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**XVII International AIDS Conference
New Insights into HIV Transmission and Pathogenesis
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GUIDO SILVESTRI, M.D.: So the first speaker of this symposium is Dr. Eric Hunter, my co-chair. Eric is a professor of pathology and laboratory medicine at the Emory University School of Medicine. Eric is also co-director of the Emory Center for AIDS Research, CFAR. And he is a Georgia Research Alliance eminent scholar.

Eric Hunter is also known as the cofounder of the University Of Alabama Birmingham Center For Aids Research, which he directed until 2004. Eric is an internationally recognized scholar in the fields of HIV, pathogenesis and transmission. And his most recent contribution to the field of aids pathogenesis and transmission has been the characterization of the molecular determinants of transmission in a large cohort of discordant couples coming from Zambia and Rwanda. So, Eric, welcome.

ERIC HUNTER, PH.D.: Okay, thank you Guido. It is our pleasure to be here, and it is great to see a good turnout for a track A presentation. It is been a bit of a struggle, I think, at these meetings.

So what I would like to do today is give you an update on some of the work that we have been doing on HIV heterosexual transmission in both Rwanda and Zambia. We started out, really, with a hypothesis that an effective prophylactic vaccine against HIV has to protect against those viruses that initiate infection of the mucosal surface, since heterosexual

transmission is the predominant mode of transmission of this virus. And that these viruses might be distinct from the bulk of variants that have evolved to survive the onslaught of the immune response of the host during their growth in that chronically infected individual.

So what is the nature of these viruses and where do they come from? As I mentioned, we are looking at heterosexual HIV transmission, and in order to be able to look at a significant number of transmission events we have been working in two HIV discordant couple cohorts.

HIV discordant couples— that is not a couple that cannot get on with one another, but rather one in which HIV is infecting one of the couple and the other is uninfected. They actually represent a significant fraction of the more than 50,000 couples tested in the capital cities of Zambia and Rwanda. In Lusaka [misspelled?], about one in five couples coming in, actually, one partner is HIV positive, the other one is HIV negative. And it is around 12-percent in Rwanda.

So these individuals, these couples represent an extremely highly vulnerable population for HIV infection. And in fact a recent paper in Lancet, Christie Dunkle and colleagues estimated that about 80-percent of transmissions in cohabiting couples could be prevented if couples voluntary counseling and testing were applied to the entire cohabiting population. And she's actually going to be speaking about this tomorrow at 11:15 in the second concordant session.

And despite counseling and condom provision, we continue to see low levels of transmission, around 7-percent in Zambia, about 3-percent in Rwanda. And that is relative to about 20-percent in uncounseled couples. And what we do know from analyzing those transmissions virologically is that about 80 to 85-percent of the transmissions are epidemiologically linked. That is, transmission is occurring within the couple.

That allows us to compare the viruses in the donor, as we call the chronically infected partner, and the recipient, the newly infected partner. Some preliminary work on this from a couple of years ago suggested that there was a severe genetic bottleneck that occurred during the transmission process. And that from this very diverse population in the chronically infected partner, in general, a single genetic variant initiated the transmission process, initiated infection in the uninfected partner, and that for a significant period of time that population was relatively homogeneous before it started, before there was a reemergence of viral diversity.

We wanted to look at that in more detail, and today I am going to report on the analysis of 21 new transmission pairs from Zambia and Rwanda, where we have utilized a p-24 ALISA 5:28 assay to identify newly infected individuals who are antigen positive and antibody negative, although in many instances by the time we get those couples back in, they have sero-converted. But we are looking in the first two to three weeks of infection.

And to avoid re-sampling our in vitro recombination, we have used what used to be called, I guess, end point dilution PCR, it is now called single genome amplification, of the envelope genes from both the transmitting partner and the newly infected partner. And we have looked and sequenced 40 individual amplicons, each amplicon being derived from a single virus genome. So that is what's giving us a quantitative look at the different variants that are present in the chronically infected partner and the newly infected individual.

And this really shows a classic example of what we see. In green you can see a phylogenetic tree. The horizontal distance between two viruses, viral genomes— let us say this one here and this one here— requires that we go all the way back to the common node and then all the way back out again. And you can see that in this case this is one-percent variation. And so some of these viruses are differing by as much as 8-percent.

So this is a broad, a very divergent chronically infected population, for the most part you can see that the plasma derived viruses and PBMC derived viruses commingle. And as you might expect, it is diverse.

In contrast, the newly infecting virus population is very homogeneous. The horizontal distances here are nonexistent in some cases, and in other cases represent less than 0.2-percent. More importantly, all of these viruses are derived from a single branch of the donor phylogenetic tree.

And that says that a single genetic variant has established infection in this previously uninfected person.

So this shows, in 18 out of 19 Zambian subtype C transmission pairs, we saw this extreme genetic bottleneck. And this summarizes 12 of those, right here. It is a little hard for you to see, I am sure, but again you can see in most cases, in each case, a very diverse chronically infected virus population, and then a single branch off the donor tree with a very homogenous population in the recipient.

In the one case that we did see additional transmission variants, you can see in this case there are actually four branches off this tree that the recipient is found in. And the diversity here probably represents, in fact does represent, recombination between the different variants that are present in that person that is infected with multiple variants.

Similarly in our Rwandan— could this be just subtype C? Is this homogeneity a subtype C anomaly? That is definitely not the case. Because here we see from 9 subtype A transmission pairs, 8 of these are from Rwanda, one from Zambia. Again, we see exactly the same picture. Chronically infected virus population that is very diverse. Single branch off the donor tree, very homogenous population.

And again here we see one instance where there are two branches off the donor tree and these viruses, although very closely related, differ by five amino acids that clearly differentiate them as independent infections.

So we can now use a tool developed by the Los Alamos national lab that is called a highlighter tool. And the highlighter tool lets us look at every nucleotide in a consensus population. And compare that to every nucleotide in each of the other isolates.

And this is an example from a Rwandan transmission pair. And actually it is hard to see, but what I will show you here is that 67-percent of these envelope genes are identical at this point in time in this acutely infected individual. Of the remaining envelope genes that we have sequenced here, 11 have a single base change and 2 have two base changes. So it is a remarkably homogenous population at this early point in time.

And if we take into account the known mutation rate for HIV, than what we know is that we would predict 76-percent would be identical at 13 days, 58 at 26 days, and so that is pretty consistent with this being a very early time point. We'd actually calculated it was about 25 days post infection.

So at this point in time, the virus is replicating very rapidly from a single founder virus and the only change we see is through random mutations imposed by the reverse transcriptase.

More recently we have now been doing single genome amplification of complete genomes, or near complete genomes—missing about five-percent of the viral genome. And we see exactly the same thing looking across the entire genome of HIV.

So each of these dots represents 9,000 base pairs of sequence, and you can see again at the very earliest time point, about a month after infection, the virus is extremely similar.

A month later, they are starting to diverge but they are very, very similar again. And it is not until six months after infection that we now start to see a more diverse population. And at this point, the bulk of this diversity, as you will see in a moment, is in the envelope gene. And this is the chronically infected partner over here.

So here you can see again, in this particular instance 3 out of 12 of the newly infecting virus genomes we have amplified are identical over 9000 bases. The majority of the others have a single base change. There are two base changes in this one and three here.

But we do see an interesting thing, here, and that is multiple point mutations within a region that is consistent with a CTL epitope in VPU. And this is what happens when CTL CTL escape occurs very early, within about three weeks of infection. And it imposes a selection on the virus population. And so we're seeing that here.

The limited additional changes at six months are concentrated around the envelope gene, and at that point we believe we're getting neutralizing antibody escape of virus, and then diversification of the population.

So in 26 out of 28 recipient quasi species, we have seen a single virus originate, a single branch of the

chronically infected donor phylogenetic tree arguing for initiation by a single virus. The recipient viruses exhibit very limited heterogeneity in both UMV and in GAG, and in fact I've shown you now across the whole genome. And in acutely infected recipients, the homogenous virus population is likely to represent the infecting virus genotype.

And preliminary analyses of genital fluids do not support the hypothesis that the genetic bottleneck is a result of very limited viral genetic diversity. So where does that bottleneck originate?

