

2000 and Beyond: Confronting the Microbe Menace
Lecture 3 – Outwitting Bacteria's Wily Ways
B. Bret Finlay, Ph.D.

1. Start of Lecture Three (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 third lecture is titled "Outwitting Bacteria's Wily Ways." And now, to introduce our program, the Vice President for Grants and Special Programs of the Howard Hughes Medical Institute, Dr. Joseph Perpich.

2. Introduction by HHMI Vice President Dr. Joseph Perpich (01:41)

Good morning. It's a pleasure to welcome the students in our auditorium and the teachers and students across the country to the second day of the 1999 Holiday Lectures on Science. We're delighted that you've joined us once again to hear doctors Finlay and Ganem continue their lectures 2000 and Beyond: Confronting the Microbe Menace. Our lecturers are truly blazing trails to better diagnosis and treatment of infectious diseases. Microbial and parasitic diseases are problems of global proportions. Humans are really ideal breeding grounds, and with six billion of us, we're a tempting target. The microbes are out to make a living, and they often do it on us. With international airline travel, infections in one region of the globe can be transmitted by travelers to another region in a very short time—as short as it takes for them to reach their destinations. The world, from an immune system's view, is filled with enemies—a mind-boggling multitude of bacteria, viruses, and parasites just waiting to pounce. The total number of bacteria on earth is estimated to be 5 million trillion trillion—a five with 30 zeros after it. Bacteria are everywhere. The microbial and parasitic invaders have perfected, over the centuries, exquisite means to end run or destroy the defenses imposed by our immune systems. Their overarching goal is to slip into the door of their target cell undetected. Once inside, they use equipment like that found in a James Bond movie to ensure their reproduction and survival. We're learning how parasites lurking in cells under the radar of our immune system can grow, survive, and transmit disease. An immunological army that cannot see the enemy cannot fight the enemy. We now have a glimpse of life of a microbe at immunity's edge that survives by outsmarting our immune system at every turn. We know that different parasites can interfere with the immune cascade at every point. Dozens of viruses, for example, have been identified that subvert our immune processes and keep one step ahead of the immune army deployed against it. The immune system defenders—the T cells and the B cells—multiply like fish in the biblical parable. They produce staggering numbers of immune system soldiers that are programmed to take on these threats. As you'll see in today's lectures, there are great opportunities in the laboratory to respond to this constant onslaught of microbial invaders. Scientists are little by little cracking the genetic code, and they will discover the chinks in the armor of these microbes and understand the different stages of their cunning lifestyles. We really are getting a close-up on several killers. Their strategies and our immune response to them is the subject of Dr. Finlay's lecture, the third in the series. Before the lecture this morning, we'll have a video again. Yesterday Dr. Finlay discussed how as a child he was always interested in how things worked, be it the lowly earthworm or life in his local pond. In the video today, he speaks of the joy of scientific discovery that is open to all students who pursue a career in the biological or medical sciences. Once again, on behalf of the Howard Hughes Medical Institute, let me

extend a warm welcome to everyone here and to all of our television viewers as we begin the second day of the 1999 Holiday Lectures on Science.

3. Interview with Dr. Brett Finalay: Joy of scientific study (05:21)

I have a wonderful job. I get to do things that many people don't get to do. I get to learn new things. I get to find new diseases. I think the best part of my job is when I wake up in the morning, I come in to work and don't know what I'm going to do that day. I'm going to learn something new. I don't know what it's going to be. I deal with intelligent, dedicated people. I interface with many different people, and I think it's a very special job. The most rewarding aspect of being a scientist has to be that feeling of discovery—when you find something that you've been searching for and working hard many late nights. Suddenly it all becomes crystal clear. You make that initial discovery. You're often in a dark room by yourself or something late at night, and you don't have anyone to shout your excitement with. But that's the best part—just that nugget of discovery when it finally becomes clear and you make that connection. I really wish that I could talk science with everyone I'm on the street with because many of society's major issues are scientific issues—things such as abortion, genetic engineering, biotechnology, engineered foods. You can't make an informed decision on public policy unless you understand these things. So I think science education is extremely important. We live in a scientific world. Everything we do, science has developed to that. And the more people that can learn more about science, the better informed society can be and use that science to their advantage. A career in science is something that doesn't just happen. You have to go about and really fashion it. In high school, what you do to be a scientist is pay attention in your science classes. Learn science. Learn how to do science. Discuss with your science teachers how you might do more about science. And just gain an appreciation for science. And I think this next two decades will be a very exciting time for biological scientists. We have genome sequences. It's exploding. Biotechnology will be the science of the future. And so just learning more about science and what the different career options are as a scientist are many, and learning those can help you go a long way.

4. Lecture Three objectives (07:29)

Good morning. Thank you, Dr. Perpich, for the introduction. Well, today I want to talk about how bacteria go about their wily ways—that is, how they actually can cause disease. We'll learn about how smart these critters are and how they can actually take you, take your cellular processes, and hijack them for their own advantages. And so we'll see that they're very clever, but we'll also see we're starting to learn things and starting to understand how they go about this.

