how in recent years, this kind of technology has allowed progress in a field that I have been privileged to be a part of in the discovery of and characterization of a pathogen that's responsible for this disease which some of you may recognize as Kaposi's sarcoma. Now, Kaposi's sarcoma is not a new disease. It's been known for over 100 years. It was discovered in the late 19th century. But it was always a very rare tumor in western Europe and America. It was common in the Mediterranean and in Africa. But in those initial pre-AIDS societies, the disease was very mild. It occurred on the feet or ankles, rarely spread beyond there. It affected old men generally, and people died with it but not of it. In the AIDS epidemic, two things happened. First, this disease all of a sudden became very common in places it had never been seen before. And second, it occurred in a particularly widespread and disseminated and aggressive form involving all the skin of the body and even the deep tissues like the lungs. When it involved the lungs, it was often fatal. So this became, in the mid-1980s, the number one tumor of AIDS patients and became a huge public health problem. This is Moritz Kaposi, who originally described the disease in elderly Mediterraneans and later Africans in the 19th century. Now,

23. Epidemiological evidence that HIV is not the sole cause of Kaposi's sarcoma (32:22)

because KS had previously been rare in our society and became very, very common in the context of the AIDS epidemic, it was a natural thing for people to want to imagine that HIV was the cause of KS. But once again, it was not laboratory scientists but epidemiologists who provided very strong clues that that was unlikely to be the whole story. They showed -- apart from the fact that we already knew that for 100 years there had been HIV-negative forms of the disease in Africa, if you looked at Americans who were HIV-positive, what you discovered is that not everybody with AIDS got KS. KS was largely the province of gay men with AIDS, 20% or 30% of whom would develop KS. If you looked at other groups of AIDS patients, particularly groups who had acquired HIV by a nonsexual route -- hemophiliacs, for example, who got it from blood products, or children with AIDS who acquired the virus from vertical transmission from their infected moms -- those folks had very low rates of KS, 10-20 fold lower than gay men, often 100-fold lower, depending on the group. That suggested that HIV alone was not the cause. Although HIV

clearly was very important in KS, there must also be some second agent or factor, chemical substance or microorganism, that's involved in KS development. The linkage to male homosexuality suggested that perhaps this was a pathogen that was sexually transmitted. That led to a search --

24. Molecular subtraction shows herpes is involved in Kaposi's sarcoma (33:51)

so that's what epidemiologists told us. Now the ball is in the court of the biochemists, the microbiologists. Using classical methods, there was a uniform failure. Nobody was able to grow the virus in the classical way by inoculation of tissue specimens into animals or culture. Success came, as you might have imagined, from a genomic detection strategy that powerfully used the technology of molecular subtraction -- not in exactly the form that I just described to you, in a more complicated and sophisticated variant of that. This was a discovery made by two very talented people at Columbia University, Pat Moore and Yuan Chang, a husband and wife team that were working on this. Through very insightful and clever work, they were able to use molecular subtraction to identify two tiny fragments of DNA, 300 and 600 base pairs that appeared to be very closely associated with KS. They were found in every KS tumor examined -- the HIV-positive forms as occurred in America and Europe and the HIV-negative forms as they occurred in Africa. And when these little bits of DNA were sequenced, it turned out that they were wholly unique. They're not part of the normal human genome. They weren't exactly the same as any other known virus. But if you lined up the sequences in a computer with the known sequences of all viruses, you could show they were members of a known virus family called herpesviruses. They named this KS-associated herpesvirus, or KSHV. You will sometimes hear it referred to by the less-informative name human herpesvirus 8, HHV8. I will call it KSHV. Now, let me tell you a word about herpesviruses, but before I do this, let me remind you that what subtraction led to here, what genomic subtraction led to, was not the whole genome of KSHV, which we have shown was 170,000 base pairs, but two little snippets of the genome. Those little snippets were the essential clue to figuring the rest of the story out. So a word