Our first question was, could it be through limited heterogeneity in the genital fluids? And that is unlikely. We do see compartmentalization of virus in the genital fluid, but these enriched populations do not appear to be the source of the transmitted virus and Debbie Barus [misspelled?] is going to speak this afternoon in the oral abstract session and provide more details on that.

Could it be occurring in the recipient? And we think either at the mucosal surface or at selection during virus outgrowth? And so we have looked at that by asking, does a single virus always or for the most part predominate? And if it doesn't, is there something other than just a lack of selection during outgrowth that is responsible for that?

We are a little bit short on time, so I will just say that in other studies, particularly in sex workers and STD clinics, the-percentage of individuals being infected by

multiple variants is much higher than what we have seen in our cohort of 10-percent.

And so we have asked, is this linked transmission in discordant couples inherently different? Could it be due to a low frequency of stds or a single partner? And we have asked what happens if we examine heterogeneity in the unlinked transmission pairs, that is, where the uninfected partner has gone outside the marriage to become infected, that 15-percent I mentioned earlier. And there we have seen quite a different result.

You can see here that in 3 out of 7 unlinked transmission cases we see very heterogeneous virus at an early point after infection, whereas in 4 cases we see the classic, very homogenous population. And if we look, again using the highlighter tool at this particular individual, you can see that there are really two populations, there. This one looks very much like I described earlier, with a number of identical envelope sequences and then random mutations, and again, a second homogeneous population that again has a few point mutations.

So why do we see that greater frequency of heterogeneity in these unlinked transmission pairs? And we went to the clinical records for these individuals and asked, is there anything that is linked to that? And what we did find was that these were, in each of the three cases, females. They

all had vaginal discharge and they all had reported lower abdominal pain at the time transmission was detected.

So it would seem that an inflammatory genital infection might be associated with this. We have gone to a much broader database now where we have looked at a total of 42 transmission pairs in both our Rwanda and Zambia cohorts and combined those. And again, now we are looking at five individuals who have a heterogeneous population being transmitted, multiple variants being transmitted. And we see again a statistical association where again a genital inflammation or ulceration was associated with the time the transmission was identified.

So to conclude, the presence of multiple genetic variants in newly infected recipients indicates that the extreme genetic bottleneck we have observed in the bulk of linked transmissions most probably occurs at the genital mucosa rather than during virus outgrowth. This is not a single virus always winning because of a fitness. And the extremity of the genetic bottleneck appears to be modulated by genital infections that could provide an inflammatory environment to increase the number of target cells or breaks in the genital mucosa allowing access to underlying target cells.

So it seems likely that when the mucosal surface is intact, a single genetic variant establishes infection following transmission, whereas in the presence of genital infections or other situations that increase accessibility to target cells, multiple variants can be transmitted.

And I say can, because in 85-percent of transmissions in discordant couples, they are linked, and they primarily occur in the absence of stds. And there it is clear that there's no requirement for a disruption of the mucosal barrier for transmission to occur.

A study like this requires a lot of collaboration. The people in my lab that have been involved in the analysis, Paulina Hawkins-Rich, Colleen Kraft and Ling Hiu [misspelled?], Debbie Mercer [misspelled?] I mentioned will be talking this afternoon. We collaborate all the time with Cindy Gordeen's [misspelled?] lab, and also with George Shaw and Beatrice Hahn.

The Asar [misspelled?] cohorts, the Rwanda Zambia HIV research group cohorts, are headed by Susan Allen. It would be impossible to do this work without her. Our field site director is Joseph Malinger, Lawrence Eldonchomber [misspelled?] Olivia Manigar [misspelled?], Etienne Carita [misspelled?], Tatiiaz Akenoor [misspelled?] and are crucial to our project. And we have also had collaborations with Betty Corbin.

Our funding is from the NIH primarily, as well as from the Gates Foundation, and I must say that we have had great support from IRV for support of the cohorts. And they are obviously supported by multiple donors. So I am going to stop there and thank you very much.

GUIDO SILVESTRI, M.D.: Thank you very much, Eric. I believe we have time for a few questions.

MALE SPEAKER: This issue of the bottleneck being a matter of the mucosa rather than fitness— have you tried to sample that mucosa? I mean, I know it is tough, but have you— tried to [inaudible] acute phage in the broken legion of the gastro-intestinal tract? So, could you begin an exploration for diversity there that is not spilling out?

ERIC HUNTER, PH.D.: You know, it is just a problem of human studies. I mean, we do not have the capacity to do those samplings at the present time.

What we have done as a sort of surrogate for that is to sample genital secretions at the time infection is detected in the newly infected individual, to ask can we find a more diverse population being produced by the genital tissue at that time. And it is preliminary data. But at the present time with fairly significant sampling of about 40 genomes per individual, we have not seen anything other than the virus that we find in the blood in the genital fluids.

SASHA HARIA: Sasha Haria [misspelled?] from NIH in the United States. Would you elaborate on your idea of CTL escape determining homogeneity of the early transmission?

ERIC HUNTER, PH.D.: In the majority of cases, we do not see the— statistically, you would not anticipate finding the same nucleotide being mutated at this very early time point. You would anticipate that mutations would be randomly distributed across the genome. And in most cases, that is exactly what we see.

But in a few cases we do find a single mutation selected multiple times. But in particular with the CT isotopes, we will find multiple mutations within a particular nimer [misspelled?] being selected for in these very early times. And we interpret that as being a very early CTL escape mutation.

SASHA HARIA: So the idea is that the CTL comes off very fast?

ERIC HUNTER, PH.D.: Very fast. And George Shaw, I should point out George Shaw and Brendan Keel have gotten very similar data to this, although they do not have the donating partner, as it were. And I've seen very similar evidence for a single virus initiating infection as well as for evidence of very early CTL escape in a small number of their subjects.

RAY CHARLES: Ray Charles, Emory University. I just wanted to know if you have actually cloned some of these viruses in details, how they found.

ERIC HUNTER, PH.D.: Yes, our first hypothesis, because these viruses tend to have shorter loops, variable loops, the ones that initiate infection, our first hypothesis was that these viruses may not require as high levels of CD4 or CCR5. what I can tell you is that both the viruses in the donor and the recipient of these clague [misspelled?] C individuals have a very high requirement for CCR5 and a very high requirement for CD4.

So they are not viruses that have been able to grow out, because they can utilize low levels of receptor or co-receptor. We are still in the process of characterizing their ability to be transinfective adendritic cells. But that is certainly a high priority right now.

GUIDO SILVESTRI, M.D.: If there are no more questions, we can move on to our next speaker. Thank you, Eric, again. The next speaker of this session is Dr. Victor Garcia Martinez.

Victor is a professor of medicine at the University of Texas Southwestern Medical Center in Dallas in the United States. He has been working in the field of HIV/AIDS pathogenesis for over 20 years.

And most recently his laboratory has achieved international recognition for developing a new mouse model for HIV infection, the so-called "humanized" bone marrow liver mouse, that is being successfully used to reproduce some key aspects of HIV transmission and pathogenics. Welcome, Victor.

VICTOR GARCIA MARTINEZ, PH.D.: Thank you very much.

ERIC HUNTER, M.D.: We have a slight glitch with the-

VICTOR GARCIA MARTINEZ, PH.D.: It is all right.

[Spanish spoken]. It is a pleasure to be here and I cherish the opportunity to be here and if you speak English you probably already know about the Dallas Cowboys.

So one of the things that Eric said is that studying HIV infection in humans sometimes can be very difficult. And in order to facilitate the evaluation of transmission events,

pathogenesis, etc., we investigated the possibility of using humanized mice for this purpose. And the simplest and most expeditious way to humanize a mouse is to simply introduce human stem cells into the mouse after preconditioning.

That is all you have to do. Take some C-34 positive cells, inject them into a mouse and he will become humanized. However, the cells will derive in the mouse thymus. And that probably would not be optimal if you are looking to study HIV and AIDS.

So we took advantage of a model that had been developed almost 20 years ago, in which a piece of human thymic tissue was implanted into the kidney capsule of a mouse and then this created a human thymus where infections could occur. So one of my grad students, Michael Marcus [misspelled?] was able to transplant these animals with autologous human, human torpedic [misspelled?] cells.

And what that did is actually created a systemic reconstitution of the mice, but now with T cells coming out of the human thymus. And we call these animals BLT, for bone marrow liver thymus, and that is what I am going to refer to from now on.