5. What is a pathogen? (07:59)

So what is a pathogen? I've put some of the things up here. Pathogens can cause disease. That doesn't mean they have to cause disease. It often depends on the state of the host. If you're—if you're young, old, starving, poor health, immunocompromised, you're more susceptible to infections. A pathogen doesn't always cause disease. It just has the capacity to cause disease when the situation is appropriate. Microbes come in all sorts of sizes and shapes in terms of their pathogenicity. Some are very virulent, meaning it takes small numbers to make a lot of people sick a lot of the time. That would be virulent. There's others you see only under rare situations, and they're not very virulent. A lot of this depends on how many of these microbes you're exposed to. This is something we call the multiplicity of infection. And basically the bottom line is the more pathogens you are exposed to, the more chance you have the disease. Some pathogens, it takes very few. 0157 E. coli—we'll talk about that later in the lecture. It takes less than 10 bacteria to make you sick to cause diarrhea. Salmonella also causes diarrhea, yet it takes 10 million to get that disease. So obviously we have differences in their multiplicity of infection. What pathogens have is basically a tool kit of things called virulence factors. Virulence factors are

proteins and accessory molecules that allow the pathogen to actually cause disease. These are not usually present in nonpathogenic organisms. Like lab strain *E. coli* won't have the virulence factors that 0157 *E. coli* will have. Think of it as the genetic upgrade that these things have these factors they can use. Now, the other point you want to make is you really don't want to kill your host. It's really hard to infect another person when you're six feet in the ground. You can't spread easily. So an ideal pathogen doesn't go about killing the host. It may cause disease, as we discussed yesterday. But often, the other thing is the disease is accidental. It shows up as what we see as disease. For example, bubonic plague—it has worked out how it lives on rats and fleas. When the flea jumps to a human, we see bubonic plague. But that doesn't want to do that. That's what it normally doesn't want to do. And so it's really kind of an accidental disease. We're the unfortunate consequence. The other thing is, pathogens may be silent for years. Tuberculosis, which is, as we discussed yesterday, the most deadly bacterium, it can be silent inside of us for 30 years. And then, given the right conditions, up it comes, and then TB flares up. So, that's a real superpathogen in that it can quietly bide its time until it knows when it will come out and do its thing.

6. *E. coli* introduction (10:33)

So as I said in my intro, diarrhea is our bread and butter. That means that we're going to talk diarrhea a little bit today and the bacteria that do it. We'll start off with *E. coli*. Everyone knows *E. coli* is a harmless microbe that we do genetic engineering with. Everyone in this room has *E. coli* in your gut. You're just fine. *E. coli* is part of the normal flora. But there are seven different kinds of *E. coli* that cause disease in people. One causes meningitis, one causes urinary tract infections, and five different kinds of *E. coli* cause diarrhea. Each has a different set of genetic tool kits of virulence factors, the proteins that says what kind of disease it causes.

7. Two types of *E. coli*: EPEC and EHEC [0157:H7] (11:09)

So, we're going to talk about two kinds of *E. coli* today—something called enteropathogenic *E. coli* and enterohemorrhagic *E. coli*. These both cause diarrhea, and as you'll see, the virulence factors are virtually the same in these, with a few minor differences. So both are foodborne pathogens. You get it by eating contaminated food or water. And enteropathogenic *E. coli* is a major cause of pediatric diarrhea—young kids less than a year of age. It kills several hundred thousand kids per year, mainly in developing countries, but we sometimes see it here. The enterohemorrhagic *E. coli* is also known as the 0157:H7 *E. coli*. This causes a severe, bloody diarrhea—more severe diarrhea than EPEC in people of all ages. And unfortunately, in a small fraction of the cases, it goes on to a more serious disease that's life-threatening, and that is called hemolytic uremic syndrome. This is a disease of the kidneys. What happens is EHEC kills cells and plugs the kidneys up, and you have this serious disease. It's thought that about 20,000 to 30,000 people get this diarrhea in the U.S. per year, and 200 to 300 children die of this syndrome.

8. *E. coli* infection via ground beef and vegetables (12:20)

So EHEC has been in the news a lot. I want to take a few minutes to tell you about what it is and some of the issues about it. So as I said, it takes a very small number of bacteria to actually infect you, less than 10, so it's virulent. It multiplies the infection. Where do we get it from? Well, cows carry this bacteria, but for some reason they don't cause disease in the cattle. The cattle are not diseased, so you can't tell. Not all cows carry it. Up to half of the cattle, 50% of the cattle can carry it, but it doesn't mean all of them do. And this is actually a disease of mechanization because how hamburger is made is, many cattle are ground up, pooled together, and if one or more of those cattle are contaminated, the whole lot gets contaminated, so we end up throwing out tons of ground beef if we find this 0157 in there. If you had a steak, for example, you're safe because in a steak you sear the inside and outside. That cooks the

microbes. But hamburger, because the fecal contamination is ground up into the meat, it's inside the hamburger. If you remember anything from this lecture, never, ever order rare hamburger. Make sure it's cooked. If you read the fine print in all your hamburgers, that's what they're telling you what to do. When you say, "I'm a vegetarian, "so it doesn't affect me," wrong. It does affect you because they use manure to fertilize radish sprouts, and that caused a major outbreak in Japan, and 10,000 kids got it. Unpasteurized apple juice caused an outbreak because of the cows grazing under the apple orchard. It impacts everyone in the society. What do they do?

9. What does *E. coli* actually do? (13:57)

This is a picture, bacteria up here, and this is an epithelial cell here, and your breakfast is churning around up here in your intestine. What this bug does is adheres to and builds this thing called a pedestal, and it will then rise up on the cell. That's all it does. It doesn't go in ripping through your body but sits there on your intestine surface and then causes the disease. The disease is diarrhea. Here's a question. Why cause diarrhea? Well, diarrhea allows—we think it allows the organisms to spread. 20 liters of explosive diarrhea is a wonderful way to introduce an organism throughout the environment. That's what we think cholera does. The other thing is it clears out all the competition so this person gets to chew on more of your breakfast and all of your normal flora because they're flushed out, so it gives them an advantage in that sense.

10. What virulence factors does *E. coli* possess? (14:47)

Well, what kind of virulence factors does *E. coli* possess? One thing we've learned is that pathogens rarely have just one virulence factor. They have a whole tool kit-- the wrenches and screwdrivers—and each has a different purpose in causing the disease. So they have several virulence factors. What these do is they actually can exploit host function, much like a virus jumps into a cell, as Don showed you yesterday, and takes over the cell. These factors, some of these proteins, go into the cell and actually do their deeds. They're not quite like viruses because they can replicate on their own, so they're just exploiting some of these host processes. You follow *E. coli*. You swallow it, and it goes down and survives in your stomach. O157 is good at surviving in the stomach. That's why it takes so few to cause infection. They go down to the intestine and do their business. You think about it. The first thing a pathogen has to do upon swallowing is stick to where it wants to go. It wants to adhere. So they have tools called adhesins, a term we use for something that allows it to adhere. The adhesins enteropathogenic *E. coli* has is called a bundle forming pilus.