25. Herpes virus isolated from Kaposi's sarcoma patient (35:46)

about herpesviruses. Herpesviruses have a complicated life cycle that involves two alternative lifestyles. Lytic infection is like what I showed you on the animation -- an infection that gives rise to progeny virus particles and kills the cell. But herpesviruses can also engage in something called latent infection, which is a cryptic form of infection in which the early events are similar to what I showed you. The viral genome is delivered to the host nucleus, but it doesn't replicate there. It doesn't produce the full panoply of viral gene products. It just makes a handful of genes necessary to sustain the genome in the cell. There is no production of infectious virus. But the whole genome is retained in the cell such that if you treat the cell with certain stimuli like hormones or chemicals, you can induce the lytic cycle from these latently infected cells and once again harvest a crop of virus. I go into that because that distinction between latency and lytic infection led to our success in the next phase of pathogen discovery here. So now remember, what we have at hand now was the genome of the virus, initially two little snippets of it, then Pat and Yuan's lab, our lab, and a couple of other labs around the world were able to clone the entire viral genome, but what we still needed is an ability to grow this virus as an agent, as a microbiological agent. We did that by taking advantage of cells that had been derived by my colleague Mike McGrath in San Francisco from an AIDS patient in San Francisco. These cells, BCBL-1 cells they're called, are shown here. They're lymphocytes that harbor the KSHV genome in a latent form.
What you see on the left panel here -- and we'll need the lights down to see this well -- are the cells in phase contrast microscopy. Over on the right, we have stained these cells with an antibody that specifically reacts with virus particles, mature virus particles. You can see that in the ground state without any treatment, there are very few cells that are producing virus. I can tell you by hybridization, every cell in this culture is latently infected. If we now treat these cells with a chemical, a phorbol ester that's known to induce other herpesviruses, for one day, two days or three days, we can see progressively now an increasing amount of viral replication happening in the culture. This electron micrograph proves that

these cells are loaded now with progeny virus particles. This represented the first ability to grow KSHV as a virus, and it was all made possible by the initial discovery that employed genomic detection. OK.

26. Herpes virus as the causative agent of Kaposi's sarcoma (38:14)

So now we have completed the cycle of new pathogen discovery, but we haven't answered an important question. Is this new pathogen whose electron microscopic appearance I'm showing you, is this really the cause of KS? Pat and Yuan had shown that KS tumors regularly harbor the genome, but that doesn't establish causality all by itself. That's to say that the agent is present at the scene of the crime, but that doesn't make it a criminal. It could equally well be an innocent bystander. How do we know? So at this phase of the detective story, we leave the police work and enter the sort of courtroom drama.

27. Koch's postulates (38:48)

How do we prove logically that the agent is really the cause of the disease? Now, this is not a new problem in microbiology. This has been thought about for a long time, initially by this man Robert Koch, who in the late 19th century isolated a bacterium from tuberculosis and was interested in showing to his colleagues that the organism he recovered was the cause of the disease. He proposed 4 criteria -- that have come down to us as Koch's postulates -- to help him wrestle with that problem. He imagined that he would be satisfied that M.T.B. was the cause of tuberculosis if he could show that the organism could be recovered from all cases of the disease grown in pure culture, inoculated into an experimental animal, and give that animal a similar disease to human tuberculosis. And if he could then go into that animal and recover from the diseased tissue the pathogen once again in pure culture -- he felt if he met all four of these criteria, he would be satisfied that the agent really did cause the disease. These are, I think, extremely rigorous criteria. The problem is, they are so rigorous, there are many bona fide pathogens that can't meet them. Let me give you an example. Suppose the organism just wasn't transmissible to an experimental animal. This is the case for many human viruses and in fact turned out to be the case for KSHV. It is difficult or impossible to transmit KSHV to an experimental animal. So Koch's postulates, although they are time honored, could not be satisfied for KSHV.

