Viral Outbreak: The Science of Emerging Disease
Lecture 3 – Fighting Viruses in the Lab and Beyond
Eva Harris, Ph.D.

1. Begin of Lecture 3 (0:16)

[ANNOUNCER:] From the Howard Hughes Medical Institute. The 2010 Holiday Lectures on Science. This year's lectures, "Viral Outbreak: The Science of Emerging Disease", will be given by Dr. Joseph DeRisi, Howard Hughes Medical Institute investigator at the University of California, San Francisco, and by Dr. Eva Harris, Professor of Infectious Diseases at the University of California, Berkeley. The third lecture is titled "Fighting Viruses in the Lab and Beyond." And now to introduce our program, the Program Director for Science Education of the Howard Hughes Medical Institute, Dr. Dennis Liu.

2. Welcome by HHMI Program Director Dr. Dennis Liu (01:09)

[DR. LIU:] Good morning, and welcome back to the Howard Hughes Medical Institute Holiday Lectures on Science. Our speaker this morning is going to be Eva Harris, and in her previous lecture you got a taste of Eva's passion and energy. I think she showed you that in Managua even the mosquito larva have rhythm. Eva's been battling dengue fever in Nicaragua for nearly two decades now, and her lab work and her community work has made a real difference clearly. She mentioned a particular problem with dengue fever, is that by having four different serotypes of this virus, it actually puts a big population at risk for the severe form of the virus. And in today's lecture, she's going to delve into some of the molecular details and her lab attempts to understand this problem at a molecular and cellular level, but she's also going to talk about how she's continued to work in places like Nicaragua in order to help the country establish its own research infrastructure so that they can carry on the battle in their own way. And now we're going to have a brief video to introduce Eva. Thank you.

3. Profile of Dr. Eva Harris (02:31)

[DR. HARRIS:] So, how different is it to work in my lab? Well, first of all when I started here at UC Berkeley I already had like six or seven people working with me. I always was terrified of the moment that people would start transitioning out because I just thought well, I have such an amazing set of people and they all get it, you know, they get the philosophy behind the work that we do. How am I ever going to replace that? And to my wonder and amazement, it actually...I have the most phenomenal people in my lab always. People kind of self-select to come to me who have a deep interest in science and in scientific excellence and have a deep interest in the scientific problem and the pathogen that we're working on, but who also have a broader vision and that want somehow a context for their work that actually has a tangible meaning in the world, you know. And so, there's many ways that that can happen. It doesn't mean that everybody is like rushing to Nicaragua, you know, at the same rate that I am, but what it means is that they want to have a context where, you know, they're doing the molecular biology or the immunology work. But our group meetings, you know, we'll have presentations on the molecular virology, the immunology, but then also there will be presentations on the clinical work and there will be somebody coming back from Nicaragua talking about a dengue epidemic or an influenza epidemic. And so, they really can feel the context, the real world context of the work that they're doing. And then my personal philosophy is very much that science is an international program and that it's about international diplomacy on many levels, and that it's also about global citizenship. And so, I think the people in my lab buy into that on many levels. Like I said, they come from people who are already versed in international work. There's people who are simply molecular biologists, but they all come because there's something else and there's like an overall philosophy that they want to be part of.

4. Immune system as potential foe (04:24)

So, great to be back with you again. I really had a blast yesterday, and it's a wonderful program. What I'm going to do today is you've heard a little bit about my research program and we spent some time yesterday learning about dengue fever and then about some of the work in Nicaragua and our cohort studies and then actually in the community how we're trying to actually intervene in this problem. And what we're going to do today in this first lecture is to look actually at some of the basic science that we do in my laboratory, and we're going to kind of go back to this question of these four serotypes and trying to understand more at the molecular and immunological level how it is that this actually can set one up for the more severe form of the disease. So, we talked about how a sequential infection with different dengue serotypes can set you up for the more severe form of the disease, and what I'd like to do now is go into how it is that the immune system can actually be both a friend and a foe. Of course we think of the immune system as being protective and it is, but actually with dengue and actually many other diseases, the immune system can actually be pathogenic as well. And so, there are several branches of the immune system which, I'll talk about this as the innate and the adaptive immune response. The innate is the response which is not specific to a particular pathogen, but it acts right away and can protect you immediately. Then there's the adaptive form which is specific to a pathogen and develops over time, and that has two branches, the T cells and the B cells. The B cells secrete antibodies. And the form of...in dengue what happens is that both the T cell and the B cell response can be both protective and pathogenic. And what we're going to focus on in this lecture is really only the B cell and antibody form of pathogenesis, but there's a lot of other research in the field that's trying to unravel the other aspects as well.

5. Animation: Dengue Virus Enters Cell to Begin Infection (06:27)

So before we get into the immune response, we're going to need to understand a little bit about how dengue actually infects a cell. And if we can roll this video, what you'll see is how dengue can actually approach a particular cell. This is -- as we saw very nicely from Joe's lectures -- what the dengue virus virion looks like in the lipid bilayer underneath the proteins, the capsid, and then within the capsid, the RNA genome of the virus. And so, this virion is floating in your bloodstream and will approach a cell, often what we call a myeloid cell which is a monocyte or a macrophage or dendritic cell of the immune lineage, and will actually attach to, this is called the cognate receptor, and we'll talk later about a different type of receptor called an Fc receptor. And what will happen is there will be an interaction through what we call receptor-mediated endocytosis. The virion will enter into this endosome which is a membranous vesicle, and what will happen is that there are pumps that will pump in protons and will lower the pH -- and you can see this by a gradient of pink -- and as the pH is lowered, there's a movement of those envelope proteins which actually will completely transform the conformation which will allow it to actually fuse with the endosomal membrane, fuse it with the virion membrane, and then allow the capsid and then within that the RNA genome to be delivered into the cytoplasm of the cell, and that will then establish infection.