11. Bundle-forming pilus attaches to host (15:55)

Pilus means hair in Latin. You can see why. Here is the bacterium and the bundle forming pilus. See all these hairlike structures? These are basically grappling hooks that allow the bacterium to adhere to the host cell surface. If you make a mutant in this bacterium so it can no longer adhere, it no longer expresses this, and you feed it to human volunteers, it doesn't cause disease anymore. So we know then by definition this is a virulence factor. So we'll now go to the first animation, and we'll have a look and see what this actually looks like.

12. Animation: *E. coli* adheres to host using the pilus (16:31)

So here is our bacterium here. And behind it will be these long strands that are the bundle forming pilus. They'll adhere to the microvilli—these wienerlike structures. This is normal intestine cell surface. It comes along here, bacterium causes these to disappear, and it nestles down on the surface, and it's locked in, and then it's going to come down, and it will set up camp right there. We know that happens because we can see this in electron micrographs and the microvilli all disappear and boom! So we're

now stuck in the intestine.

13. Demonstration: Virulence factor injected into host cell (17:06)

At this stage, I'll ask for a volunteer to come up and give me a hand. How about you right there? -Hi. I'm Brett. -I'm Shannon. Come on over. We're going to have fun here. What does this look like to you? -A bagel. -Wrong. This is a mammalian cell. Here is the nucleus. My finger poking through. So that's what I've been eating for breakfast. You're a pathogen. You have a choice of causing bubonic plague, dysentery, whooping cough. What's your choice? How sadistic are you? I've been coughing today, so we'll go for whooping cough. Your mission is to put virulence factors into this mammalian cell. Any idea how to do that? Go in through the portals used to--for the cell to get food. These things are more sophisticated than that. I'll give you a clue. Part of your genetic tool kit virulence factors is a syringe. You have a syringe. That's full of virulence factors, blue ink virulence factors. Don't stab me with this. So you can now infect this cell, inject your virulence factors. Now, don't squirt anywhere. It's coming out the side here. OK. Good. So she's just put her virulence factors in here. You want to crack it open and see where these things—break it open. It's a tough one. Actually, I want you to break it where the ink is so we can see what's going on in here. OK. Why don't you hold it up and see what you've done? Beauty. So we've now injected virulence factors. Thank you, Shannon. Here is your UBC T-shirt. Thank you. Many pathogens can do this. They can squirt virulence factors into mammalian cells.

14. Type III secretion system (19:07)

And they all have this kind of a syringe. This is something we call a Type III secretion system, a fancy name. Think of it as a syringe for this lecture. And they have the ability to put proteins into the mammalian cell. Now, these Type III secretion systems. You can imagine-- the syringe is the same, but the ink is different. You want to cause different diseases. Some of the diseases that have these syringes are whooping cough, dysentery, bubonic plague has it, and it's in bacteria that causes disease in plants. They have this syringe system to inject bacterial proteins into the plant cells that cause disease in plants. It's found in gram negative bacteria--the one that have the two envelopes here. And when the bacterium are living, they don't squirt any of the ink out in the environment, so they have a plug here. When they contact a mammalian cell, they push the syringe, and in go these proteins into the mammalian cell, where they will go about causing whatever disease they want to cause.

16. Virulence factor modifies cell signaling (20: 11)

So the *E. coli* have one of these syringes, too, and it does a neat function. It actually fires its own receptor into the host cell. This is something we figured out a couple of years ago and caught us by surprise because up until now, we'd always assumed bacteria adhered to preexisting structures on the surface. Both *E. coli* we're talking about, they shoot this protein, this thing called Tir, for translocator inter-receptor, into the host cell. It then goes into the host cell. Then there is another molecule in the bacterial surface--this thing called intimin-- that locks on with Tir and binds tightly to it. So you think of it as a harpoon. If I was to fire a harpoon into this wall here, then reel myself in and lock on to it, that's what this bacterium has done. This really, as I said, took us by surprise. We were—it was unexpected. Knowing that, it's led to all sorts of neat things about this organism.

17. Animation *E. coli* injects receptor protein into host and binds to it (21:05)

One of the things these virulence factors do is cause signals in a cell. You know there's many signals going on in the normal cell. What these bacteria do is kind of like ringing the doorbell. You know if you walk up to a doorbell, someone will answer the door, and you hope it's—usually, you assume it's someone you want to talk to. Well, these bacteria know what doorbell to push. They modify these

signals, and in the case of the *E. coli*, they actually cause phosphorylation of the tyrosine residue of a protein, and that signal is absolutely critical for getting these things going. Here we have bacteria stained in bluish purple up here. Underneath it we've stained with phosphotyrosine. You see it—the tip here. That's where all of the signaling is taking place. And then, as we'll see in a moment, this is cytoskeletal – this is actin. It actually forms a pedestal on it. At this time we're going to the second video and pick up where we left off with the bacteria adhering to the cell.

18. *E. coli* changes host cell cytoskeleton (21:57)

Bacteria are here. We'll zoom in. Here is our Type III secretion system. Right now, this thing will poke out. We know a protein comes out. We know this protein. And then inserts two more proteins that come out and down this tube and they insert into the host membrane. These green proteins will sort of form a doughnut core. This is the tip of the syringe. We have to fire in the receptor, the Tir molecule. You'll see this red molecule coming down the chutes, and it gets fired into the host cell. Then it goes into the host cell. We don't know if it goes through the cytoplasm or goes straight in the membrane. We haven't figured this out. You'll see a blue dot. That's the tyrosine phosphorylation event. The Tir gets modified. Then it ends up in the host membrane. So here we're piling the receptor into the host cell. And then we have to dock Tir with intimin, so we think this tubelike protein retracts. What will that do? It will bring the bacteria down into contact. Here is intimin up here. Blue Tir here. Houston, we have contact. We have now landed. Bacteria is stuck on this cell. So...