So if you have never seen a humanized mouse, the first thing that would strike you is the peripheral blood of the animals. So in essence, if you look at the panels on the top of this slide, that is blood from a humanized mouse. And at

the bottom, this is blood from as normal of a human as you can find in your laboratory.

And if you compare panel to panel what you see is there is virtually a one to one correlation between the cells that are found in a human and the cells that are found in a humanized mouse. So that gives the impetus to begin to address some very important questions regarding the reliability and the robustness of the system.

And so going from peripheral blood, we started looking at virtually every single tissue in the animal. And this is the summary of the panels of animals where we looked for the presence of human cells. And what you can see is that virtually every tissue contains human lymphoid cells.

So in essence now do you not only have a thymus but you have a lung, a mucosal surface, etc. , all composed of human cells, that are relevant to study HIV.

If you live in Dallas, and you make humanized mice—this is exactly how these mice look. [Laughter] So, if you lived somewhere else in the world, they might look different. But at least in Dallas, that is how our mice look like. And they move very, very fast.

So in order to prove that this system is actually performing the same way that a human would, we perform a very simple test. And we injected the animals with a very powerful stimulus. In this case, something called toxic shock syndrome, Toxin 1. And what it does, it actually activates a subset of

human T-cells very, very specifically, and it makes them proliferate in the absence of proliferation of virtually every other cell from the body.

And what this slide shows is that indeed, administration of the toxic shock syndrome Toxin 1 results in a very furious expansion of these sub cell T cells, indicating that both the dendritic cells, the myeloid cells, the B cells and the T cells are perfectly functioning in this response.

But what is characteristic of when a patient shows up in a hospital with toxic shock is a cascade of inflammatory cytokines, all of human origin. So we asked the question, is this resulting in the same type of event? And yes, indeed, there is a time dependent production of human cytokines that is literally 1:1 with what you would see in humans, both in magnitude and in breadth. So, again, the immune systems of these mice seem to be working appropriately by these two tests.

And then I guess the third and final test that we did is to ask are these cells capable of mounting a similar response that would prevent pathogenesis from occurring? And then in order to do that, we did something that is very close to the clinic, which means is we infected the animals with Epstein Barr virus, that in the absence of human cells would create very large tumors in these mice, and asked if there was any humanoid response.

And the way we measured that is we measured the cytokines present in the different organs of the challenged

animals. And actually, if you look at this slide, on the right hand side, there are some yellowish pinkish spots. That is actually an immunoresponse to the virus that infected the animals. So this is a specific T-cell response. And it is not only a specific but it is also restricted to class 2 and class 2, suggesting that the type of immunoresponses that you observe in these animals closely map what you would see in humans.

Well, with this is in hand, we started to ask some questions about pathogenesis. And we actually took advantage of a classic experiment done in the laboratory by Ron Desrosiers to evaluate the role of NEF in HIV infection.

And what they did is they infected monkeys with viruses that had or did not have this important auxiliary gene of HIV. And to make a long story short, what they saw is that the animals were infected with viruses with NEF or were capable of fixing the gene to become functional were unable to survive and they died. But if you took the NEF gene down those survived, and they were literally just vaccinated by the virus.

So we repeated this experiment, in this case we used the humanized mice, and challenged them with wild type virus and with a virus that did not have functional NEF.

And what we saw is very interesting. This highly pathogenic virus is capable of depleting the CD4 positive cells present in the animal. So you can see, it is a very dramatic drop in cells in the course of two weeks. And there is no

recovery. Once cells are gone, they are gone. They are not going to come back.

However, when we look at the animals that were infected by the virus that did not have NEF, as you can see there is a much longer survival of the CD4 positive cells. So the animals were resistant to the infection for weeks. But then suddenly, after a while, the CD4 cells disappear, and pathogenesis has begun.

So we wanted to know what was happening here, and we looked at that specific point right before the CD positive cells dropped. This is the sequence of the original virus, the pathogenic virus on the top. And at the bottom is the sequence of the virus that was missing NEF. Okay?

So what we were able to get out of the animals had the following sequence: so in essence, in vivo, the virus was able to repair this open ended frame suggesting that there was a very strong selective pressure for the presence of a functional NEF gene correlating very well with the previous forms that had been done in non-human primates.

So based on these results, we started looking at another aspect which has to do with pathogenesis and that mainly is the fact that in humans, the gut is a primordial site of virus replication and depletion of human CD4 positive T-cells. So it was important for us to characterize the gut and to ask, can the humanized mice serve as a surrogate system to evaluate this type of pathogenesis?

And so in this slide I have a summary of why the gut is important. In essence, it is a very important site where many CD4 positives are present, and there are memory cells that are present in the gut that are particularly susceptible to depletion after infection. More than half of all the cells in the body are there, and importantly for therapeutic development, there is a discordancy between the depletion of the cells in the gut and the recovery of the cells of the heart in HIV infected patients.

So whereas you see significant increases in the numbers of CD4 positive cells in the periphery, you do not seem to see that in the gut. And we need opportunities to be able to intervene and try to see if we can remedy that.

So this is a slide that was given to me by one of my grad students Paul Denton that describes the tissue. In essence, it is a little bit complicated. You have lymphocytes that that are in the outside of the gut. You have lymphocytes that are inside the gut. And then all these lymphocytes seem to go and wash out in the mesenteric lymph node.

So what he did is he went in and took the gut of a mouse and cut it into small intestine, large intestine, and took the lymph nodes that were next to the gut, and asked the question, are there human cells there? And do they look like the cells present in the human?

This is actually a very busy slide, but nevertheless, it represents a summary of an amazing amount of work by this

grad student. And what it shows is that every part of the mucosal surface in the gut of the humanized mouse closely recapitulates the same cells and populations and phenotypes that you have in a human. Namely, you have human T cells, you have human P cells, monocytes, macrophages. And the predominant CD4 positive cells in certain parts are present. In other parts are not.

And most of the cells are memory cells. In essence, the cells have seen antigen. They are no longer naive. And we know that this is actually not a mistake, because if you look at the bottom of the slide, where the detailed analysis is for the mesentery lymph nodes. What you can see is that there are plenty of naïve cells in the lymph node, whereas there are none in the rest of the gut.

So based on these results, we started to ask questions about the transmissibility of viruses by the mucosal surfaces into these animals. And this is what Pauline Syn [misspelled?] did, was to challenge humanized mice with HIV intra-rectally. And after single exposure of virus to these animals, what you can see again is that after infection, there is a peak of viremia that coincides or precedes the depletion of CD4 positive cells in the peripheral blood.

And in this particular virus, it is an r5 tropic virus, which are the vast majority of viruses transmitted in humans, and what you can appreciate is that there is a stabilization of

the levels of the cells in the periphery. In essence, the c4 cells are maintained, there's a set point.

And the same thing happens with the virus. The virus is controlled and the levels are certainly maintained. This is one type of virus.

We also looked at another type of virus, namely, a virus that uses a different co-receptor, X4. They are known to be much more pathogenic in humans. And what happens is very different. This is a control mouse, and you can see has very stable levels of CD-4 positive cells in the periphery. But after infection, after a peak of viremia, again all of the cells, all of the CD-4 cells disappear from the peripheral blood.

And together with the presence of virus, you can actually also observe the presence of antibodies against specific proteins of HIV. So in essence it shows you the T-cell response of EBB, now I am showing you a human response to HIV, and the depletion of the cells, which is highly pathogenic virus in the periphery.

But what happens in the gut? Well, what happens in the gut actually stays in the gut. In essence, virtually all the CD4 cells present in the gut are gone. They are disappeared; they are destroyed. And as you can see, there is an abundance of CD8 cells in the gut after infection, meaning that this is not a nonspecific effect.

And also if you then look at what type of cells are where, what you can see is in the left hand side is the control animal which was not exposed to HIV. And on the right hand side, you actually looking at an infected animal. And every brown dot on the first panel on the right is a CD8 positive cell.

So there's an influx of CD8 cells to the tissue, and then there are the appearance of markers that indicate that these cells are not only activated but are probably mounting a robust immune response against the virus in this specific tissue.