6. Antibodies neutralize viruses and tag them for destruction (08:00)

And so as I sort of mentioned, we have these different receptors that can mediate the entry. And I'm going to talk about this a little bit more through this next set of slides because what we'd like to understand is how is it that it's different to have a single infection versus a second infection with the same serotype versus a second infection with a different serotype. And so, we're going to kind of look at that at this cellular level. So, what happens is that we can have the dengue 1 here in red. It's going to come in and will actually interact as we mentioned with a specific receptor on the surface and will enter this endosome, again through the receptor mediated endocytosis, and then will have the pH drop and will be able to change confirmation of the envelope proteins fused, deliver its RNA into the cytoplasm, and establish infection, like we just saw. Now, I'm going to show you an example of how the antibody

response can be protective. In this case we then have a second infection with the dengue 1, the red virion. And in this case we already have red antibodies. So, we've got antibodies that are directed to that dengue 1 virus, and they have a variable region and a constant region, right? So, every antibody has its variable region over here and that's going to interact with the specific antigen, and then the constant region. A constant region can interact also with what's called an Fc receptor. So, let's just go through this. Now, we have the red antibodies are coating the red virion, and they were coating it so much that we say that they can neutralize it because they can get in the way of its interaction with its own receptors. So, it's blocked and it can no longer interact with the cell and cannot get into the cell. So, we say that the virus is neutralized. Now, the antibody though, because it's got this Fc portion of the antibody connected to the virion, it's actually what we call an immune complex. And this part can actually interact with Fc receptors on these cells -- which we call of myeloid origin -- and then they can actually get endocytosed or phagocytosed as we say, but what happens is that in a regular situation, these antibodies will actually...when there's many of them, they'll activate the cell. And so, what you have is that they'll come through into an endosome, but there are these little vesicles called lysosomes which are chock-full of like nasty enzymes and different chemicals which are going to go and attack a microbe that's coming into the cell, and these lysosomes will then fuse with the endosome and will actually kill the virion. Okay? And so, this virus will no longer enter the cell. The other option is that these neutralizing antibodies can not only block entry into the cell, they can actually bind to the virion so that that crazy transformation of all those proteins can't happen. And so, it's locked into this initial conformation and it cannot move into its fusion competent state. Okay? So, it's stuck in the endosome.

7. Antibody-dependent enhancement (ADE) of disease symptoms (10:49)

Now, in contrast I'll show you what happens when we have this kind of pathogenesis, this pathogenic response. And in this case, we've got our green dengue 2 virion that's coming in, but there's red antibodies. So, the dengue 1 antibodies will bind, but they actually bind ineffectively and they don't necessarily block the sites of recognition to the receptors. So, in other words even though there are some antibodies, they're actually not neutralizing and this guy, this green virus can just go in, go do its fusion conformation, and then dump its RNA into the cytoplasm and infect the cell. But that's one way. The problem is that when you have this what we call antibody- dependent enhancement or ADE, we have the antibody which will then interact with the Fc receptor, again via this Fc portion of the antibody, but because there's only a few antibodies, it's only going to weakly activate the cell. And so, the endosome does not get the lysosomal fusion and the antibodies don't prevent fusion of the virion with the endosomal membrane. And so, it can actually deliver its RNA into the cytoplasm. So, the problem we have here is that we actually have two infection pathways. So, now that you have antibodies, not only can you come in through the cognate, but you can also come in through the Fc receptors, so that the previous infection is actually helping the virus to get into its cell of choice, into its target cell.

8. ADE leads to physiological changes that cause severe dengue (12:11)

And so, what we think happens then is that it gets into the target cell, which is a monocyte or macrophage, and it activates the macrophage. And then what happens is that you get T cell activation, and then you have T cells which are going to secrete cytokines, and the cytokines are inflammatory cytokines -- we call some of them TNF alpha or tumor necrosis factor alpha and other cytokines -- which actually will work on the endothelium, the capillary endothelium and will pull apart the tight junctions and will allow plasma leakage out of your circulatory system, and that will then reduce your blood volume and you can go into shock and die. Okay? And so, that's kind of how we think the severe dengue actually happens in a human.

9. Maternal antibodies can cause ADE in infants (12:53)

But in terms of trying to understand how is it that these antibodies actually can make this happen, we're very limited because we can either look in cells, and cells are fine, but you can't look at immune response or a disease outcome in a cell. You can do that in a human, but of course you can't do experimentation on humans. You can do clinical observations. And so, the idea that ADE is important in dengue actually came from clinical observations in humans where we see a big peak of hemorrhagic fever and shock syndrome in infants, and we think that that's due to maternal transfer of antibodies from the mother into the child, into the fetus, into the baby through the placenta. So, when the infant is born there are a lot of antibodies at the beginning that can protect, but then those antibodies kind of gradually get destroyed over about a 10 month period, and then they actually get to a level where there is too few of them and they become enhancing. And you actually see a peak of severe disease in infants from five months to 11 months. So, it exactly correlates with the antibodies going away, but that's just correlation. It's interesting, but it doesn't prove it.

