

**Conference: 15<sup>th</sup> Annual International AIDS Conference  
Special Lecture: Pathogenesis of HIV Disease:  
Current Concepts  
July 15, 2004**

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**MALE VOICE:** I'd like to start a special plenary lecture by Dr. Tony Fauci who is the Chief of the National Institute of Allergy and Infectious Diseases. He will be introduced by my co chair, Greg Gonzales who is a leader of the community and has played a great role in the movement to scale up access to care and is also fighting for cheaper and simpler diagnostics and monitoring tools. Greg.

**GREG GONZALVES:** Thank you. It's my great privilege to introduce Dr. Anthony Fauci who I've known and worked with 15 years now. I've known him both as a policymaker and a scientist and he's been superb at both. So Dr. Fauci is the Director of the National Institute of Allergy and Infectious Diseases and he's also one of the leading immunologists studying the interaction between HIV and the immune system. And I think it's going to be a great pleasure today to hear his lecture on the immunopathogenesis of HIV and I think we'll also have some time probably at the end for some questions from the audience because I think people don't get often the pleasure to hear Dr. Fauci talk in person. Tony?

**ANTHONY FAUCI, MD:** Thank you very much. It's indeed a pleasure to be here with you this afternoon to talk about the pathogenic mechanisms of HIV disease. This is an area that myself and my colleagues, not only in my own lab but in a number of laboratories throughout the world have been pursuing

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in order to gain insight into how we might counter these rather perplexing pathogenic mechanisms with therapy, immunological reconstitution and a variety of other mechanisms that we'll get a chance to discuss during the talk. I want to first start out by saying that I'm going to make as my major theme something that has been of interest to our laboratory for now over 20 years, and that is the role of immune activation as the underlying driving engine in HIV pathogenesis. Something that we and others felt was an important issue back when we first started working with HIV infected individuals and even before we knew what HIV was. But it's only been over the last several years when we've had the opportunity through a variety of techniques, molecular and otherwise to look at the very subtle aspects of aberrant immune activation which as you'll see as I go through the talk is truly the common denominator of many of the abnormalities that we see that contribute to the pathogenesis of HIV disease.

Now through the years we've often heard camps of people that say it's the virus or it's the immune system. And when people talk about pathogenesis, not infrequently they talk about viral pathogenesis somewhat separately from immunopathogenesis. When in fact as we now know as we learn more and more about this illusive virus, they really are in many respects if not one in the same thing, they certainly are very closely related. And as I mentioned just a few moments

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ago, immune activation is the common denominator. But there's something very special about HIV that many of you in the audience I know are quite familiar with, but for those of you who are not, any microbe or any antigenic stimulus will elicit an immune response. HIV is no exception, but HIV also has a very unique capability of directly activating the immune system itself by virtue of receptors on immune competent cells to which the virus binds. And there's been a number of very elegant pieces of work by a number of laboratories throughout the years that have shown that. So what I'm going to pursue with you for this talk is the very, sometimes obvious and sometimes subtle ways that immune activation plays major roles in the propagation of HIV pathogenesis. So since there's such a tremendous amount of work has been done by so many people over the years in this, let me try and summarize it for you, particularly for those of you who are not deeply involved in this field. For those aficionados in the audience, I apologize for just giving you the broad concepts, but I'll show you some very specific data in a little bit.

Now HIV infection, as I said several times already leads to a state of generalized immune activation. There's increased T-cell turnover, both increased production and increased destruction. There's increased activation induced cell death, not only of T-cells that in fact come into direct contact with the virus as we knew early on, but now a number of

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laboratories have shown that the general broad expression of activation with cytokine secretion often leads to bystander activation induced cell death. Not only of T-cells, but also of B-cells and other cells. This leads to a decline in the size of the CD4 positive T-cell pool and then ultimately, which is the expression of disease a state of what we refer to as activation induced immune deficiency. Now the advent of antiretroviral therapy not only was an extraordinary breakthrough with regard to how we treat and care for HIV infected individuals, but it also allowed us to dissect out more precisely the pathogenic mechanism that are associated with the process of virus replication. So if you insert antiretroviral therapy into this scheme, what you see and a number of labs have now very precisely measured this a decrease in T-cell turnover, increased T-cell survival, a natural consequence being the expansion of the CD4 positive T-cell pool, and then a very interesting phenomenon that we're just starting to appreciate. Associated with the reversal of this activation induced immune deficiency is not only the spontaneous resolution of opportunistic infections, such as people who are not able to clear CMV who once they're reconstituted in fact do it quite well, but on the other side of the coin we've now seen immune reactivation syndromes where the return of the immune response and its functional capability actually has at first what appears to be a negative pathogenic effect.

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Now I want to spend just another minute or so on the whole issue, and again I'm simplifying it but I want you to walk away at least with the broad concept of how this fits into the entire situation of lymphocyte turnover. This here is a slide from Cliff Lane's group in which he labeled lymphocytes in individuals prior to treatment in the blue and then after they have become aviremic in red. And he did this both looking at peripheral blood and as you see in the right hand side of the slide also with lymph node labeling. There's a very dramatic change in lymphocyte turnover after the initiation of antiretroviral therapy. We'll get back to this at the end of the talk because this is just one of many manifestations of how turning off the initial source of the immune activation has a number of heterogeneous but very profound effects.