Now, the other advantage of these animals also is the fact that you can not only infect them rectally but you can also infect them vaginally. And that allows us to evaluate things like the pathogenic potential of viruses in therapeutic interventions.

And the point I would like to make with these particular slide is that if you look at the panels on the top—that is the pathogenic virus—that shows a depletion of the CD-4 positive cells. But the panels on the right, for both inter-rectal and inter-vaginal infection, shows you that the CD cells are not completely lost, indicating the less pathogenic potential of these viruses throughout.

And like before, what we saw with the highly pathogenic viruses, depletion throughout the tissues, is not the same with the R-5 virus. So in essence what you see here is that the CD4

cells are maintained. And this is focusing now on the virus and not on the host.

This is extremely reproducible, something that you can see with a multitude of donors. So in essence the ability to deplete the cells is an intrinsic opportunity for the virus to affect the host, not the other way around.

So in summary, just on tissue of mice, implanted with human thymic glandular tissue and C-34 positive cells, results in very long term systemic repopulation with human topietic cells. All kinds of human topietic cells. Humanized mice develop a functional immune system and are capable of mounting a specific immuno-restricted responses.

Humanized mice are susceptible to infection by HIV, permitting the in vivo study of fundamental issues regarding pathogenesis as well as the evaluation of novel approaches aimed at the treatment and prevention of HIV disease. I would like to end by acknowledging our extremely talented group of investigators in Dallas and our colleagues in Minnesota, Jacob Estes and Ashley House, and I would also like to show you a picture of my lab. Thank you all for coming.

[Applause]

GUIDO SILVESTRI, M.D.: We have time for a few questions. Mario?

MARIO STEVENSON, PH.D.: Victor, in your animals that were infected with [inaudible] F, and compared [inaudible] CD4

counts were dropping off, but P 24 stayed low. So why were the CD4 cells being lost if it wasn't [inaudible].

VICTOR GARCIA MARTINEZ, PH.D.: Okay, in case you did not hear it, Mario's question— the question is, how do you see a drop in CD4 cells in the periphery with low levels of virus in peripheral blood? And actually, that has been the case in every experiment that we have done with this. Now if you let the experiment go longer— we sacrifice the animal at that point. But if you let it go longer, then the virus completely recovers, you see peripheral blood P24, and you can see the further depletion of the cells.

MARIO STEVENSON, PH.D.: So is it just replication on the gut, then, that you are—

VICTOR GARCIA MARTINEZ, PH.D.: Actually, in this case there is internal replication, as you can see. And then eventually you will see it in the periphery if you carry the experiment longer. Thank you.

MALE SPEAKER: Have you tried using very low doses of the challenge to see what happens?

VICTOR GARCIA MARTINEZ, PH.D.: I am sorry, but I do not understand your question.

MALE SPEAKER: Like using a specific dose of HIV, right, for infection?

VICTOR GARCIA MARTINEZ, PH.D.: Yes?

MALE SPEAKER: Have you tried, like, maybe 100 times lower or 1000 times lower amount of virus to see what happens to the response?

VICTOR GARCIA MARTINEZ, PH.D.: No, we have not.

JOEY ROD: Hi, I am Joey Rod from Chapel Hill. I wonder if you have done the same experiments that Eric Hunter or [inaudible] or George Shaw have done, and looked at the virus in those vaginal/rectal transmissions to see if you're actually getting a single variant transmission as they observed, to see whether the situation is comparable to the human transmission.

VICTOR GARCIA MARTINEZ, PH.D.: I think that is a great question, and we have not done experiments with mixed populations of viruses of yet. We hope to do them soon, and we will let you know how it goes.

GUIDO SILVESTRI, M.D.: Yes, sir?

FEMALE SPEAKER: Do you see the abnormal immune activation characteristic of HIV infection in the BLT mice?

VICTOR GARCIA MARTINEZ, PH.D.: I am sorry. Can you define it? Because—

FEMALE SPEAKER: You know, pathogenesis is associated with abnormal immune activation, the activation markers, individual levels of cytokines. And could you— do you see this in the mice? And could you get this among pathogenic models?

VICTOR GARCIA MARTINEZ, PH.D.: Okay. So if I understood the question, it is, do we see abnormal immune

responses. Actually, the responses are just what we see in [inaudible] before. But the activation levels of the cells are very different before and after infection. So in that case, yes, we do see higher levels of activation markers.

We have not measured cytokines in these animals. Samples are there; they just need to be run. Thank you.

MALE SPEAKER: I also have a question, Victor, if I may.

VICTOR GARCIA MARTINEZ, PH.D.: Sure.

MALE SPEAKER: So, part of the pathogenesis of AIDS is the repopulation of several components of the immune system in particular tissues like the gut that you mentioned. And presumably some of this repopulation your system is affected by the fact that you have murine molecules dictating the homing of human T cells. And do you want to comment on that?

VICTOR GARCIA MARTINEZ, PH.D.: I am sorry but I did not understand what you meant.

MALE SPEAKER: Do you want to comment on how your model could miss some of the aspects of the repopulation of T cells that occurs since you have potentially homing molecules that are murine, trying to work on human T cells?

VICTOR GARCIA MARTINEZ, PH.D.: That is a great point. How does it differ from what you see in a human? And we actually see virtually no differences. So in essence the T cells present in the different organs, in particular for example in the gut are literally the exact same ones that you

would see in a human. So so far it is so good. So hopefully as we go and investigate this in more detail we might see some specific changes, but so far the correspondence is one to one. Thank you.

JASON HOPE: Hi, I am Jason Hope from the University of Washington. Could you talk for a minute about the donor tissues? Does it have to be from the same donor in various components of the model?

VICTOR GARCIA MARTINEZ, PH.D.: Okay, that is actually a great question. And in essence, it depends what kind of questions you are asking. In our case we want to ask questions that are relevant to a normal immune system. In an immune system the donor tissue and the stem cells have to be from the same type.

So we try as hard as we can to do the same donor for all our mice, and then these multiple donors for each experiment, so we can see the type of breadth and variety that you would see in the human population. Does that answer your question?

JASON HOPE: Yes. Thanks.

VICTOR GARCIA MARTINEZ, PH.D.: Sure.

ERIC HUNTER, M.D.: I think we need to move on to the next speaker. So, our next speaker doesn't really need much of an introduction at all. It is Dr. Mario Stevenson. And Mario is from the University of Massachusetts Medical Center, where he is a professor in the program of molecular medicine.

And he is currently the Debbie Freelund [misspelled?] Chair of AIDS research, as well as being director of the Center for AIDS Research at the University of Massachusetts Medical School. Mario's research is and has been aimed at uncovering the functions at viral accessory genes. The mechanism of viral persistence and the immunopathogenicity as well as cellular factors that influence virus-host cell interplay.

As most of you know, he has made a significant mark on understanding molecular biology of virus host interactions with HIV. It is a pleasure to have him speak today. He is going to be talking to us about host restriction in T cells and macrophages.

MARIO STEVENSON, PH.D.: Good afternoon. It is actually very difficult for an Englishman to say nice things about Scotsman. So Eric is to be congratulated for going against the grain.

If any of you have problems with my Scottish accent, Victor Garcia is going to translate for you. [Laughter]

ERIC HUNTER, M.D.: [Inaudible] stiff upper lip.

MARIO STEVENSON PH.D.: Let us not go there. So, I am going to talk to you about cellular restrictions. I am going to present evidence for a novel cellular restriction. That is manifest in macrophage and if time allows talk about some early efforts in small molecular drug discovery in the harnessing some of these restrictions as antivirals.

So apparently antiviruses commandeer cellular cofactors in order to replicate, because lenteviruses have a simple genetic makeup. So it is not surprising that they have to usurp cellular functions in order to complete certain aspects of the life cycle.

However, it is now apparent that cells contain proteins, what we call cellular restrictions, that antagonize rather than help viral replication. So I am summarizing some of these, one was identified this year by the groups of Paul Binash [misspelled?] and John Gertelli [misspelled?] is a protein called Tetherin or BST2.

And what tethering does is it anchors viral particles on the cell surface and prevents them from detaching from the surface of the infected cell, thereby truncating spreading infection. And then in an infected cell there is a cytokine deaminase called APOBEC3, and what that does is it perturbs reverse inscription by causing catastrophic cytokine deamination in viral C, D and E, thereby preventing it from going on to complete the viral life cycle.