10. Engineering a mouse model for dengue research (13:58)

So, what was really needed was a mouse model, essentially an animal model that we can actually prove this point and then come up with ways to prevent it. So, what happens is that obviously humans are very complex. Right? Everybody's different. They don't inbreed, and so therefore the genetics are very complicated and we can't do, obviously, experiments on humans. So, what do we do? Well, we can do experiments on cultured cells, and again that's limited because we can't look at immunology or disease pathogenesis. So, in very carefully justified circumstances, we do work in animals, and I have to emphasize that the use of animals in research is very, very carefully considered and we write huge protocols and we justify every single animal that's being used. So, you know, it is being used so that we can then develop therapies and vaccines for humans. And it's really important in dengue though because we need to understand how is it that the immune system can sometimes be good and sometimes be bad? And we can only do that in the context of an organism. And there's very important implications for vaccine development as we sort of discussed yesterday. So, the problem with the virus though is it's what's called host specific, meaning dengue doesn't usually infect mice in the wild. So, to be able to create this system, we actually had to modify both the virus and the mouse for this system to be able to work and to actually reproduce disease that looked like human disease. So, what we did is we passed a virion so that it was modified at the genetic level, and it turns out that this new virus can then cause plasma leakage and all of the features that look like disease in mice, but in fact when we sequenced the whole virus, we found that actually there were only two amino acid differences. Of the 3,500 amino acids in the virus, only two were different, and the result was simply to have the virus stay in the blood circulation a little bit longer. So, it wasn't a crazy different virus. It's just a very slightly different virus that can stay longer in the periphery and cause disease. On the mouse side, what we had to do was I told you about the innate immune response being very effective. Well, interferons are a big part of the innate immune response, and so if you mess up the interferon system a little bit in the mice, then they become more susceptible to dengue. So, what we see here now is this...and I'm just comparing the human disease with what we see in what we call our AG129 mouse model, and we can see that there's increased fluid leakage, we have low platelet counts in both situations, increased cytokines, we have internal bleeding, lethality within the first week, etcetera. And so, you can see that there's quite a number of features that are similar in the two situations.

11. Severe dengue can be induced by ADE in the mouse model (16:32)

So, what we wanted to know about using now this model that we've now created that can actually reproduce features of human disease, we wanted to say, let's look at this question of antibody-dependent enhancement. Can we actually reproduce this and prove that this in fact happens? So, can antibody alone enhance disease? Is this enhanced disease associated with increased viral burden? Does it have the same pathogenic mechanisms? And then if we can show this, then let's work on methods to prevent this. And

so, what I'm going to tell you know is a kind of series of experiments, and a lot of them are going to have a similar experimental setup. So, what you see is we have a mouse that we call control serum, is that we just took serum or plasma from a regular mouse and administered it to this recipient mouse, waited 24 hours, and then we challenge with dengue 2. And what you see here is called a Kaplan-Meier survival curve. So, on our y-axis what we see here is the amount of survival, the number of animals that survived. And so, what we see is that when we bring in different levels of virus, when you administer 10 to the four, 10 to the five, 10 to the sixth plaque forming units per milliliter, nothing happens. The mouse is perfectly fine. When we get to 10 to the seven though, we get this lethal phenotype with the vascular leakage. But now let's look at this situation. What we did is that we infected a completely different mouse with dengue 1, the red virus, and then we collected the serum from that mouse six or eight weeks later. So, that serum now does not have immune cells, no B cells, no T cells, but it does have antibodies. And then we see does that polyclonal mix of antibodies against dengue 1, if we administer that to a new mouse and then challenge that mouse with a different dengue serotype with that green dengue 2, then what happens? And boom, now suddenly all of these mice that before had a sub-lethal infection now they all die. So, that shows that just the administration of an antibody against a different serotype can actually kill this next mouse, and therefore we show that we can have lethal antibody-dependent enhancement.

12. ADE mouse has high viremia and elevated cytokines (18:33)

And now that we have this system, we can then see well, what's behind it. And we say well, we look at the sub-lethal infection, we have a certain amount of virus -- this is virus in the bloodstream -- but then when we have this ADE or just a lethal infection, we can see about a 20 to 100 fold increase in viral load. So, now we can say okay, so that lethality is associated with more virus. We can also look and we can show that it's associated with these increased levels of tumor necrosis factor alpha, which is actually what mediates the inflammation that causes the disease. And so, what we see is again a certain amount in the sub-lethal infection and then here, but both the antibody- dependent enhancement and the lethal infection, we have a much increased level of this inflammatory cytokine. I'm not going to show the data, we also see a concomitant decrease in platelet levels. Okay? So, what that's all showing us is that yes, now we have a model where we can prove that antibody alone can enhance the disease, that that enhanced disease is associated with an increase in the viremia which will then trigger other pathogenic mechanisms. Now, we wanted to see whether the increase in the viremia was similar to what we see in humans or in monkeys, non-human primates, and in fact it's exactly the same type of viremia, about 10 to the 20 or 100 fold. So, it's the same increase in viral load. And then when we look at the pathogenic mechanisms, we see that it's the same tissue and cell types. I showed you data about the cytokines being elevated, the platelets depleted. And so, we've actually now created a model that will actually reproduce this antibody-dependent enhancement. And now what we can do is try and prevent it. That's why we do this research.

13. In vitro cellular model of ADE (20:16)

And so, what we are moving back to now is this concept of ADE, and I want to show you how we measure this in vitro, meaning in cells, and then how we are going to measure this in vivo, meaning in our mouse model. So, the Fc receptors as we saw before are on cells, and what we're using in this particular assay are these purple cells that actually don't have that cognate receptor. They only have the Fc receptors, so the only way that a dengue virus can get into a cell is if there's antibodies. So, when we add dengue virus with no antibodies to these particular cells, they can't infect the cell. But if we add dengue virus with antibodies, then we can get infection through the Fc receptor, and I'll just show you what that looks like. The way we graph this is that we have on our y-axis the percent of cells that are infected. And so, if you have no antibodies, then you have this green line where there's no infection. When you have antibodies at a highly concentrated amount, they will block infection. As you dilute the

antibody though, you get this peak of enhanced infection, and then after a while you dilute all the antibodies, nothing's there, and nothing happens. Okay? So, you get this little bell-shaped curve which is the ADE curve in vitro.