19. Animation: *E. coli* causes pedestal formation (23:07)

I now return to the slides. We'll talk about the cytoskeleton that occurs in the host cell. Now actin, as you know, holds up cells. This is a major way of holding up cells. We know that the *E. coli* actually pirate this actin to build those structures on them. In this one, we stained red for actin and green for bacteria. And bacteria obviously polymerizes actin to form this long projectile under it that will then form these pedestals these bacteria will then rise up upon to rule over the cell. A common theme in pathogenesis is the ability to use this actin for the pathogen's advantage. We'll see two more examples in this lecture on two different pathogens who use it differently. We've been studying not only bacteria but the host in trying to understand the cytoskeleton. You can do experiments like take a specific mutant cytoskeletal protein that has a tag on and label it purple. That's why this cell is purple. You can then put it into this cell and ask, can it form those pedestals? On the right, the green staining is actin. The cell on the right is an untransfected cell. It has normal cells, and you see all those green dots on there. Those are actin horseshoes under the bacteria. If we put a mutant protein in, in the purple cell, you completely ablate that process. And by doing that, we can start to understand which of these cytoskeletal proteins do what and which were necessary for forming the pedestal. With that in mind, let's go to the next video. That will show us the actual actin polymerization. We'll pick it up here. Bacteria comes down.

20. Electron micrograph of pedestal in vivo (24:40)

And now we'll go inside the cell. And we're going to start to see the face of Tir that's—recruits the cytoskeletal proteins, these little doughnut-shaped things, that we are starting to understand more. You will see yellow beads come flying in. These are actin monomers. They then get together much like a string of pearls. They polymerize--they are flying in and start to get longer and longer. Actin does this for a living— forms long projections, strings of beads. What that will start to do is start to force, put a force on this thing. You will then start to see the bacteria raise up on the cell. It's because of this actin pushing underneath it that pushes it up into the cell. There it goes. It starts to push up. Bacteria starts to raise up. It's now building its throne. I'm gonna sit up on top of this thing and rule out over it. If this doesn't want to make you be a microbiologist, I don't know what will. Isn't this a thing of beauty? It continues to rise and rise and rise. The end result is we then end up with the bacteria sitting up on this

throne, shall we say, to rule out over the mammalian cell. And that's what we see in disease. OK. We'll now return to the slides. OK. So a major question— most of these studies are done in tissue culture. That means that we grow mammalian cells in flasks, and then we can add bacteria to it. But if this has something to do with disease, you should see it happening in disease. That's when we turn to animal models--mice and rabbits. In those things you can see the pedestals just like we see in tissue culture. On the left is this--this is in a rabbit model bacteria. You can see the pedestals under it here. Another pedestal here. The same processes we see in tissue culture occur in disease. If you make a mutant of any of that syringe pieces, you get what's shown on the right. The mutant. It can stick because it's still got the bundle forming pilus, but it can do nothing. This honeycomb-shaped thing here, shag ruglike thing, that's normal. This cell is not affected by these bacteria. The one on the left is. These animals will get diarrhea, and the ones on the right won't. Another key virulence factor. And all that process— building the pedestals— is absolutely key for the organism to cause disease.

21. Toxins as virulence factors; Shiga toxin in enterohemorrhagic *E. coli* (26:55)

So if I was to ask you, at the beginning of this lecture, "name a virulence factor," you'd probably say toxins. That's one most people have heard of. What is a toxin? Well, a toxin is a secreted molecule— usually a protein— that's secreted by these bacteria that can toxify a mammalian cell-- that is, cause some drastic effect on it—usually kill it. And enterohemorrhagic *E. coli*, but not enteropathogenic *E. coli*, produces a toxin, this thing called Shiga toxin. Shiga toxin is found in shigella dysenteria. Got named by a Japanese microbiologist named Shiga. The structure represents the doughnut inserted in the cell. The second half of the toxin flies through that hole and into the cell, where it then kills the cell. Now, it turns out that this toxin is the same as in shigella dysenteria, which causes dysentery. And it's also structurally similar to cholera toxin. So there is this whole family of virulence factors that do this. What they do is, they go to the cells, and they kill the cells. The cells float up, debris is filtered in the kidney. They plug up the kidneys. That leads to hemolytic uremic syndrome. That's why we don't see HUS in EPEC disease, and we do in enterohemorrhagic *E. coli* disease.

22. Evolution of pathogenic *E. coli* (28:14)

So a major question comes, where do these bacteria come from? Where have these pathogens arisen from? And EHEC was first described in 1982. It was due to some--a major hamburger producer was very careful about aliquoting the meat into lots, and they could trace the contaminated meat back to where it was and isolate it. It's a really neat epidemiology story. When you look—go back in time and look at diarrhea samples, that bacteria is not there. We haven't seen that organism prior to this time. It's a new pathogen. It has come from somewhere. The pathogen hasn't come— what has come from somewhere is it acquired genes, these virulence factors—cassettes, shall we say, that say "you cause disease." We know that all the bits that are necessary to cause these pedestals are in one tiny fragment on the chromosome, this thing called locus of enterocyte effacement— less than one percent of the chromosome. And if you take that and clone it with nonpathogenic *E. coli*, you can then build those pedestals and things like that. This is what we call a pathogenicity island because it's a cluster of virulence factor genes that have all clustered together. This has come from somewhere. It's a different G plus C content—that means the DNA is foreign— it picked it up somewhere. Within that region is our Type III secretion system, our syringe, plus all the ink of the syringe encoded within that. OK. Now, so we have that one cassette. We know EPEC has that. EHEC has another cassette, its toxin. That, too, is a mobile genetic element carried by a bacterial virus that hops from bacteria to bacteria. So we think the way that EHEC arose is it acquired two genetic elements, the one being that locus of enterocyte effacement, and the other Shiga-toxin phage. When we put the two together, voila, a new pathogen. That's where we think it came from. And pathogens are always evolving. We're always going to see new pathogens. And we now know, using genomics, that the enterohemorrhagic *E. coli* has greater than 20% more DNA than the lab strain *E. coli*, and a big question for the future is what is this DNA doing, and

how much of it is needed for the diseases?