So you would predict, on the basis of these very potent cellular restrictions that human cells should be resistant to viruses like HIV-1. However the virus has evolved counter defenses in order to protect itself from these potent cellular restrictions. So in a case of APOBEC, the virus protects itself from the cytokine deaminase by using an accessory protein called VIF or viral infectivity factor. And what VIF

appears to do is target APOBEC3 proteins for premature proteasome destruction. Ubiquitination [misspelled?] and proteasomal destruction.

And then in the case of tetherin, tetherin, although the details are being worked out, tetherin apparently is a cell-surface protein. And while so on the surface it is preventing the formation of the sessile [misspelled?] bond at the time it detaches the viral membrane from the plasma membrane, and when that happens, the viral particles, instead of being released into the extracellular fluids are being targeted to an intracellular compartment. And what VPU is doing apparently is targeting tetherin away from the plasma membrane, thereby preventing it from interfering with the detachment of infectious virus.

So what about the other accessory proteins? My lab has been interested in the Vpr/Vpx proteins for some time. And one of the themes that has emerged from research in a number of groups is that these proteins, we have evolved to promote macrophage infection. And that is illustrated on this slide.

So what I am comparing is the phenotype, the infectious phenotype of a wild type virus in SIV that lacks Vps or an SIV that lacks Vpr. And the effect of it are being compared in macrophage and in cell lines. And the upshot is that a virus lacking Vpx is not infectious for macrophage.

Now that virus maintains wild type infectivity for other cell types as well as human and monkey lymphocytes. And

that peculiarity of the phenotype of these viruses is that they are noninfectious in vivo. So if you infect monkeys with our Vpx deleted virus, that virus has a profound phenotype. It does not spread whether it is introduced indirectly or intravenously.

So one of the clues as to what these proteins might be doing in the viral life cycle is revealed by the fact that they are variant proteins. So the virus packages these proteins in to particles. Now if the virus is going to lengths to package the protein into the variant, it means that it needs that protein prior to de novo synthesis of new viral proteins in the infected cell. So it must be acting early in the viral life cycle.

So there are a couple of models we must explain what these proteins must be doing in the viral life cycle. The first is that macrophages contain a restriction. And that this restriction interferes with the infection process. Vpx and Vpr have evolved to counteract this restriction and prevent it from interfering with viral infection.

An alternative model is that macrophages have a positive factor which is inactive or inert, and that this has to be modified perhaps post-translationally by a protein such as Vpx, thereby allowing it to cooperate in promoting viral infection. So to distinguish between those two possibilities, we use a strategy that was previously used to show that the VIF

proteins of primate lentiviruses antagonize a cellular restriction.

So we used heterokaryons in order to reveal whether macrophages had a positive factor which had to be activated by Vpx or whether they contained a restriction that had to be neutralized by Vpx. So the schema for that, the rationale for that experiment is here.

Permissive cells would be cells in which VPX is dispensable for viral infection, such as cos cells, hela cells and primary lymphocytes. Nonpermissive would be primary macrophages. So by fusing these two to make heterokaryons, if Vpx is counteracting a restriction, you would predict that the hetero carriers should remain resistant to infection by a Vpx deleted virus.

And that is essentially what happened. When we mate heterokaryons— mating heterokaryons with primary macrophages is not trivial. But the way to do it is to express the mutagenic proteins of mucosal disease virus in the hela cell. And when you do that, those cells fused readily with primary macrophages. And when we challenged those heterokaryons with the wild type virus, the virus was infectious. However, when they were challenged with the deleted virus, it was still noninfectious. So the take home message from my experiment is that Vpx counteracts the restriction that is manifest specifically in macrophage.

So I do not have time to summarize the research, but what I believe is that there is a novel restriction that is expressed in macrophage and that primary antviruses have evolved accessory proteins Vpr and Vpx to counteract this restriction. We have actually evidence that the restriction is active against HIV-1, HIV-2, SIV and murine leukemia virus. In fact, we believe that some of the peculiarities of oncoretrovirus and lentivirus biologies dictated by this restriction.

In other words, oncoretroviruses normally do not infect non-dividing cells. If you neutralize this restriction, those cells become highly sensitive to murine leukemia virus infection. So we believe that the reason viruses such as MLV do not infect non-dividing cells is because they are unable to counteract this restriction that is normally counteracted by the Vpr and Vpx proteins of primate lentiviruses. We believe that these proteins counteract the restriction in the same way that VIF counteracts APOBEC.

It targets the restriction from proteasomal degradation. And to do that, we have evidence that the virus commandeers a cellular protein called DDB1. This is a protein that is activated in a DNA damage response and it uses that protein to target the restriction for proteasomal degradation.

And I guess the point I want to make is, here we have, three of the four viral accessory genes have involved to counteract cellular restrictions. So the virus seems to have

gone to a great deal of genetic effort in order to protect itself from these cellular antagonists.

So based on that one would predict that these would be good targets for drug discovery. Small molecules that interfere with the biological action of these accessory proteins would theoretically allow these cellular restrictions to once again manifest their antiviral activity against primate lentiviruses and this conflict between the virus and the host would tip the balance once again in favor of the host.

So with that in mind we developed a cell-based small molecule inhibitor screen that was designed to look for small molecule inhibitors of the accessory protein VIF. We chose VIF because there is a lot of biochemistry on it and we know a lot about how the virus uses Vif to antagonize the antiviral activity of APOBEC. And it is a simple cell-based assay.

Basically APOBEC3 is fused to a fluorescent protein. Now normally in cells when expressed in cells, that protein is stable. If one coexpresses VIF, VIF then targets the fusion protein for proteasomal destruction and you lose the fluorescent signal in your cells. So basically you screen those cells for small molecules that stabilize the fluorescence.

So we did this with our diversity 30,000 compound library. We turned up a number of hits which were screened for nonspecific fluorescence and for nonspecific inhibition of proteasomal function. And the ones that made it through the

secondary screen were then examined for antiviral assays. And we were looking for compounds that had a very specific antiviral activity.

Now VIF is only required for viral replication in cells that actually express APOBEC3 proteins. That would be primate lymphocytes, primary macrophages, and some cell lines. However in some cell lines the virus replicates very efficiently without VIF because those cell lines, such as MT4 and CESS do not actually have APOBEC3 proteins. They have chromosomal deletions that remove the APOBEC3 gene locus.

So what- if we have a specific Vif antagonist, we would be looking for small molecules that specifically inhibit viral replication only in cells that contain APOBEC. Only in cells in which VIF is required for viral replication.

So one of the compounds that came out of this screen, which recall RN18, the antiviral activity of that compound shown here. So on the right hand panels, this is the replication profiles of viruses which are wild type or which are deleted with regards to VIF. So in what we call nonpermissive cells, cells which express APOBEC, the wild type virus replicates fine, but the virus lacking VIF is defect. Because that virus can not antagonize the APOBEC3 protein expressed in that cell.

However, in cells that we call permissive, that do not contain APOBEC3 proteins, you do not need VIF. The wild type and the delta Vif viruses replicate indistinguishably. And if

we now look at the antiviral activity of this small molecule, in nonpermissive cells, we see a boost in inhibition over spreading viral replication. However, in permissive cells in which Vif is not required for replication, there is no antiviral effect of that compound arguing that this small molecule appears to be specifically antagonizing Vif.

Now one of the characteristic features of APOBEC antagonism is that it leaves a characteristic signature in viral C, D and E. APOBEC is a cytosine deaminase that acts during reverse transcription to de-aminates viral C, D and E. And as the result of that, in the nascent C, D and E's are synthesized in the target cell, there is extensive G to A hyper-mutation. So that is a characteristic feature of the antiviral activity of APOBEC3 proteins.

So to prove to ourselves that what we were looking at was a specific Vif antagonist, we examined whether this small molecule inhibitor would be able to increase the extent of everything in viral C, D and E. And that is what is shown in this slide.

So in the presence of wild type virus, in the absence of drug and permissive cells there is no editing. If you delete Vif and look at nonpermissive cells, you see extensive G to A hyper-mutation. If you now take that same virus and now make it in the presence of that small virus antagonist, you see an indistinguishable level of cytosine deamination in C, D and E.