14. Modified antibody can prevent ADE in vitro (21:26)

So, what we now do is we say okay well, if we think that the Fc portion of the antibody is so important, then let's get rid of it and we should be able to prove that we don't get enhancement if we don't have Fc receptors. So, the first thing we did was we cut off the whole Fc receptor, the whole Fc portion of the antibody. And so, this should then no longer be able to interact with the Fc receptor, and in fact that's what we see in cells or in vitro. So, you have here with antibody, we have our bell-shaped curve, and then when we add this modified antibody lacking the Fc tail, boom, there's like no infection in cells. And when we did this in our mouse model, we also found that there was no enhanced disease. But, you know, that's kind of messy, right? I mean it's just like you're not...you're essentially cutting off like your whole arm to be able to look at what your fingernail does, you know. So, we said no, no. This is too messy. So, we collaborated with one of my former post docs who had a very elegant system where you make a single amino acid change, one amino acid change, and that will essentially obliterate what's called a glycosylation site, and this antibody can no longer interact with the Fc receptor, but it's a very specific change. Now let's look at what happens. Well, we have the antibody. It's a normal antibody. We get our nice curve of enhanced infection, and now we have a modified antibody, but only that one amino acid that can't interact with the Fc receptor and bam, we completely eliminate the ability to enhance infection.

15. Modified antibody can prevent ADE in mouse model (22:50)

And now let's look at what happens in our mouse model. So, when we take here our standard protocol, which you've seen before, we've injected anti-dengue 1 antibodies and then we've challenged with dengue 2 and we get our lethality, and now we're going to contrast that with modified antibodies that can't interact with the Fc receptor and boom, now we get 100% survival. So, just that one little change in the antibody will block the interaction with the Fc receptor. It will eliminate ADE and will completely protect these animals, okay? I should mention one more thing. This is really important because not only do you get survival, but you actually get a decrease in the viral load. So, now we can understand why we have this increased survival. We showed you here when there's no antibodies, we have a purple bar. When we have enhancing antibodies, it increased, and now when we use these modified antibodies, not only do we not enhance but we drive down the viral load all the way down to the floor. And so, this is showing you how you can get this protection. But the problem is that when...you don't know when you're going to get dengue, right? I mean you only get infected and then go to the doctor and then you want to have some kind of what we call therapeutics.

16. Modified antibodies work as therapy (23:57)

So, what we wanted to test was the therapeutic efficacy or how well does this antibody work after the infection. Not before, but after the infection. Could that actually be something one could use in a clinic? And so, what we see here is that we set up the animals with this anti-dengue 1. We then challenged with dengue 2. With no treatment, again we get a 100% mortality, and then we wait 24 to 48 hours later and then we come in with this modified antibody like pretend, you know, it could be say a drug after you go to see somebody at the clinic, and boom, it's 100% protective. And so, this shows you that this concept of trying to understand first what is it, what's the mechanism behind this disease, and then can we mess with that so that we can actually come up with a therapeutic modality? And so, what we're showing you here is that in fact yes, this will be possible. And so, now there's a couple of groups that we're working with that have what we call humanized mouse antibodies and then we have actually human monoclonal

antibodies that they've put into cocktails that are going to be tried in the clinic to see whether we can actually after people start developing hemorrhagic fever, can we actually prevent that. And so, the important part here is again showing that when you have this increased survival, we are again knocking down the viral load and importantly we're knocking down the levels of that inflammatory cytokine TNF alpha. And so, now we can actually show not only just the survival, but we can actually get down to the mechanism of how is this actually protecting the animals and therefore we hope protecting the humans.

17. Using the dengue mouse for basic and clinical research (25:31)

So, what we're doing now is using this model to be able to really dissect what are the other elements about the antibody, not just the constant region in this ADE, but what are the epitopes? What are the serotypes? What are the domains that are important? This is kind of getting at what parts of the virion are important. Which antibodies work, don't work? And then what we're doing, we get certain answers from our work in the laboratory, and then remember that cohort study I told you about yesterday? Remember, that we were able to get serum before the kids were sick and before they got infected? So, we can actually say okay well, from our mouse work we think that this and such antibodies are important. Now let's look in our human populations. Did the kids that never got sick have more of this and such antibody? So, now we can actually move from our laboratory work into our human populations, which of course is still correlative but we're actually being able to have a hypothesis that we test with, you know, real outcomes in real humans, even though that's still an observational study, but we can come in with the mechanistic hypotheses derived from the laboratory. Okay? And then we also...several other projects that we have are looking at the role of elements of the memory immune response like you might have heard of B cell memory. And then also as I mentioned a little bit, the mechanism of action of other kinds of antivirals because now we have a really nice system that can actually reproduce a lot of the features of human disease. And the question here is can we help them along through pre-clinical testing so that then those drugs can go on into monkey trials and then out into human trials. But then we can actually learn from them about the pathogenic mechanism and about the viral life cycle as well.

18. Q&A: Can the modified antibodies be used as a vaccine? (27:08)

So, I'm going to end this section and take some questions, and then we'll go onto the second section after that. Yes?

[STUDENT:] This serum that you have like created that decreases like the mortality of the mice, could this like become a vaccine for dengue basically?

[DR. HARRIS:] Yeah, so one thing I should just mention...so, it's actually, these are monoclonal antibodies that you can genetically engineer which is the serum has a mix of antibodies and a monoclonal is you clone a single antibody. And so, I just technically should let you know that, but yeah, the idea in this case is that this research is allowing us to understand what a good and bad immune response would be, which would then help us understand what a good or bad vaccine would be, but the actual antibody would be used more like an antiviral, like a drug. So, this research actually helps you both with vaccine development and with actually developing an antiviral drug.

19. Q&A: Can injected antibodies compromise you against other serotypes? (28:04)

[DR. HARRIS:] Yeah?

[STUDENT:] My question is, if you inject me with these new antibodies and let's say I leave your hospital, they're still going to be in my bloodstream, and then I get a new infection. Could the supposed antibodies you just gave me to cure me of dengue kill me with the next thing?

[DR. HARRIS:] Well, the idea...first of all they clear after, you know, a period of two or three weeks or so. So, you won't have these antibodies for very long, but remember those antibodies will never be able to increase an infection because they can't interact with the Fc receptors. So, no matter what next dengue you get infected with, that antibody will never be able to increase. It will only be able to decrease the infection.

[STUDENT:] What about non-dengue?