23. Protein therapies to fight bacteria (30:25)

That takes us to knowing all this, how do we come up with potential therapies? This is where the basic science can translate into using this to improving our lives. Well, by identifying the virulence factors, you have new targets to go after and places to think about making antibiotics. One example would be, why not plug that syringe so it can't inject the virulence factors? Because that syringe is in so many pathogens—plague, dysentery, whooping cough, sexually transmitted diseases, blindness, things like that. We would have a broad spectrum— at least for gram negative— target to go after. That's one area we're working hard on with various pharmaceutical companies. There's a neat concept here. Antibiotics kill bacteria. If we block that syringe, you won't kill the bacteria, but you will block its pathogenicity. And this is a concept that has been slow in coming with the antibiotic production people because they— normally we thought we'd kill them dead. You want the bugs dead. So maybe, we block virulence, we'll then block disease. And the other thing is, because we're not putting a selection pressure, you'd predict there should be less resistance arising, so it makes it a good target to go after. The other way is to go after vaccines. We talked about vaccines yesterday, and we're doing this knowing the molecules of the bacteria use its receptors it inserts into. We're working on two fronts coming up with a childhood vaccine using Tir and some of the other effectors. And we're also looking at the idea of vaccinating cattle so if the cattle can't carry it, that would improve our meat supply. Those are some of the lines we're going after to pursue this. OK.

24. Introduction to *Salmonella* (32:03)

Continuing with diarrhea, we're switching to different bacteria and talk about salmonella. This is another pet in our lab that we work on. Salmonella causes two major diseases in people. One is something called typhoid fever. This affects 16 million people a year. About 600,000 deaths a year. The good news is, typhoid fever has a vaccine that works, so typhoid fever is actually decreasing. Salmonella typhimurium is one you hear more about. This is food poisoning— gastroenteritis, nausea, vomiting, bloody diarrhea. This is one you see a lot in food poisoning cases and doesn't usually cause a life-threatening disease unless the host is in a state of immune compromise such as HIV patients. They suffer from serious complications. As we saw yesterday, like most pathogens, the antibiotic resistance is going through the roof. You can see antibiotic resistance in Salmonella typhi. This continues to rise in Asia. In India, greater than 90% of typhi is resistant to most normally used antibiotics.

25. Microscopic view of *Salmonella* (33:08)

Let's have a look. Let's meet salmonella. And I'm going to the microscope here. And if we could have the microscope display up, here we have some salmonella. Now, the salmonella in the microscope. You can see them swimming around here. Hopefully there's some that come wiggling in here. These things are motile-- that means they swim along. They'll come crashing into cells. Every once in a while, you'll see one flying through here. Here comes one guy. They're camera-shy today and a little cold. It's best if you have them warmed up. You can see them wriggling around here. They're sniffing around to see where they can travel. That's what salmonella looks like. This is real life under this microscope.

26. Virulence factors of *Salmonella* (33:52)

So what kind of virulence factors does salmonella have? Let's go to the slides now. And just like E. coli, it has to have some way of sticking in the gut, so it has these pililike things. And if you look closely, you can see it has these hairlike structures bridging the bacteria to the cell. We don't know a lot about this yet, and we haven't figured it all out, but there's at least six different kinds of adhesions that stick to the

cell. But salmonella is sneakier than *E. coli*. It wants to go into the cell and live as an intracellular parasite. So just like *E. coli* and other pathogens, salmonella, too, has a syringe system. It actually has two syringe systems we'll talk about. The first one, it injects factors into the cell to rearrange the cytoskeleton like *E. coli*, but the effect is very different. Instead of the actin building a pedestal, it actually causes the mammalian cell to grab the bacteria and yank it into the cell— that is, it invades. It ends up inside the cell.

27. Demonstration: Jell-O simulation of *Salmonella* invasion (34:53)

At this time I'll take another volunteer. Come on up. You like Jell-o? Good. We have liters and liters of it up here. -I'm Brett. -I'm Chris. Come on up. Chris, we have a gram negative salmonella, right? Pink. It's supposed to be pink. Looks like a marble, but we won't tell. Here we have a mammalian cell— cytoplasm of a mammalian cell. See it jiggle? That's the consistency of cytoplasm. I put a piece of Saran Wrap on it to represent the membrane. You are a salmonella. You have the dirty deed of invading that cell. Go for it, Chris. Drop it and see what happens. Here? Well, that sucked. I want to go in the cell. Come on. Pick it up? No. Invade. Push. Push. Push. There she goes. A little bit of force. Let me rearrange the membrane a little here. You're still not inside. You're a lousy invader. Go for it some more. There we go. Now we're invaded. Very nice. Have a T-shirt for your effort. Thanks. OK. That's what salmonella does. It goes into the mammalian cells by pushing through it. Now, at this time, we're going to show on the video a real-life salmonella invading a cell. So go ahead and run the video. Thank you.