That is imparted by the wild type virus. Arguing that in the

presence of this drug the biological activity of VIF has been nullified. In other words, VIF is no longer able to antagonize the cytosine deaminase activity of APOBEC3 protein.

So the message I will leave you with is that these small molecule inhibitors are the research at this point on viral accessory proteins is now getting to the stage where we can start to develop small molecule inhibitor screens to come up with biologically interesting compounds that can antagonize the action of these accessory proteins in a way that I have described for VIF.

And I would also like to leave the message that it has been said that many times that lentiviruses have not been vanquished. And that is not strictly true. Because the human genome is populated with endogenous retroviruses. And some of these viruses, when made exogenous, illustrate that these viruses are actually sensitive to some of these restrictions such as APOBEC.

So it is quite likely that our ancestors were faced with similar pathogens, but those pathogens were vanquished by some of these cellular restrictions. And for that reason I think there is a strong rationale to mount a serious drug discovery effort targeting these accessory proteins.

I would like to acknowledge the people who were engaged in research, in particular my Myala Vitalashirova [misspelled?], Mark Sharkey, and Zian Ansu [misspelled?], who did most of the work on the restriction. And on VIF

antagonists, in collaboration with laboratory of Tardik Reiner [misspelled?] and the Department of Biochemistry. Brigit Bier [misspelled?], bioqualm [misspelled?], Keith Mansfield, New England Regional Primate Research Center.

I would also like to acknowledge the support of the National Institutes of Health, particularly NAID, and NIMH, and also support and discovery support from the U Mass Center for AIDS Research. Thank you for your attention. [Applause]

ERIC HUNTER, M.D.: The talk is now open for questions.

MALE SPEAKER: My question is about the Vpx story, not the VIF story. You mentioned that Vpx counters a DNA damage protein and [inaudible] proteasomes. What is the role of the DNA damage protein in viral infection?

MARIO STEVENSON, PH.D.: The DNA damage protein is actually not the restriction itself. It is actually something the virus is using as an adaptor to target the restriction for proteasomal destruction.

DDB1 is actually turning out to be an interesting protein. It is used by a variety of viruses to interact with the interferon response. Because it targets that proteins for proteasomal degradation. So SB5 polyoma viruses are actually using DDB1, are commandeering DDB1 to interfere with start activation. So this might be a general principle.

Now I am not saying that Vpx and Vpr have evolved to neutralize the interferon response. That is a model that we are pursuing. But it is simply an adaptor that links the viral

protein Vpx and Vpr to this restriction. We do not know what the actual restriction is, yet. We have some candidates. Hopefully, if I get invited back to this conference in two years we will have the answer.

MALE SPEAKER: Just a followup: What happened to the old story of Vpx as a protein which causes nuclear import?

MARIO STEVENSON, PH.D.: That is still a valid model, because the restriction could be acting at the level of import. Now the readout that we use is just D and E synthesis. There is very low D and E synthesis in the presence of this restriction. It is a very potent antagonist somewhere between virus entry and synthesis of viral C, D and E.

But the research is revealing, actually, that reverse transcription and import are actually linked. And the reverse transcription is not actually completed until the virus makes it to the nucleus. So we may actually be looking at a nuclear import phenotype, but the readout is reverse inscription defect because reverse inscription is completed in the nucleus. Thanks for your question.

FEMALE SPEAKER: Yes, as regard Vpr and Vpx, other groups have described and published a relationship and fixation on dekafe 1 [misspelled?]. Could you tell me what is the relationship between DDB1 and dekafe 1, please?

MARIO STEVENSON, PH.D.: The question is, there is about, a protein called dekafe that links proteins to ubectin

ligase [misspelled?] into ligase complex. And there are a number of substrates that have been identified for decaf.

The most likely explanation is that DDB1 is acting as a decaf to link Vpx to the proteasomal machinery. We do not have any evidence for that. That is the most likely explanation. Vpr has been shown to bind to dekafe 1 in order to induce cell cycle arrest. My own feeling, and based on some of the recent research in my lab, Vpx probably does not need dekafe 1. DDB1 is a substitute for dekafe.

MALE SPEAKER: Mario, can you— This restriction is only occurring in macrophages. What is going on in replicative cells such as T cells with regards to the oncoviruses versus lentiviruses?

MARIO STEVENSON, PH.D.: So, if you neutralize the restriction in macrophage, they become sensitive to oncoretrovirus infection. Actually, if you neutralize the restriction in monocytes, monocytes become sensitive to lentivirus and oncoretrovirus infection. So I think this restriction is playing a big part in the cell cycle dependence, the so-called cell cycle dependence of retroviruses and lente viruses in general.

In T cells, we have some evidence that if you transducer quiescent T cells with Vpx, they become sensitive to infection. Now there are many theories as to why quiescent T cells are refractory to infection by viruses like HIV and SIV. So it is actually— I suggest it might be low levels of DNTPs,

blocks at the level of nuclear import, lack of cofactors necessary for reverse inscription.

Our data would suggest that it is more simple than that. It might just be a restriction that is regulating the ability of the virus to come in and- if the virus can not antagonize it, it is unable to establish an infection in that cell type. Does that answer your question?

ERIC HUNTER, M.D.: Thanks very much, and we will go on to the last speaker of the session, although I would like to bring all of the speakers back at the end, since I think we will have time from our generalized discussion at that point.

So our next speaker is Guido Silvestri. Guido is an associate professor of pathology in laboratory medicine at the University of Pennsylvania in Philadelphia. He is director of the clinical laboratory in the University of Pennsylvania hospital.

Guido has been involved in HIV research for many years but is really focused in the past few years on the role of nonspecific inflammatory responses in media and in pathogenesis of HIV and SIV infection and being responsible for the depletion of T cells. He has done pioneering studies on the natural SIV infections, particularly in Sooty mangabeys, and continues to do that. And today he is going to be talking about the immune basis of HIV pathogenesis. Thank you, Guido.

GUIDO SILVESTRI, M.D.: Thank you. Thank you very much Eric for your kind introduction. I guess it must be much

easier for an Englishman to say good things about an Italian than a Scotsman, so I will take it any time.

So what I am going to do in the next 15 or 20 minutes is try to walk with you some of the complexity and the intricacies of HIV pathogenesis, particularly at the level of the interaction between the virus and the host immune system.

And I think this is important, because it is my firm conviction that really one of the main reasons why we do not have a cure for AIDS and we do not have a vaccine that works against HIV, is that we do not understand completely the interaction between the virus and the host immune system.

Now when it comes to the pathogenesis of AIDS, there is something that I like to refer to as the classical model of infection, of pathogenesis, which is in a way very simple. And it probably is deceptively simple. And it is based on the fact that you have a virus that infects CD4 T cells. And the CD4 T cells are progressively depleted from the lymphoid organs and the blood of the infected individuals. And the depletion of these cells predict the development of AIDS.

And in fact when you block virus replication with antiretroviral therapy, CD4 cell count usually recovers and the risk of AIDS diminishes. So in this model, the role of the new system in conducting a response against the virus is to protect from virus replication and to protect from disease progression.

Now in reality, it is much more complex. And I will try to convince you of that. And I am just showing this slide.

I am not going to go through it, but it is a part of what I like to refer to as embracing the complexity of AIDS pathogenesis. This is the last figure of a recent Journal of Experimental Medicine paper published by Louis Picker, who is a famous expert in AIDS pathogenesis. And you can see that the CD4 depletion that is associated with a lenteviral infection is becoming appreciated as a complex event that is different based on what T cell, what CD4 cell subset that we are looking at, what body compartment, what surface markers are we interested in and so on and so forth.

So in reality, despite 25 years of studying HIV and AIDS, some of the— there are just some basic questions that are still unanswered. And particularly what is the relationship between virus replication and depletion of CD4 cells, and what is the role of the HIV specific immune responses?

And there are two key features of HIV infection that I want to emphasize that make it really difficult to apply the basic immunology concepts, the one on one immunology type of concepts that the classical model of AIDS pathogenesis foresees. And these two key features are, one, that HIV infects activator CD4 T cells. This means that pretty much every single time that we are trying to elicit a response against the virus, we increase the number of targets for virus replication.

The second feature is that the virus has the ability to change its genetic structure because of the error prone

features of the reverse transcriptase. And it also has an incredible structural plasticity that allows to change the structure and become resistance to immune responses and still function.