[DR. HARRIS:] Oh, yeah. Non-dengue won't be affected because the specificity of that variable chain is only going to be looking at dengue. It's too far away from like West Nile and other things to actually cause this problem.

20. Q&A: Can this treatment be applied for other viral diseases? (28:55)

Yes?

[STUDENT:] I'm wondering could this same treatment, this antiviral treatment be applied to other viruses?

[DR. HARRIS:] Well, so potentially if they have the same mechanism. So, this is interesting, like I mentioned how we can look at different types of antivirals. For instance, you could look at an antiviral that would interfere with...with entry of the virus into the host cell, and that maybe could work for various different viruses. Or for instance, you could look at an antiviral which messes up a cellular process necessary for viral infection, and that might work for many different viruses. This would work for viruses...this is kind of looking at a different aspect of the disease which is that interaction with the Fc receptors. So, there are like other types of diseases, feline leukemia, there's a couple of other viruses that can do ADE. In that situation, maybe this would work, you know, with a similar concept. Obviously the antibody would have to have a different specificity.

21. Q&A: Wouldn’t the dengue RNA be degraded in the cytoplasm? (29:53)

Yes?

[STUDENT:] So when dengue, its genome is only a few strands of RNA, right? So, wouldn't these strands of RNA be degraded by host enzymes when it was injected into the cell? How is that enough to, you know, to like replicate and actually like, you know, continue the life cycle?

[DR. HARRIS:] Right. So, we were actually debating on how far to take that little RNA through the life cycle in the video, but essentially what happens is it's not naked RNA. There are several ways that the virus deals with this. First of all, it gets coated with proteins, and another is that it folds up. It's not just like this little thing going like this. It actually folds up into all of these secondary structures which are much more resistant to any kind of degradation. And the third is that it actually goes into certain vesicles within the cell. It increases the cell...the membranes within the cell start proliferating like crazy and they form these specialized vesicles which is where the virus goes to replicate. So, it's not just replicating. It's in the cytoplasm, I mean it's not in the nucleus but it's actually not just in the cytoplasm. It's in these membranous structures that are essentially protected from the proteases and the nucleases and all of this that float around in the cytoplasm. Very good point. Okay, I'm going to go on to the next part of the talk.

22. Building research capacity in developing countries (31:08)

So, this is...we're switching gears here because as you've all seen, we have rather a broad program. And what we talked about and I was hoping to show you is how at the scientific level in my research program, what I've been trying to do is set up questions in the laboratory that will essentially bring up hypotheses that can then be tested in the field, and then again how we can make observations from our field research that can then be tested in the laboratory. But what I'm going to focus on now is the other mission in my life, the other side of me, which is building scientific capacity in a broader sense in many developing countries. So, in many developing countries there's limited material and financial resources, poor infrastructure at many different levels, lack of scientific careers, like there's no HHMIs in many countries to support scientific development and careers, and a whole array of different challenges which exist in developing countries that we actually don't necessarily run into here.

23. Strategies for enhancing local scientific capacity (32:07)

And so, what happened is I spent several years of my life, about five years, working with a number of, especially Latin American colleagues and colleagues from other developing countries to come up with a program that would work for them to be able to respectfully partner with scientists in developing countries to bring them training, what we call material aid which is essentially equipment and supplies that they can then use in their laboratories, and then use small grants to be able to support their scientific research on locally relevant problems. We also have been building networks and partnerships of what we call our peers. So, this is what we call South-South transfer or partnerships. We have North-South and South-South. We try and make lots of different types of linkages between scientists in different countries. And then we support scientists, essentially research projects and efforts that I'm going to tell you about, dengue -- we've already started talking about that -- and then also tell you a little bit about the influenza work that we're doing in Nicaragua kind of as an example of how you can really follow forward this work and actually support efforts in the long term. I'm also going to give you an example of how this has kind of moved out of biomedical sciences and into health informatics, so information technologies applied to health. And so, all of this is actually part of this non-profit organization called Sustainable Sciences Institute that was officially started in 1998, but actually in a virtual sense began in 1988, and we've been working kind of with the same mission for the last over two decades. So, the idea here came from this initial foray into Nicaragua, which we started talking about yesterday, when I was just kind of overwhelmed by the magnitude of the problems and the paucity of the resources that were there. So, there was a number of issues in terms of laboratory skills, but you know soon we found that, you know, the laboratory skills were really important, but it would be even more important if you could apply them to the epidemiology or the reality, the study of one's own population. So, linking the lab with the study of the local population, the lab and the epidemiology became really important. But that's all great except that you need money. So, you need money to support research. And so, we started teaching people how to write their own grants. And then they came to us and they said you know, now we have these nice projects and we've got all this data, but we actually have never been trained in how to write scientific publications. So, then we started a whole series on manuscript writing, and then they were like you know well, actually working with human populations is pretty tricky and, you know, we need...so we started a whole series of courses on bioethics. So, we're always like listening what is it that people need, and then we create the response to that need. And so, the idea in all of this is actually leading from essentially scientific research but that has a public health impact in those countries.

24. Transferring technologies and alternative techniques (34:58)

And so, just to get a little bit more in the detail of how this works, a lot of times when we think about technology transfer in this country, it's about developing a novel technology, say in a U.S. lab at a university and then transferring that out into a company, but for me the idea of technology transfer is