28. Video: Living *Salmonella* invading a cell (36:22)

Here is a mammalian cell. You can see these salmonella and see them flying in. It causes a big ruffling event. Bacteria into the cell, engulfs it, and it ends up inside the cell. And you can see the cell attracting—see the movement up here? And down here is your nucleus, and there is a clump of bacteria up here that have come into the cell. They come in and end up inside. So we're now going to go to an animation that if you don't believe real life—I know you're of the video age and like animation, so we'll go to that one when that gets cued up and have a look at that. What you will see—

29. Animation: *Salmonella* invading a cell (36:58)

run it full speed. Nice motile salmonella screaming along here. Here she comes. Boom! It's got a Type III secretion system and squirts in proteins and triggers a major event. Here she comes. Ahh. Satisfied, right? Haven't taught these videos to burp yet. OK. Bacteria is inside the cell.

30. *Salmonella* needs to survive and multiply inside the host cell (37:20)

That's what salmonella does. We've seen this real-time and live. We know this happens. That brings up the next concept. What do you do inside the cell? That brings up a whole bunch of other difficulties because we know that salmonella have to survive in the cell. Now, when things go into cells, the cells basically have pockets of poison called lysosomes that are designed to come along and dump a load of toxic substances onto organisms and particles and chew it down to nothing. So salmonella knows it's going to hit this and has to figure out a way to get around it, and it does. So here is an actual electron micrograph we took, and you can see the ruffling of the mammalian cell waving out bacteria inside a membrane-bound vacuole, and then the bacteria is in here. That's what the Saran Wrap represented. The bacteria never left the enclosure of the Saran Wrap. It's pinched off and surrounded by that Saran Wrap. Once inside, it has a neat way of actually knowing it can't fuse with this because it will die, so it avoids this lysosomal fusion, this delivery of toxic poisons. So every good pathogen, your number one trait is increase in number. You want to multiply. If you don't multiply, you're a lousy pathogen, you're not going anywhere. Salmonella has to learn to multiply inside the cell. We spent a fair number of years

studying this, and we know salmonella grows inside this vacuole increases in number, and keeps growing in number. The other thing it does is makes these strange structures, these tubular structures. The bacteria are where the arrows are. It makes these things called salmonella-induced filaments, which are key for the bacteria growing in there. If you knock out the gene of the bacteria that makes these things, it can't cause disease. We know it grows in there and has the ability to grow in there, and that's one of the areas our lab works on is how does it do that and get nutrients across the Saran Wrap into the bacteria? Now we have another video that will show us from inside the cell. Go ahead and roll it.

31. Animation: *Salmonella* inside the host cell (39:20)

Here's the vacuole coming in. This is Chris' finger pushing the salmonella in. Here's our bacteria. The yellow stuff is actin pulling the bacteria in. It's absolutely needed. We then have fusion with things called early endosomes. This is a normal event. Then we're going to zoom in on the bacteria and we see a second Type III secretion system, a second syringe that will put proteins into this vacuole. Now we have a protective coating. Now bring on the lysosomes, bring on the toxic delivery particles. Bacteria doesn't care. You'll see these come in. They glance off. It blocks the fusion of these things so it won't die. Not only that, it starts to divide and divide and divide. And eventually it will divide so much that this thing will actually probably explode and then release the bacteria. We don't know anything about the final event, and that's why I wouldn't let the video animators draw the cell exploding because we don't know it happens. We guess it does, but we haven't seen it. OK.

32. Bacterial strategies for intercellular survival (40:18)

Let's turn to the concept of, how do you live inside a cell? Why do you want to live inside a cell? This is a great place. It's warm, it's full of nutrients, it's got all the stuff you need. But you've got to get around those lysosomal fusions. So intracellular pathogens, those that live inside a cell, know they have to figure this out. And there's three ways these pathogens do that. So they all go into that vacuole like we've seen with salmonella, a standard sort of generic way. Then there's a few macho pathogens, and they say, "I don't care if you fuse lysosomes with me. I'm tough enough to take it." There are a few that survive and actually thrive in this environment and grow. Those are rare, though. You don't see many of those. The majority of intracellular pathogens do like salmonella. They go in the vacuole. They'll put on the sort of, the armor around the vacuole. They block the toxic package delivery. And then they grow. That's salmonella, that's tuberculosis. That's chlamydia. That's many, many pathogens and parasites, too. The third process I want to talk about in a second with another pathogen does something really neat. It goes in, but then it makes an enzyme that chews out the Saran Wrap vacuole and puts it free in the cytoplasm. What a wonderful place to be. It's got everything. It's like a little nirvana, from a microbial point of view. Because once you are out of this package, you can go anywhere you want. These pathogens do this. There's listeria, shigella, rickettsia. These things chew it out, and then they do something really neat. They grab actin directly. They take that actin and push the bacteria inside the cell. Why do they do that? Because they want to go to the next cell. Then they jump to the next cell. They have done this.

33. Introduction to *Listeria monocytogenes* (42:08)

So the organism I want to talk about is listeria monocytogeny. This is a gram positive organism. So, the other color stain, or the gram stain. What this does is cause a disease in the very young, especially newborns— a terrible disease in newborns. Also in older patients. This is something we call an opportunistic pathogen, meaning it only jumps into hosts that are basically compromised. A pregnant woman is immunocompromised. Her immune systems are down. In an older person, it jumps in there. How do you get it? Unpasteurized milk, goat cheese, soft cheeses. In Quebec, they make a lot of soft cheeses. They're actually trying to pass legislation in Canada banning all unpasteurized soft cheeses

because of listeria. Listeria is everywhere. You can go in the field and find it everywhere. But just to show you what happens. So that would be like Camembert and Brie and cheeses like that. You'll see some of the traits of the virulence factors similar to what we've talked about. It's got adhesins that allow it to adhere. It's got invasins that allow it to get in just like salmonella. Then we pick it up here where it's different. The bacteria degrade the vacuole. Then they're free in the cytoplasm, and then they can move around the cytoplasm.