Now when you look again at the pool of lymphocytes and how they enter into the T-cell repertoire, as we all know stem cells go into the thymus and then there are primary thymic emigrants, which lead to a pool of cells that are naïve and apparently resting. Notwithstanding HIV if an antigen, be it a nonmicrobial antigen or a microbe such as HIV perturbs that pool of cells you have a natural, normal response. The pool is perturbed, it increases turnover, it becomes activated in a positive way to respond to the antigen, and that naturally removes the antigen. If the antigen is not removed you would have a very aberrant cyclic activation that would lead to a

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deleterious effect as opposed to the beneficial effect of what the immune response does most of the time when we're confronted with an antigen. When the antigen is removed naturally or through antimicrobial agents the cells that have recognized return to the resting pool and that's where you develop memory or an accumulation of your antigenic experiences. In many respects that's exactly what happens to HIV as shown in the bottom of the slide. But with HIV the antigenic load and turnover is not only extraordinary in its degree, it's persistent. So you have a very aberrant state of lymphocyte turnover and only when effective antiretroviral therapy comes along do you turn off this process, which then leads to a situation that we would have with a normal antigen being removed. The problem is that during that situation many HIV specific T-cells instead of going into the memory pool have been destroyed because of the unfortunate situation that they have receptors on their surface that are very high affinity receptors for the virus itself. And again, we'll get back to that in a moment.

Now having said that as a broad general issue, I'm going to look at 3 very specific pools of cells and how over the last few years we've been able to get greater insight into how, not only the virus itself but the consequential immune activation greatly perturbs both phenotypically and functionally these pools.

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Let's start off with the CD4 positive T-cell reservoir. A few years ago, actually in 1997, after we had the opportunity to observe patients on studies who had been successfully treated with antiretroviral therapy, and by successfully I mean bringing the level of virus in the blood to below detectable levels. At first that was 500 copies, but now we get down to 50 generally or less. And 3 separate laboratories, ourselves included found something that was very sobering. And that had to do with the reservoir. Our group, Doug Richmond's group, Bob Cilicano and others demonstrated that even in people who were aviremic for up to 3 years, when you went back and looked they had in their system a latent reservoir of virus generally contained, but not exclusively contained in the CD4 positive T-cell pool. We did an empiric experiment soon thereafter because of this sobering news and we stopped therapy in a group of patients that we followed very closely at the NIH. And much to our dismay, virtually every one of the individuals when we withdrew therapy despite the fact that they were healthy with good CD4 positive T-cell counts, they rebounded with their viremia essentially back to what their original viral set point was. In hopes not too optimistic, but in hopes that we might be able to in some patients eliminate that reservoir we treated a few patients with cyclical activations of the immune system to try and, as it were, flush them out. This reservoir of HIV infected cells. And we published this in Nature a few years ago

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in individuals who to the best of our somewhat crude techniques we could no longer detect a reservoir either in the peripheral blood or in 2 patients in which we had the opportunity to do biopsies. Now these blue bars here on the right are the measurable reservoir and when you go below the dotted line, that's below detectability. Again, much to our dismay we found that when you stopped therapy as in the others not only was there a rather robust rebound of viremia, but the latent reservoir was almost immediately replenished. So since this reservoir has now become a very important obstacle to total elimination of HIV, it became something that was the subject of intensive study by a number of laboratories. These are 2 papers that appeared in the New England Journal and Nature Medicine, not from our group. One from David Ho's group and one from Bob Cilicano, which showed a broad range of time frame with which if you perfectly suppress the virus you would likely, be able to perhaps see elimination. The lower number of several years was from Ho's group, but Cilicano's group showed that it would take probably 60 years or more. In other words, it was likely impossible to eliminate this reservoir. Because of that we became very interested in looking at the physiological state of that reservoir and in a commentary in PNAS we referred to it as the major obstacles to the eradication of the virus.

So now that we had the opportunity to look in a much more precise way at people who were viremic and hence, as I've

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shown you just a few moments ago with a number of slides, had this degree of what were referred to as aberrant immune activation and compared them either longitudinally with themselves when they were no longer in a state of activation, when the therapy actually eliminated in the plasma identifiable virus and compared it to the time of immune activation. And we found some very interesting things.