actually taking existing technologies and then actually transferring them out into the field, into the community where they can actually make a difference in the public health of a particular country. So, a lot of places, like I said, don't have fancy equipment. And so, this is just an example of this wonderful laboratory in Bolivia where we worked in the late 90s. So, they had actually come up with all kinds of innovative solutions. So, they didn't have money to buy a centrifuge. They made what we call a blenderfuge, which is a blender with a little aluminum bowl and then these are the widgets that you would put at the end of faucets and boom, they had a little centrifuge. And then they took a record player and then translated that circular motion into a horizontal motion and made a lab shaker. So, they made all kinds of things because they couldn't actually afford to buy it. You can invent it. Another important thing is to look at alternative techniques. So, this is really important and this actually harks back to what Joe was talking about yesterday in the polymerase chain reaction. So, what you saw yesterday here on the bench and what he showed you are, these are thermocyclers. And so, these are expensive pieces of equipment. They're about $10,000 to $15,000. And what you do is you put your reagents into a tube and you put them and you program the machine and then you move the temperatures, those three different temperatures around the tube, but if you understand that what PCR is about is moving a mix of chemicals around three different temperatures, then instead of having the temperature move around the tube, you can make the tubes move around the temperatures, right? So, what you do is you make three different water baths and then you can manually move from 94 degrees to 55 degrees to 72 degrees, and you will do exactly the same thing because you understand what that biophysical process is. Now, this is actually funny, if I have a moment to tell a story because we actually did this in Quito, Ecuador. And so, we did a lot of workshops in many different countries, and the idea was always to bring the technology to the place. So, you know, we were setting this up the day before and this stupid water bath wouldn't work. It just like never got up to 94 degrees, and so we said oh great, you know, thank goodness we're at least doing this a day before. Got a new water bath, it still wouldn't go past 89 degrees, and we're like. what's going on here. Does anyone have any ideas?

[STUDENT:] Not enough power?

[DR. HARRIS:] No. It turns out Quito is 9,000 feet above sea level and water boils at 89 degrees. So, we were never going to get 94 degrees. So, like this is why you need to go to the place. And so, what we did was we put...actually we ended up with a layer of oil on the top which kind of approximated a closed system and we got up to 92 degrees. So, that was like, yay. Anyway, so you've really got to do it in the place that you're going because me in Berkeley would not have thought of that. Another alternative technique is just, you might have heard of issues with the cold chain where you have blood that you've taken for a test and you need to keep it cold, but a lot of these countries are very hot. And so, how do you deal with this cold chain problem? And one way is to just...well, you can just spot blood onto a filter paper and dry it, and then actually your proteins, DNA, and even RNA can be stable and you can just put them in a little bag and move around with that. And so, that's a way you can get around it. Another way is to just use saliva for instance instead of blood, and you saw that we used that in our field study yesterday. So, using all this together and all those different workshops and working over time, we, you know, have a number of accomplishments and we've had many onsite workshops and trained, you know, 1,400 scientists, and a lot of those scientists have published their articles and gotten grants funded, and maintained a lot of collaborations.

25. An RT-PCR test to identify dengue subtypes (38:38)

So, what I want to give you is a couple examples of how that actually has worked. So, we've already talked about dengue. And so, what I want to show you is kind of where this went. We started in Nicaragua with a very simple concept, which was just to detect, to diagnose dengue. And there was very kind of awkward ways of doing this using serological techniques or viral isolation that takes a long time, and since PCR was being invented right at that time, we thought wow, why don't we apply this?

Actually there was an existing publication. What we did was we simplified it dramatically so that we could actually make this happen in a single day, in a single reaction. So, remember we have our four different flavors, and then this is an RNA virus. So, the first thing we do is we extract the RNA and then we reverse transcribe it to DNA so that we can then amplify, but what we do is we do this all in one tube, and the clever part is that we can add a single primer on the 5' end which is common to all four serotypes, and then we have a 3' primer which is, each one is specific to a different serotype and it's in a different position on the genome so that when you amplify this you get four different amplicons as they're called, but they're different sizes. And then you don't need all this fluorescent fanciness and sequencing actually that Joe says. You can actually just do something very simple, which is to run it on what's called gel electrophoresis. And what you do is you pour a gel which is agarose which is really like Jell-O, and then you put in the different reaction mixtures. And if you have a small product...this is DNA and it's negatively charged, and so what you're going to do is run it from the negative to the positive pole, and it will just squiggle through that Jell-O and the little guys will squiggle faster than the big guys. And so, you can actually just separate them according to size, and what you see here is dengue 2, 3, 4, and 1 based on the size of the product. You can do this quite simply in many different laboratories. And this is what it actually looks like. So, this is dengue 2, 3, 4, and 1, and this actually is me. So, I actually had dengue 3.

26. PCR test successes in the field (40:31)

It's actually kind of a funny story because this was the first time that we had ever been able to do this kind of typing in Nicaragua in a single day. They usually had to send it to the CDC and wait about six months to get an answer back while the epidemic was raging. And so, here I was...we'd done it and it was so exciting, so I gave this talk to the entire epidemiologist in all the country. Everyone was so excited, and that day I get dengue. But it was actually very good because we were just establishing it. So, I thought this is great. Take my blood. Put this at 23 degrees, 37 degrees, and we can see the stability of the virus, et cetera. So, it was actually very useful, but it was actually...and this has been used now, you know, for 15 years as the national diagnostic system and is very useful for our studies. So, you know, this started with just detecting dengue, but then we were able to use it, or I should say they, the Nicaraguans were able to use this in a number of different outbreaks, not only in Nicaragua but in a number of other countries as well. So, we were able to export this, you know, simple technology to Paraguay, Peru, a number of other different countries. And the scientists there were able to apply it in real time. When an epidemic was starting to happen, they were able to right away be able to identify it and then go out and do mosquito control in the areas where those cases were detected and prevent the spread of that outbreak. So, it's really important to have the capacity to identify a particular outbreak, but it's actually also important to be able to identify that it's not dengue. So, for instance in this case there was an outbreak in the north of Nicaragua and everyone thought it was dengue, but when they did the PCR it was all negative, and they were like Eva, oh my God, it's negative. What happened? And I was like well, then it's not dengue. And it turns out that it was leptospirosis and it was the first time that this had actually been identified. This is a bacterial disease that looks like dengue, but since it's a bacteria, what can you do? You can treat with antibiotics and you can treat early. So, it's really important to know when something is or is not dengue or another pathogen, and you need to know this in real time, and the only way to do that is to have the technology in the country ready to go. And then they also used this in a respiratory outbreak of respiratory syncytial virus and had everything worked out before the CDC team actually got to the country. So, there's a number of reasons.