34. Demonstration: Jello-O simulation of *Listeria* invasion (43:21)

This time I want one more volunteer. I don't think I picked from this side. How about you over here? You guys are good volunteers. You know it's free T-shirts. OK. Hi. I'm Brett. Karen. Do you like Jell-o? Yeah. You're in real trouble now. You have a nice, fresh bowl of Jell-o coming up here. OK. Here we have a nice gooey mammalian cytoplasm. Here we have listeria. Gram positive, so it's a purple marble here. OK. You have to invade this cell. No Saran Wrap this time. Beauty. Now, not only do you have to invade, you've got to move around inside that cell. Congratulations. Start moving. Beauty. You get into this great. You do like Jell-o. Gorgeous. All right. A hand for her. Excellent. Here are some Kimwipes. Clean yourself up. You can have a T-shirt, which I won't give—you can—there you go.

35. Intracellular lifestyle of *Listeria* (44:26)

That's what listeria does. It plows around inside the cytoplasm. The bacteria here grabs the actin, polymerizes on the bacterial surface, pushes the bacterium around, provides a force, then leaps into the next cell. Why does it want to do that? It doesn't want to ever come out of the cell because then it doesn't see antibodies, antibiotics, macrophages, things like that—this is a great place. So it tracks around from cell to cell doing this.

36. Video: Live *Listeria* moving inside the host cell (44:53)

We're going to the next video. This will show actual listeria. This is from Julie Theroit in Stanford. What she did is took a picture of a mammalian cell, a nucleus here, listeria, and photographed listeria moving. Roll the video, please. See the actin comets behind it and bacteria here? These things just cruise around. Watch this guy. He's going to smash into this one, sniff it. "Nah, I don't like it. I'm outta here." Keep going. They do move. This is not animation. This is real life in a cell. See them plowing along in here doing their lengths. I told you cytoplasm was a great place to be. You'll see them follow along the surface here. Gorgeous actin comets. They make a protein at the end of bacteria that causes actin to bind and polymerize actins present in the normal cell. You can see some of them bang into the side, form these projectiles, and then come out. OK. At this time, we can stop the projector. OK. So let's go to the slide, please.

37. Regulation of virulence factor expression (46:00)

One last concept I want to get across. You don't want to express virulence factors all the time. There's no point in expressing virulence factor when you're in the dirt when you want it inside human. These things have ways of sensing. They kind of have antennas that say, "I know when I'm inside a host cell." Can any of you come up with some kind of an idea of what it might take to actually sense? Go ahead. ...let them know when they are on the cell. Maybe there are other proteins or something that can hook up to a cell. OK. That's a complex answer. The idea is, he's saying maybe they recognize mammalian cells. I'm going to give you this candy cane here. Good idea, but these things are simpler than that. We have them documented. We think that occurs, but we haven't shown it yet. They measure their environment. What's an environmental cue that you're now inside my intestine? -Temperature. -Perfect. What temperature are we? 98.6. 37 degrees. We work in Celsius in science. Temperature is a

good cue. One more. From way up in the back there. pH. Absolutely. Stomach pH. I'm not going to wing this this far. You can pass it back. Hopefully he'll get it. All right. Good idea. They do. The other thing is that they actually can sense—they have something called quorum sensing. They basically wait until they've got their numbers up to the stage where they then said, "OK, if we attack now, we can win. "If we attacked earlier, we couldn't." They wait until they get to the stage when there's enough of them and they reach a quorum. Then they turn on the attack genes, boom! They go at it, and that's how they also regulate virulence factors. If you turn them on too soon, you know the host will get mad at you.

38. The future of infectious bacterial diseases (47:45)

OK. Where are we going in the future?

Well, as we said yesterday,

we're never going to be in a germfree environment.

We will always be dealing with this--new organisms arising, genetic exchange, environmental changes, new pathogens coming out.

Research is coming along. We've got genomics.

We're finally starting to understand things.

We understand cell biology

and how these things go about their dirty deeds.

So there's lots of neat things there.

New disease models-- these are coming all the time.

We can now genetically modify animals to have a human gene.

A human pathogen can be used in an animal.

Recently we and others have been using worms as models for infectious diseases

because they have some things that can cause disease.

From this, we then use that new information

to think about making new therapeutics in our constant battle with the microbes.

I leave you with the concluding statement

there's no way we will outdivide them,

but we can absolutely outsmart them.

The last slide, I need to thank a lot of the people

that are involved in doing the stuff I told you about.

This is my lab. And science is great.

You get to work with really neat people from around the world, many people from around the world. It's all their hard work and effort that allows me to be up here today.

39. Student question: Treatment differences for gram-positive and gram-negative bacteria (48:56)

OK. We're now out of time.

Now we're going to questions,

and we'll start with a house question.

Speak loudly, please.

I'm from West Springfield.

I was wondering, like, is there any difference in treatments between gram positive and gram negative bacteria?

Yes, there are.

Gram positive and gram negative bacteria--

some treatments are the same, and some are different.
Some antibiotics work better on gram positive.
Some work better on gram negatives.
Some vaccines are designed against a specific pathogen.
There are differences. There's a few similarities.
Diseases are treated based on disease.
The number of--diarrhea, a lot of different bacteria,
but usually one disease is caused by one organism,
and you have a treatment for that.

40. Student question: Why don't we have a cure for the common cold? (49:40)

We're going live to Miami. Go ahead, Miami, please. I'm Chelsea from North Miami Beach Senior High. I would like to ask you, why haven't we come up with ways to fight the common cold? Why haven't we come up with ways of fighting the cold? We are coming up with ways, but the common cold is caused by many, many viruses, not just one. It's like why we haven't cured cancer. It's multiple diseases. We are coming up with ways. And one of the ways, much like bacteria, is to block the receptor that adheres to it, so we know it binds in a thing called a canyon, and if we can plug up the canyon, it won't be able to adhere. There are therapies that are coming like that. I think the answers will come. So it's a good question.