And to summarize those two features, I have made a couple of cartoons. So this is a typical antiviral adaptive immune response, with helper T cells that see the antigens, become activated and produce cytokines. And these cytokines will be helping T cells in developing anti-HIV antibodies and also will help CD8 positive T cells in developing a cytotoxic lymphocyte type of antiviral activity.

Well, the problem is that every time we have activated CD4 T cells around, we create new targets for virus replication which translates in turn into more virus being produced.

The second problem with HIV specific CTLs is that a lot of lines of research suggest that the structural plasticity of the virus is such that the virus can escape these responses. And in fact can still replicate effectively having mutated the parts of the AIDS genome that are not recognized by CTL. So in this cartoon, I add in the vertical axis the fitness and the CTL resistance. So towards the bottom line, you have viruses that are— how do I go back? I was trying to go back.

Okay, this is the one that I wanted to look at. So in the vertical axis we have, towards the bottom line, viruses that are susceptible to CTL and have high fitness. These are

viruses that are recognized by CTL. CTLs kill the cells that are infected with these viruses.

But what happens is that you generate mutants. And these mutants tend to have lower fitness but also to become more CTL resistant. And it is just a selecting pressure.

And what happens is that the when the virus ramps up in this hill of increased resistance and lower fitness, the CTLs usually can follow it to a certain extent but then cannot really reach it. It is almost like they were tethered by the inherent limitations of the T cell receptor plasticity. That can not really allow them to chase the virus.

And what happens is that in this relentless effort to thwart virus replication they get exhausted. And much emphasis has been placed recently on the fact that in fact CD8 T cells get exhausted during HIV infection, and the exhaustion can be tracked by looking at specific service markers like PD1 and others.

So in this context of adaptive immune response that seems to fail to control virus replication and on top of that may create new targets for virus replication, the big question becomes, is it possible that sometimes these responses actually are pathogenic and they contribute to the disease progression? And this idea has led to what I like to refer to as the immune activation hypothesis.

The immune activation hypothesis stems from the observation that during HIV infection, the immune system is

chronically hyper activated, and this hyper activation can be observed as increased expression of markers of proliferation of cell cycle and activation of T cells as well as V cells and K cells and other immune cell types. And it is interesting that the fact that HIV infection leads to an immune deficiency has associated paradoxically with immune activation was actually observed in the very first report of AIDS as a new disease back in 1981.

And so the hypothesis of the immune activation is that this chronic stress on the immune system actually plays a pathogenic role and favors disease progression. And there are two main lines of evidence in favor of this hypothesis. One is that immune activation has been shown by many clinical studies to be a strong and independent predictor of disease prevention.

The second point is that when you treat patients with antiretroviral therapy, according to some studies, what predicts their recovery of CD4 cells even better than suppression of virus alone is the suppression of the immune activation. In other words, the immune system responds better to blocking out its implication if the immune activation is also blocked as well as blocking virus replication. The third line of evidence that I will discuss later on is the fact that nonpathogenic infection of African monkeys are typically associated with high viral load but no immune activation. And we think that because of this lack of immune activation they actually remain healthy despite high viraemia.

And I want to show this slide from a recent paper and I think it is very interesting, by Steve Dix and Peter Hunt. They were looking at the lead controllers. The lead controllers are those blocking HIV factor in affected individuals that maintain virus replication below detectable limit. What they discovered is that even in this cohort of patients with visibly no detectable virus replication, there is immune activation. In fact, if there is immune activation you do see CD4 depletion and you can see progression to AIDS, really suggesting that the link, one of the key pathogenic links is this phenomenon occurring at a level in the immune system.

Now we will spend the last few minutes of my talk discussing what we have learned in terms of immune activation hypothesis studying the African monkeys that are infected with SIV. As you all know in this room, the SIVs that infect African and nonhuman primates are the origin of both HIV 1 and HIV2, with SIV CPZ of in chimps being the origin of HIV 1 and SIV SMM of Sooty mangabeys being the origin of HIV2. And also the origin of the SIV mark that are used as the key primate model to study AIDS pathogenesis and AIDS vaccines.

Now what is interesting is that all SIV hosts seems to have an infection that is nonpathogenic despite chronic virus replication. Particularly, my laboratory has been interested in the Sooty mangabeys.

Sooty mangabeys are west African monkeys. They are commonly infected with SIV both in the wild and in captivity. And interestingly they virtually never progress to AIDS. And they usually maintain normal CD4 cell count and normal immune system despite chronic virus replication and despite the fact that the virus is perfectly capable of killing CD4 T cells in vivo.

Now interestingly, when you look at the virus specific T cell responses in these animals, you find a somewhat paradoxical result that these responses are actually lower in these animals than what you find, using the same techniques, in humans infected with HIV. In other words, the system has not evolved to have stronger immune responses that control virus replication but rather seems to have evolved to tune down these responses and let virus replication occur and preserve the system from the detrimental effect of having a chronic immune activation going on for years and years.

In this slide here I want to show you an experiment that we have done years ago that was published a couple of years back where we depleted a CD8 T cells in SIV infected Sooty mangabeys. As you probably know, when you deplete CD T cells in the pathogenic model of SIV infection in macaques there is a major increase in virus replication. In this model we found only a mild increase in virus replication, and that would be the blue line on the bottom of the graph, again

suggesting that the determinant of viraemia in the nonpathogenic SIV infection is not the antiviral host immune response.

So what is it that determined viraemia in these animals? We did an experiment about a year ago that was recently published in the journal of critical investigation where we depleted the CD4 T cells in SIV infected Sooty mangabeys. And what we found is that the virus actually follows the number of available target cells.

In other words, when we deplete the CD4 T cells in vivo in this animal with monoclonal antibodies, viral load goes down. And then when the CD4 cells start cycling, likely as a homeostatic mechanism, in order to repopulate the population of the peripheral CD4 cells, new targets are created and viral load follows the number of target cells that are present in the system. When the system reaches a new equilibrium of CD4 T cells activated, then also the set point viral load is achieved again.

So what I think is that what these monkeys are telling— and similar data has been shown in African green monkeys and mandrills— so what these monkeys are telling is a reality check. The fact that evolution has worked not by creating a strong immune response that blocks the virus, but rather by sort of tuning down the immune response and creating other mechanism that preserve the ability of these animals to maintain their immune system despite a virus that kills their CD4 cells.

So the hypothesis that my laboratory is entertaining is that in fact Sooty mangabeys and other natural hosts maintain their healthy immune system because they avoid this strong immune activation in response to the virus. And this identifies actually a major difference between these animals and the so-called long term non progressor or lead controllers.

Those are the lucky humans that, as I mentioned before, are able to control virus replication. And the difference between the natural hosts, the healthy immune system of the natural hosts and the healthy immune system of the long term nonprogressor is that in the natural host, viral replication continues unabated, while it is suppressed in the long term nonprogressors. And the cellular responses to the virus that seem to be very strong in the long term nonprogressor, or actually low in the natural host.

So the model that emerges is almost like an all or nothing type of picture. In other words, to stay safe from a primate retroviral infection what you have to do is one of two choices. Either you have you mount very strong immune responses that bring virus replication below a critical limit, and in doing that probably also allow the system to rest the level of immune activation to a very low level, or you ignore the virus or mount radically weak immuno responses. Let virus replication occur, but at the same time preserve the system from the damage created by the chronic immune activation.

Anything in between, when you have a strong immune response that is also ineffective, usually leads to AIDS.

So what are the final questions? If this model is true, what are the implications in terms of therapy and what are the implications in terms of vaccines? So when it comes to vaccine, as I said before, the main problem is that every time we try to mount an immune response against the virus, we are also creating new targets for the virus itself.

And in a way it is not that surprising what happened with the Merck STEP trial, with the so-called paradoxical results that the vaccinated individual actually had a higher risk of contracting infection. If you think along those lines, of the immune activation, it makes perfect sense that that is exactly what happened.

So what can we say to conclude this talk? I hope that when you think about the infection in a slightly different way, at least considering as much more complex the interaction between the host immune system and the virus. Well the first conclusion is that clearly there is so much that we still do not understand between how HIV, SIVs and immune system interact with each other.