28. Other methods for determining causality (40:13)

Do we have another way to approach the problem of causality? Obviously I wouldn't be standing here talking to you if we didn't, and let me tell you what that method is. Remember that the whole notion that there was a virus involved in KS derived from epidemiology. So we return to the roots of the problem, the epidemiologic roots, to ask the following question -- is KSHV the virus predicted by KS epidemiology? That is to say -- in order to answer that question, you have to develop a test for viral infection and then apply it to many human subjects and ask if the virus behaves in the right way. Is it frequent in people you know are at high risk for KS and infrequent in people at low risk? To answer that question, we developed an antibody test to the virus. This is a test that measures not the virus itself but the host immune response to the virus. Since antibodies regularly arise following infection, they are very sensitive and long-lasting indicators of infection. We used our BCBL-1 cell line as the source of virus and viral antigens that we used to test for antibodies. That's why the development of the cell culture system was so important here. And rather than drag you through all the technical details, I will show you two slides that embody the central part of the answer.
 So in this study, Dean Kedes in my group used our antibody test to examine 5,000 human patients from various risk groups. Over here, we looked at the general population as mirrored by people who show up to donate blood. These are HIV-negative people.
And in the general HIV-negative population, rates of infection are very low. Now we know they are around 5%. However, if you look at HIV-positive individuals, you get a striking picture. HIV-positive gay men, 30% to 60% of them are sero-positive. But in other HIV-positive groups like hemophiliacs or

infants, infection is rare. We have yet to detect an HIV-positive infant who is infected with KSHV. This antibody reactivity directly parallels the known risk, shown in red, for KS that epidemiologists had earlier described and strongly suggests that this virus is, indeed, the one predicted by the epidemiology. How do we know that KSHV is the cause of the disease and not the result of it? What if KSHV just likes to grow in an established KS tumor? Then infection would happen after the tumor, not before. And our lab, and Pat Moore and Yuan Chang's lab in New York, showed that that was not true. The infection antedates the development of the neoplasm, and in fact, as I show on this study, which is a large study that we conducted in San Francisco and in Washington involving over 1,500 gay men who are positive for both HIV and KSHV, if you enroll people who don't yet have KS but who are infected by both viruses and just follow them over the next 10 years, by 10 years' time, 50% of these men have developed Kaposi's sarcoma, indicating that if you're infected with both viruses and you receive no effective treatment for either, you have an astronomical risk of developing the tumor that I showed you earlier.

29. Summary of Kaposi's sarcoma (43:14)

So to sum up this part of the argument, all KS lesions harbor the viral genome including the tumors that are HIV-negative. In populations, infection tracks with KS risk. In individuals infection precedes the development of the tumor and predicts an increased risk of tumor development. And finally an experiment I haven't shown you, it infects the basic tumor cell of the tumor itself. We conclude from this that KSHV is necessary for Kaposi's sarcoma. If you are not infected with KSHV, you are not going to get this neoplasm. We're confident about that. But there is also embedded in this epidemiology an even more important conclusion, which is that although it's necessary for the tumor, we also believe it is not sufficient for the tumor all by itself. Why do we think that? We think that because 5% of the general population, the HIV-negative population, is infected, and yet those people virtually never develop Kaposi's sarcoma.

30. Herpes virus needs cofactors to cause Kaposi's sarcoma (44:10)

Our conclusion is that Kaposi's sarcoma is a multihit event, that KSHV is an absolutely essential hit in this process but something else has to happen, some other cofactor has to be present. In the AIDS-related form of the disease, that cofactor is very clearly HIV, although it's still not clear exactly what HIV donates to this process. But in the classic form of KS discovered by Kaposi himself, where HIV is not a part of the picture, we are still deeply in the dark about what the second event is, what the cofactor is. Are they host genetic factors, are they hormonal factors? Because KS occurs much more frequently in males than in females all across the globe. Or are there some environmental factors in Africa and the Mediterranean that are responsible? We really don't know. We do know that KSHV is a part of both processes.

31. Future directions for research in Kaposi's sarcoma (44:58)

So where do we go from here? Obviously now we have left the part of molecular epidemiology, and now we're going on to pure virology to try to ask the questions: what are the viral genes that are expressed in KS tumors? How do they act? What is the interaction at the atomic and molecular level between HIV and KSHV, and how does that dual infection conspire to generate the neoplasm? And finally, from a practical point of view, having both agents in hand, can we begin to contemplate strategies for vaccine development that would protect the population? So this is where we are going, and these are the people who will take us there. These are the folks who work with me. Most of them are only a decade older than you are. They are an extremely talented group of people who are in the stages of committing their lives to science, as I hope some of you will do. And perhaps if we are lucky, maybe in a few years' time, some of you will join them. Thank you. I would be happy to take your questions. Are you mesmerized or narcotized? Yes.