27. The impact of the Sustainable Sciences Institute (42:53)

What we've learned is how to actually be able to apply these techniques and work with people through workshops and have it be their own, have them take ownership of it, have them understand, have regional instructors, but you know, it's not enough that people learn and say hey, that's cool, you know,

see you later. We actually want them to be able to use this in their own daily life. And so, that's also happened and they've been able to apply this to many projects and that's wonderful, but we actually don't want to stop, you know, at the publication. We want to go on and apply this to public health practice. And so, the wonderful thing is that many...pretty much everywhere that we've worked, our partners have actually taken this and applied it into the daily life ongoing beyond the project. So, you know, say we trained someone in tuberculosis PCR. We come back and they're doing chlamydia, gonorrhea, and many other diseases, and they've trained people in other parts of the country. It's called a multiplier effect, and it's a really wonderful thing to watch that in action. So, what I wanted to show you is all of this work actually started with a simple idea of bringing PCR to a country so they could do their own diagnosis, but then it spread. In Nicaragua you saw how it spread in the lab, and then we worked in hospitals and then in clinics, and then from there on to communities -- like the project we talked about yesterday -- and then actually on to other countries as well.

28. Video: Impact of Nicaraguan Dengue Project (44:15)

So, what I wanted to do was have you hear in the words of the Nicaraguans, just, you know, what this has meant to them.

[DR. NARVAEZ:] The important thing about this study is that we have the ability to know if the patient has dengue or not within 24 to 36 hours after coming in. We take an acute sample, it's sent to the lab, and in a short time we have the result, which helps us as a clinic to see which patient really has dengue and which patient doesn't have dengue.

[DR. PEREZ:] This is really a project that has supported the hospital with training, technical resources, with organization, patient care. We've also had the opportunity to generate publications and bring what Nicaragua does to the forefront.

[DR. BALMASEDA:] I think you could say we were pioneers in applying molecular techniques to a dengue epidemic. The laboratory went from being a virtually unknown laboratory to an institute of excellence in the region.

[MS. MORENO:] I'd say the main benefit I received through this organization was to earn a Master's in epidemiology. The experience and all the knowledge I acquired in this Master's degree will help us to monitor the various vectors we have here in Nicaragua.

[DR. GONZALEZ:] The change that we've had due to the effort of this technology transfer that Dr. Harris has done at the center has been amazing. We're now applying them to food monitoring, we're going to apply them to medicines, we're working on medical entomology, as well as molecular biology techniques. All of this has been possible due to this tenacious effort, this dedication that Dr. Harris has. The enthusiasm she does it with, the honesty she does it with, with the love for our people so that we can really bring health to the people of Nicaragua.

29. Large cohort study reveals impact of influenza in Nicaragua (46:34)

[DR. HARRIS:] So, this is what we've been able to do in the area of dengue for instance, and as you can see it's moving out from dengue into a lot of other areas, wherever the Nicaraguans feel that that should move. And so, what I want to show you, this may look familiar to you because what this is is that same design of the cohort study. Remember, we talked about that yesterday? So, this is the design of the dengue cohort study, but then we noticed something. We had 80,000 medical consults for these children and we had 350 cases of dengue. So, something else is going on out there, right? And so, we thought wow, we've got all this clinical data and all these samples. We can actually look at other diseases as

well. So, for a number of reasons we started looking at influenza, and we found that if you look at the Ministry of Health, there's kind of nothing really happens across the year with influenza, but when we look at our own data we could start seeing these seasonal peaks. Because the data is squeaky clean and we have all of this information about the children, and because of that we can see things that you otherwise can't see. And so, we said well, we think this is influenza. Now we better start looking for influenza. So, in 2007 we laid on top of the dengue study, we just started testing for influenza virus, and what we found was really striking. One is that when we actually were able to calculate the burden of laboratory confirmed influenza, it was the same in Nicaragua as it is in the United States, around 21 cases per what's called 100 person years. And this is considered a huge problem in the United States, and yet it was not considered a problem at all in Nicaragua simply because it wasn't known. And the other thing we saw which was very striking is that this does not look like a flat line, right? You definitely see a seasonal peak, and in fact every June 1st we get our first case of influenza. And so, you see this huge seasonality, and this is important because it's right over...June is right over where the Southern Hemisphere influenza peak is. And sometimes we see a peak in December and that is right under where the Northern Hemisphere influenza peak is. So, if you're going to do global pandemic modeling and you forget about the tropics in the middle, you think that's kind of a problem, right? And so, nobody was thinking about this because nobody had this information. So, we went from a place where vaccines were unnecessary because there was no influenza, we didn't know what the disease burden was, what the seasonal variation was, there was no coordination with global pandemic planning. Now they're actually starting to get vaccines because influenza is in fact a problem. We know the burden of disease, we know that there's seasonality, we are starting to look at risk factors, and what was really important is that we were able to set them up for that influenza, the pandemic influenza scare. As we know, it was identified in April of 2009. By May we had already set up the lab to be able to do the RT PCR and the real time RT PCR to capture that. And in fact, the first case that was captured in the country of Nicaragua was in our cohort. And so, we're able to really work with them on this, and now we just got funded to do a five year study to continue this work to really understand how is it that influenza is transmitted within a household in Nicaragua. What does this look like over the long term? Let's sequence all of these viruses to see how they relate to the different geographical places where influenza is going on. And then we've been collaborating with Joe and Nathan, his graduate student or our graduate student who's actually looked at our negative samples, so influenza virus negative samples to look at first known viral agents that Joe will show you later, and then unknown viral agents. And that's the story that Joe will finish with today.