The second point is that yes the immune system, the immune response have the capability of controlling virus replication in a lot of systems has been shown. But also have this potential to damage immune system by creating more targets

for a virus and also by inducing a chronic exhaustion, a chronic detrition of the ability to respond.

And this has been shown actually in some experiments where compounds like IL15 or antibody against CTLA4 were given in an attempt to boost the immune responses against the virus with the paradoxical effect of seeing an increase in virus replication occurring. And that is why I am a little bit skeptical for instance of proposing to block PD1 or PD1 system as a way to suppress virus replication in vivo.

In terms of an AIDS vaccine, I think that in order to have an immune system that can control HIV we might need some major breakthroughs. And I identified a few areas that I think would be of interest. One is the possibility of eliciting CTL responses or antibody responses that are less dependent of having a large number of activated CD4 cells in mucosal tissues. I think a second area of interest would be, can we disconnect CD4 activation and CCR5 expression? In other words, can we make the cells that are CD4 activated that would be good targets for the virus, virus resistant by somehow manipulating the expression of CCR5?

And finally, can we find ways to increase the flexibility, and the structural plasticity of the T cell receptor repertoire, so that that can chase the virus more successfully than it does now?

And, some of this work was done with a large number of colleagues. I particularly want to thank the NIH and other

sources of funds. And thank you all for your attention.

[Applause]

ERIC HUNTER, M.D.: So the talk is open for questions.

MARK WAYNEBURG: Mark Wayneburg [misspelled?], McGill University. A very interesting talk and very provocative, Guido. You mentioned that you think that the viruses that survive CTL attack have diminished fitness. What do you suppose would be the mechanism for that?

GUIDO SILVESTRI, M.D.: Yes? I am sorry. I was trying to look at you. So, I think that it is not necessary that the virus that escapes CTL has a lower fitness. I think that we know that in some situations it has been shown in vivo, for instance in classical work by David Walkins [misspelled?] that if you engineer a virus with escaped mutant and you put it in the background where the CTL does not control the wild type, the escaped mutant usually reverts to the wild type.

And also there is data on transmission suggesting that when a CTL escape transmission in humans is passed to a different HLA background, you have a reversion to the wild type. So those two models in humans, in observational transmission in monkeys, by engineering an escape virus suggests that indeed there was a loss of fitness when the virus escaped from the CTL responses.

MARK WAYNEBURG: And at the end of the talk, you almost seem to be suggesting that if we could figure out how to prevent immune activation in humans to begin, with we might

somehow have the situation in which pathogenesis would not exist. Do you really think that that is potentially the case?

GUIDO SILVESTRI, M.D.: I think we ought to explore that direction. I think that it is complicated because HIV is an immunodeficiency so blocking the immune system, the response in the context of immunodeficiency is a risky business. That is why we need to understand more.

But if there is a specific pathway of immune activation that is associated with this chronic immune activation and it is not and you can block it without blocking immune responses to other pathogens, that could be a therapeutic target, yes.

MARK MILANO: Mark Milano [misspelled?] with Dicria [misspelled?] and Health Gap. I absolutely think there is a way to deal with immune activation. Your talk is really deja vu. I have been to every AIDS conference since '89, and at virtually every conference someone speaks about immune activation as being the cause. Anthony Fauci has spoken about it a number of times.

And I always get up and say, I have been living with HIV since 81, I controlled it for 26 years with no antiretrovirals by using alternate day prednisone since 85 for a different condition. And I had very high viraemia, over-close to a million for 5 years and yet my CD4 counts stayed completely stable at 4 to 500 just like a Sooty mangabey and with no side effects whatsoever, a clear case, I think, of

immune suppression, low grade, controlling immune activation and yet for 15 years I have said, let us look at this.

No one will study it. People refuse to give a drug like prednisone or any other immunosuppressant to people with HIV. They will not do it. How do we get this studied when I know there are more people than just me who have been taking prednisone and have been able to control their HIV pathogenesis without antiretrovirals? How do we get a study? What do we do? It is very frustrating. You are not the first person to say this.

GUIDO SILVESTRI, M.D.: Amen. That is all I can say. I agree with you, obviously you need to understand that the regulatory agencies like FDA are cautious. There have been some studies. Mike Letterman did some cyclosporine experiments. Jeffrey Pantilare [misspelled?] did some microphentilate. There are obviously some caution by regulatory agencies when it comes to giving potent immunosuppressants to individual with an immunodeficiency. But I agree with you, I think it is something that ought to be explored.

MARK MILANO: Some day.

MALE SPEAKER: I have a question. I have read most of your work and I know about your model. I have if you can state for me one aspect of it. Viral replication is very high in the naturally infected host most of the time? Knowing that transcriptase induces mutation, [inaudible] lot more than a

human would have or unnatural host would have. How the immune system does not respond to that? Because it is a physiological event? It has to respond to it? So what you speculate? What is happening?

GUIDO SILVESTRI, M.D.: Yes, I think the reason – this is actually, if you read my work you know that we have done a lot of work on trying to understand this question. The problem is that the causes of immune activation in HIV infection are complex.

So HIV triggers it, and the responses to HIV, the adaptive immune responses to HIV are certainly a big part of it. But then there are other ways of potential cause of immune activation including cytokine production including deficit of regulatory T cells, including microbial translocation, including probably other effects of HIV that is not directly mediated by TCR, classical TCR activation of T cells.

So we are sort of exploring one by one this hypothesis in the Sooty mangabeys and African greens. And I can tell you that we are in the process of identifying some differences. But you are right, that is exactly the key question. Why, in the presence of all this antigen and different antigens the response is actively tuned down.

ERIC HUNTER M.D.: We will take two more questions, then we need to finish.

RON TODDSTRUM: Ron Toddstrum [misspelled?], Chapel Hill. I want to challenge you on the idea that nature has only

solved this problem by suppressing immune activation. Because we really would not see the opposite.

There are a number of examples where if you take a virus and put it to a different species of primate you can get transient expression but then it is gone. That would not allow enough time for transmission, so it is possible that all the nonhuman primates species that do not have lenteviruses actually have solved this problem in a different way.

GUIDO SILVESTRI, M.D.: No, I agree. It is a good point. What I can say, Ron, is that of the ones that we know that have high virus replication, and there are a large number of species now where we are trying to put our hands on more and more species. Obviously for them it appears their immune system did not choose that route.

Now we have African monkeys that do not seem to be infected with SIV now. Whether this became that way because they were able originally to control some sort of ancestral pandemic and then they— that is a good point. It is speculation right now because we do not have direct evidence that that actually happened, but—

ERIC HUNTER, M.D.: Last question?

MALE SPEAKER: [Inaudible] Nice talk Guido. I appreciate the [inaudible] of the slide where you compare LTLP and your natural infection, because this really highlighted the differences. And given your general scheme, you could substitute the Sooty mangabeys with the rapid progressors,

because in HIV infection, rapid progressors exactly are doing that, high viral load and low immune response. So we must be careful in making analogies between human and monkeys.

GUIDO SILVESTRI, M.D.: Yes.

MALE SPEAKER: I find we commonly think the only bias that I found in your talk is that immune activation is a package. There are different types of immune activation under different types of stimulants. And I think what is fascinating in looking to therapy is that you can really uncouple activation from virus replication.

So I share a lot of common things with you, but I think you must be careful in not making single package for this.

GUIDO SILVESTRI, M.D.: I always welcome, when someone invites me to be careful, because it is an important part of science. I think in terms of extrapolating from monkeys to humans, the point I wanted to make by comparing long term progressors and Sooty mangabeys is that you can get to the point of dealing with the retroviral infection either by controlling it or by letting it go. And a lot of stuff that happens in between is what puts you in trouble.

When it comes to the complexity of immune activation I fully agree with you. There are probably many mechanisms of activation, many molecular pathways responsible for immune activation. There are a lot of things that we do not understand. But I think ultimately what is important to

understand and to internalize as a community is that we need to start thinking about acting on the host immune system.

Which does not necessarily mean suppressing it. It could mean modulating it. It could be finding a way to respond in a different way to certain antigens. But not just thinking that the only way to go is killing the virus.

ERIC HUNTER, M.D.: Thank you Guido. We are out of time, and so I would like to thank the speakers and also the audience for their participation.

[END RECORDING]