32. Student question: Latent infection (46:05)

I was wondering, can you go over latent infection one more time?

Yes. The question is, can I tell you a little more about latent infection? Latent infection with herpesviruses is an outcome in which the viral particles enter the cell, the genomes are delivered to the nucleus, but rather than undergo the full replication program as I showed you on the animation, they repress a lot of their genes and express only a minor subset of genes. The net result of that is the viral DNA hangs out in the nucleus, replicates once per cell division. So that if the cell should divide, both daughter cells will get a copy of the genome, but by and large, no virus particles are produced from that cell. So it's a cryptic, or silent, infection. Since the whole genome is present, if you can somehow stimulate the cell to express all those viral genes, then you can once again get virus particles back out. Is that clear? Yes.

33. Student question: Evolutionary advantage of latency (47:00)

 I have two questions. What do you think is the evolution advantage to the virus of latent infection? And what facilitates the development from latent to lytic infection?

Those are great questions. Let's take them one at a time. What is the evolutionary advantage? What is the survival advantage to a virus of establishing latency? Of course, it's difficult to know for sure, but you can make a couple of models. One of the great features of a latently infected cell is because it is expressing very few viral antigens, it is not targeted by the immune system as expressing foreign proteins. Some viruses, like herpes simplex, which is distantly related to KSHV, express few or no detectable proteins on their cell surface. In that regard, the immune system can't tell the difference between an uninfected cell and a latently infected cell. And that, of course, promotes the ability of the virus to maintain itself within the microorganism. That's certainly an important potential advantage for having such a mode of infection. The second question was?

34 Student question: What activates a latent agent? (48:03)

Oh, yes, what are the things that trigger the switch from latency to lytic reactivation? This is a very active subject of research in our lab. We know from other herpesviruses that signaling events, bio-chemical events like phosphorylation of transcription factors, activators of gene expression, can induce lytic reactivation. In our lab in San Francisco and in George Miller's lab at Yale, both of our groups have converged upon a single viral gene which is the product of gene 50 in KSHV that is a transcription activator, and that protein -- the expression of that protein will trigger the switch from latency to lytic reactivation. This is all controlled at the level of the control of gene expression. And cellular signal transduction pathways involving phosphorylation of viral proteins are the mediators of that switch.

35. Student question: Herpes virus diversity (48:54)

Let's take a question from one of our remote sites in Miami.

My name is Lisa. I'm from Miami Northwestern Senior High. My question is how similar genetically is the herpesvirus found in Kaposi's sarcoma to genital herpes?

The question is, what's the relationship between KSHV and herpes simplex virus type two, which causes genital herpes? These are very distantly related to one another. They are all identifiable as herpesviruses, but they are not closely related to one another. I don't have in my head the exact homology figure in terms of what percent of base pairs are identical between the viruses, but they are quite diverged. They are

recognizable as members of the herpes family, but they are very, very distinct from one another. That's mirrored by their biology. KSHV grows in B lymphocytes and endothelial cells. Herpes simplex grows in epithelial cells and neurons. Herpes simplex establishes latency within the nervous system, whereas KSHV establishes latency within the immune system. So the very large differences in their sequences are mirrored in very large differences in their biology. You needn't fear that being infected with one would put one at risk for the other disease.

36. Student question: Cell surface receptors (50:15)

Any other questions in the house? Yes, in the back.

Several years ago, I heard on the news that some white males lacked the receptor for the AIDS virus. How would this kind of discovery help, maybe the cure -- to finding the cure or some other way to maybe help people with the AIDS virus?