41. Student question: Lysosome avoidance by *Listeria*-type invaders (50:27)

All right. We'll go back to the house now. How about you right up there? Speak loudly. I'm Brian from Woodson High School. I was wondering—when you were talking about the intracellular survival, you mentioned something about the third kind, where the vacuoles are chewed up and they're free, and the bacterium is free in the cytoplasm. How would they avoid the lysosomes? The lysosomes are two membranes, two pieces of plastic. When they fuse—they do what's called fuse, sort of melt together. And then what's in the lysosome gets delivered to that vacuole. If you're free in the cytoplasm, lysosomes have no way of fusing with you, so they can't come there and deliver the contents, so that's why those listeria were happily zipping around. They won't get dumped on. They've got to go through the first phase, where they're in the vacuole, and most are killed in that stage, but once they get out, they're free and clear.

42. Student question: Is the actin used by bacteria the same as actin/myosin (51:27)

OK. We'll continue with a house question. Up here somewhere. I'm Ben from Flint Hill School. I was wondering what the relationship, if any, there is between the actin utilized by the bacteria and the actin that bonds to myosin in muscle contraction. Actin is a normal part of our cells. It plays many roles. It causes cells to move and divide. It causes cells to hold up. And so what the bacteria does is, it basically plugs into what they know will happen, and they say, "Over here, I want you to do this." They make molecules that can hijack the system and go in and make it do it. It's the same actin. Actin polymerization occurs as a normal process. The bacteria just said, "OK, you do it here"—either at the tail of listeria to push it or under the E. coli to build a pedestal or to cause the salmonella ruffling. It's the classic example of exploiting a preexisting pathway.

43. Student question: How do some pathogens mutate faster than others? (52:19)

Let's go to Miami for another question. Hello. I'm a student at Miami Western. I would like to know if there's a genetic explanation why some pathogens mutate at a faster rate than others. The pathogens mutate at a faster rate than others? I don't think that that's a true statement. It's just that we see them

arising. All bacteria are constantly mutating and evolving. This genetic Internet we talked about doesn't have to pass virulence factors but passes different things. They're changing fast, and by growing so fast—every 20 minutes or whatever—they change fast. And we see it selected for in pathogens, but all bacteria do that, and bacteria are genetically plastic, shall we say.

44. Student question: Are there harmless bacteria in humans that harm animals? (53:04)

Let's go to the house for a question. How about between the cameras up there? I was wondering—cows have developed different strains of *E. coli* that harm humans. Could humans be developing different strains of bacteria that harm other animals but don't harm us? Delicious question. If 0157 doesn't harm cattle—I don't know. I can't think of a disease that we know that happens. We tend not to pay a lot of attention to animal diseases and human transmission to animals. We have seen that human behavior has changed diseases and brought new diseases out and, for example, by cutting down the trees, you then release the deer that have the ticks on them and get Lyme disease and things like that. So human behavior has no doubt given us pathogens. I don't know of any example of a human where we just carry it that causes a disease in animals. I can't think of one right now. Good question. Great question.

45. Student question: How do bacteria survive host cell death? (54:00)

We'll go for another house question. OK. I'm Shannon. We already met. The Jell-o lady. I was wondering—once the toxins are released by the cell and—not by the cell but by the bacteria and kill the cell, how do they get out of the cell before it dies, and how do they survive if—A toxin is a protein, right? So the bacteria are just sitting on your gut and spewing out all this stuff. So it doesn't matter if the cell dies with the toxin. That's just kind of like me throwing candy canes at you guys. If I kill you, I won't be affected. The candy cane might be, but that's OK. So it's a protein. So it sort of goes, we think it goes with the cell and gets cleared out that way. Neat idea. OK.

46. Student question: Jungles as a source for antibiotics (54:48)

We're now going back to Miami for another one. Go ahead, Miami. Hi. I'm Betty. And I'd like to know, where would you suggest antibiotic hunters look for antibiotic agents? Is the jungle a good place to look for the antibiotic agents, and if not, why not? The question is, why don't we look in the jungles for antibiotics? That's how antibiotics were first discovered and how we found most of our antibiotics, by screening soil organisms and various things. And if we cut down the rain forest, we lose that vast biological reservoir that has all these compounds—that we might find new compounds. There's been no real new antibiotic class developed in 30 years, so unfortunately we haven't—that strategy is not really working, so instead we're trying to use science and combinatorial chemistry where you throw in a test tube and make all these different compounds and all these variations. We just want to make a broad, diverse number of reagents. And so there's more going that way or looking for new targets. There will be some in the jungle, and I'm sure as we continue to screen, we'll find them. But we're trying to do other things. OK. Our time is up. I'd like to say thank you very much. You've been a terrific audience. And remote as well. I'll turn the floor over to Joe. Thank you.

47. Closing remarks by HHMI Vice President Dr. Joseph Perpich (56:12)

Thanks, Brett, for that impressive presentation on your work. You've given us the first pictures on how the wily bacteria skulking around our bodies and our cells outwit our defenses. But what you've heard this morning from Brett, we're developing an understanding. And with that understanding will come the promise of effective strategies to thwart and defeat every trick in the book that these cunning foreign invaders employ. The fourth and final lecture of the series is "Emerging Infections: How Epidemics Arise." Dr. Don Ganem will discuss the forces that create and shape epidemic infections and how

clinical medicine and public health measures respond to that challenge. As Dr. Choppin, the president of the Institute, noted in his remarks yesterday, we especially encourage you to visit our Holiday Lecture web site and check out the Institute's web site, too. It is becoming a 24-hour, all-news channel on research and education and the biomedical and medical sciences. The grants web site increasingly, we hope, will be a science education resource for teachers, students, and their families. Many of the animations you've seen here and in prior lectures are also on our web site—demonstrations, virtual labs and the like. Please come to the site, visit it, and let us know how we can improve it. And we look forward to having you all join us in one half-hour for the concluding lecture in this series. Thank you very much.