30. Improving clinical processes with technological advancements (50:03)

So, the last story I'm going to tell you to finish up this part is that it's not just the biomedical sciences. What turns out happening is that our Nicaraguan team wanted to use a number of different informatics tools to be able to track these 3,800 children over time. And so, they started using PDAs and barcodes and fingerprint scans and all kinds of interesting things including geographic information systems. It's a way of just using satellite to identify a place, and this is actually really important in Nicaragua because there are no addresses in fact. And so, it's actually a lot easier if you have a GPS device to find the house. So, that was very useful in our study. So, the other thing that was very useful was that this is the cataloging system. So, we have 4,000 children coming in and we have to have this squeaky clean data, you know, that's going to be this international studies and trial, and the way they had it organized was by persons' last name, but in many Latin American countries, there's many last names. So, Lorenzo de Vallecido de...And so, then they're like, well, where are we looking? So, that wasn't good. So, then they said well, we're going to do it by birthdate. That's another option except that the mother can probably get it exactly right, but if the grandmother comes in, they'll probably get the week or the month right, but if it's the father bringing in the kid, they'll probably be like this year, that year, you know. So, it ends up being that these kids had like six different medical records, and we were like, you know, no, no, no. This is really not going to work. So, it took six months of negotiations, but we finally got a method where

they just come in and do a fingerprint scan or a barcode scan on their ID card and bam, it brings up their fingerprint or their record using the barcode, and then boom, you just go right to the number. So, of course being the scientist I had to measure everything. And so, I wanted to know exactly how good is this. So, we measured, you know, on 593 records, how long does it take, and it turns out that using this system it takes five seconds instead of 173 seconds to find your medical record. So, this is actually very useful.

31. Taking the fight against the diseases to the front lines (51:53)

But then what was really cool though, is that, you know, we were using this for our study, but since we work really closely with the Ministry of Health, they were like, you know, how can we apply all of this technology? And just the ability to collect information and know it in real time through computerized programs and data entry, how can we use this to other big public health problems? So, now we've expanded. We have a lot of projects in prenatal care, in vaccination efficiency of the children, and these are spanning from the field health workers into health centers, at the state level, and all the way up into the Central Ministry, so that you can actually really make an impact with these technologies, and in fact now our team which used to be like one or two informatics people, we have now over 15 people in our informatics team in Nicaragua. And the coolest part of this is they started off, a lot of them as data entry people and then they became programmers. So, now they're really moving up and they're being their own programmers, their own system designers. And so, all of these areas, electronic health care records are actually more advanced than the United States. It's really impressive. And the laboratory information management systems, everything has become electronically controlled. So, the quality has gone way up, but the point of all of this is to show you, and I'm hoping that I've been able to give you an example of how in the biomedical sciences or using information technologies in health, or if we remember back to yesterday with the community-based projects, how the idea is simply to work with people and develop local talent because, you know, we started out yesterday and my first sentence was about these big inequalities we have in the world in wealth, in opportunity, and access to science, but you know what's not lacking? Intelligence. People got the smarts everywhere and the only thing you need to do is partner with them respectfully and bring the tools and the knowledge and the resources, and then magic happens. And I can just say it's been really a privilege and a honor for me to have been along and to continue to be along for the ride.

32. Q&A: Where does your motivation come from? (53:48)

So, I'm going to end with that and take questions. Yes, there was someone in the back?

[STUDENT:] You said that something rushes you back to Nicaragua and you've done all this amazing work. What's your motivation for all of this and what keeps you going?

[DR. HARRIS:] To change the world. I mean I want to use science to make this a better place and I just feel really privileged with my educational opportunities thus far and I want to share it. And I happen to go to Nicaragua, but I mean I've been to many countries and, you know, pretty much have fallen in love. The people are phenomenal everywhere, and people working together to solve health problems and further education in their own countries, I mean that's a really amazing bunch of people to work with and it's truly inspirational, and that's what kind of keeps me going through the trials and tribulations of science.

33. Q&A: How long does manual PCR take? (54:34)

Yes?

[STUDENT:] You said that you had to like make alternative machines to work in the labs. How long does that take and how long do the experiments take when compared to the places with actual machines?

[DR. HARRIS:] Right. So, you start...I mean the idea is you can do manual cycling and it's two minutes, one minute, and so after a while it's a little tedious, even if you put on the salsa and you take turns. But nonetheless, what we did was we used that in Nicaragua for instance, we used that to get enough data that we could then get a grant application together, and then with that we were able to buy a thermocycler. But then we also use it, though, when we have workshops in other countries to first introduce the technology because it's really important that people understand physically what the biophysical and biochemical processes are behind a technology and behind a technique because then they can troubleshoot it themselves. So, the idea though is to...when you can't afford other equipment or...you find a way to innovate. And then if it's going to work better in the long run to eventually buy an expensive piece of equipment, then hopefully you can actually get the grant using your data that you got in your own innovative fashion. But it also goes backwards because now Joe is busy using that 3D printer I've just found to recreate all of the centrifuges and all of the gel combs and everything, you know, in his own lab for eight cents. And so, we're actually going back down the other side.

34. Can dengue virus remain latent in the body? (56:01)

One more question. Yes?

[STUDENT:] When the virus enters the cell and like it replicates and then the person undergoes treatment, can that virus remain latent in the cell and continue to reoccur through the person's life?

[DR. HARRIS:] Not with dengue. Dengue is an acute disease and it's actually cleared from the system within about five to seven days. Yeah, from onset of symptoms. So, it's a little bit longer once you have the infection, but from the mosquito will be about two to seven days, and then once you get the onset of fever, the virus will be cleared within about five days. There's some others. For instance West Nile, there are some reports of persistence, but dengue, it's an acute illness.

35. Closing remarks by HHMI Program Director Dr. Dennis Liu (56:54)

[DR. LIU:] Thanks, Eva, for another fantastic lecture. It's really inspiring to see, you know, the very best science and then this effort to try and get that same science going in a place like Nicaragua. For the next lecture I hope that you all join us again. Joe DeRisi is going to put on his virus hunter's hat and tell us three fascinating stories of viral discovery, and he's going to try and open up a window on the near future of biotechnology.