The comment is that many patients with HIV -- at risk for HIV -- have been shown to be resistant to HIV infection because they lack one of the two molecules on the cell surface that's necessary for the entry of HIV. Although I depicted in the animation a single protein on the surface as being the receptor, some viruses use two molecules on the cell surface, so-called co-receptors. For HIV, they represent the molecule CD-4 and a chemokine receptor. That pair of molecules on the cell surface is involved in the entry of HIV into the host. It turns out what the questioner is alluding to is that some patients in our population -- some people in the general population have mutations in the co-receptor, in the chemokine co-receptor, that lack this receptor altogether in a functional form. Those patients, it turns out, are highly resistant to HIV infection because they can't internalize the virus. The question is, does that give us any clues about the therapy of HIV? The answer to that is yes. Because these chemokine receptors are known molecules, pharmaceutical companies and academic investigators are attempting to develop drugs and chemicals that will block the function of the co-receptor in HIV uptake. Such molecules would be expected, based on the results of people who harbor the mutant protein, to protect against HIV. Whether that will turn out, practically speaking, to be a useful strategy, we don't know. But certainly theoretically it is. And those human mutants provide very strong evidence that this approach can work if we get the right compound.

37. Student question: Viral DNA interaction with human DNA (52:11)

Shall we go to Miami again? Let's take another question from our remote site.

Hello. I'm Wesley from North Miami Beach Senior High School. I was wondering, how does virus DNA interact with our DNA in order to reproduce?

The question is, is there any interaction between herpes viral DNA and human DNA? Or viral DNA in general and human DNA? The issue there depends on -- it is different on a case-by-case basis. In retroviruses, of which HIV is an example, there is a very intimate relationship between viral DNA and human DNA. The virus establishes a persistent infection in human beings by integrating its DNA directly into our chromosomes. That way they become like our own Mendelian genes. It provides a very long-lasting and stable interaction between the virus and the host. Once that happens, the host can never get rid of that genome, except by killing that cell. Herpesviruses, on the other hand, and hepatitis B, which I worked on for many years, have a different mode of persistence. They persist as circular DNA molecules in the nucleoplasm. So they don't integrate into the chromosome, but they replicate once per cell cycle and distribute their progeny genomes to the progeny cells. Those are two different modes of association between viruses. There's no single answer to your question. In the case of herpesviruses, however, there is no evidence that integration into the host chromosome is important.

38. Student question: Going from fragment to whole viral genome (53:41)

Let's take another question from the house. Yes, in the back.

I'm Nate Curtis from Flint Hill School. In the genomic method of identifying the pathogens, you said the ultimate goal was cloning the DNA or RNA of the pathogen, but in the molecular subtraction, all you wound up with was one or two small strands of genetic material. How do you then go about cloning the DNA and RNA if the original standard methods of culturing were not effective?

If I understand the question correctly, it's, how do you go from the little snippets of DNA that the genomic subtraction methods give you to the whole viral genome? Is that the question? I'm gonna need -- do we have a blackboard capability? No. OK. Well, let me try to do it with my hands. Let's suppose that the genome is 10,000 base pairs. And you have only 1,000 base pairs from the subtraction method. How do you get the rest? What you do is go back to the original infected tissue and prepare large DNA fragments which you clone in a plasmid or phage vector. You might hope to have 5,000 base pairs on a single fragment. How do you detect those fragments? You detect them by hybridizing them to the little snippet that you do have. That'll hybridize to one extremity of the fragment, and if the fragment is big enough, you can get 5,000 base pairs from that, OK? So then what you do is you sequence that whole 5,000 bases and go to the opposite end of the clone, and you pick a little snippet from that end of the clone. Then you go back to your collection of large DNA fragments and hybridize again, looking for things that hybridize to that fragment of the genome. This is called chromosome walking. You are bootstrapping your way with little snippets of DNA along the entire length of the genome. Is that clear? I'm sorry. It is difficult without a blackboard. Do you get the fundamental concept? It's based on hybridization. You go and make big pieces of DNA and capture them with the small piece that you have. OK? I think that will be it for now. Let's take a break. We will reconvene shortly. Here is Dr. Choppin with a message for you.

39. Closing remarks by HHMI President Dr. Purnell Choppin (55:54)

Thanks, Don, for a terrific opening lecture. We are going to take a break now. When we return, Brett Finlay will present our second lecture. He will shift the focus from bacteria -- to bacterial diseases and explain why they continue to be a major health problem in North America and especially in the developing world. For those of you in the auditorium, we have exhibits outside which we invite you to look at, after which you will have a few minutes to stretch your legs
 or visit our Holiday Lecture web site before we begin again. That will be promptly on the half-hour. We will see you then.