First of all, we're going to now show you several slides that are going to be viremic individuals compared to aviremic individuals. First of all if you look at the morphology of the resting CD4 positive T-cell pool in either the aviremic or the viremic patient, they are identical. We looked and looked. It doesn't look any different at all as you might not expect. It's a very gross way of looking at things. However, if you look at certain receptors that are what I will refer to for the remainder of the talk as the classical, universally used surface receptor markers of activation. In this case, CD25, CD69 and HLADR. And for those of you not looking at cytofluorometric analysis, I would just point out the important quadrants to look at. For those of you who really get excited by these dots and things you could have fun with it, but for those who don't the important issue here is that if you double mark CD4 against the activation markers and compare one of our patients, the viremic patient above with an aviremic patient, patient 22, and look in what would be the upper right

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quadrant, which would be the activated CD4 positive T-cell, there's very little activation and essentially no difference by classical surface markers of these 2 types of cells. However, when you take a little bit more subtle look at what I will refer to as the physiological state of that pool, putting cells in culture and just seeing what they spontaneously do, as you see on the left hand side of the slide in viremic individuals there is spontaneous release and secretion of HIV virens. Look on the right to the comparable group on the media alone. Not so with the aviremic individuals. You put cyclosporin in, no effect. You put a combination of antiretroviral drugs, no effect. Which means we're not looking at cell-to-cell spread, we're looking at the spontaneous production of virus from the infected latent reservoir. Atinomycin D, which is an inhibitor of RNA as you would expect does block it. The positive controls with anti CD3 in both of viremic and aviremic are there for your observation.

We wanted to take it a step further and over the past year or two we've looked at a technique which is now very commonly used, which more helps you to form hypotheses than to solve the hypotheses. And that is looking at micro array analysis or gene expression. It was at first quite complicated. There's more difficulty in interpreting data in this because you get reams and reams of data. Again, for those of you familiar with this, there's just a simple, commonly used micro

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array where you can analyze the expression of clusters of genes when cells might look either morphologically like I showed you or even with gross protein expression as in the receptor data I showed you, you get a little bit more subtle when you just look at gene expression. And this is a typical micro array. For those of you in the back of the room, if it's a darkish black that means no change. If it's green it's a bit down modulated, but if it's red that means the genes are expressed differentially among the different groups. And as you can see on the bottom left of the slide we're looking at HIV negative normal donors and aviremic treated individuals compared to viremic. And notice that in the viremic individuals despite the fact that we couldn't show any difference in the expression of surface markers, there were clusters of genes transcription regulators, RNA processing and modification genes and protein trafficking and vesicle transport genes all of which are associated with a permissive state for virus replication. Clearly a major difference in the resting CD4 positive T-cells of the patients that have the continual aberrant activation.

So we conclude from this part of the discussion that indeed there's a fundamental difference in the resting CD4 positive T-cell pool when you compare those groups of viremic vs. aviremic patients. We've heard a lot of talk about true latency with HIV. It doesn't really exist when you have a continual source of activation because the cells are

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continually poised to express virus. However, when you look at the aviremic individuals, there's much greater stability of that pool and this may represent, perhaps transient, a truly resting reservoir that only when you perturb the system can it then start to spontaneously express. And so active virus replication which is the source of this immune activation really has a significant impact on the physiological state of that pool and in turn allows the release of HIV without obvious exogenous activation stimuli. So we're getting from gross measurable things to very, very subtle differences and it all has to do with immune activation.

Let's turn our attention for a few minutes to V-cells. And this is something that we had been historically interested in for a very long time. At the time that we first started taking care of HIV infected individuals and our wards at the NIH prior to the recognition that HIV was the cause of AIDS since we had been working with B-cells and immune mediated and infectious diseases, the natural thing that we did first was take a look at the B-cell repertoire in HIV infected individuals. And talking about feeling getting old, this is a paper that we published more than 20 years ago when Cliff Lane was a Fellow in the laboratory. And what Cliff showed is that when he looked at B-cells from patients with this disease, even though we didn't know what the virus was, there were some very striking abnormalities of B-cells that seemed paradoxical

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because they were all, not all, but a substantial proportion were expressing what we called aberrant activation. Measurable by spontaneous secretion of immunoglobulin, but the inability to [Inaudible] respond to a new antigen. Now almost 20 years later Susan Moyer, again in our group, took a look in a much more subtle way at the phenotypic and function of perturbations when an individual is in a chronically activated state. And rather than show you all the data in this particular paper that we published in 2001, this is what we saw. There was an interesting loss of an expression of one of the receptors, the complement receptor on B-cells, CD21. I'm going to get back to CD21 in a second because that allowed us to look more carefully at this abnormal subset. We noticed the cell did not respond de novo [misspelled?] to proliferation as Cliff had noted years previously there was increased immunoglobulin secretion, but also there was an interesting change in morphology. And I know it's difficult to see in the audience, but the far left is an HIV negative individual's B-cells and if you look at the CD21 positive, which is normal the way B-cells should be, the cells morphologically look okay. But if you look at the CD21 negative, the activated cells that have lost the expression of an important receptor, they look very much like plasmacytoid cells or they appear to be pushed towards differentiation. And because of that we started to look at this population of cells more closely and in the next series of slides is work that

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we've just completed that is now in press in the Journal of Experimental Medicine and that is looking at the subtle expression of genes in the activated group, i.e. the viremic individuals versus the relatively unactivated group either HIV negative individuals or people who were aviremic. So we reverted back to the same technique of looking at gene expression. And again, rather than try, which you can't to read every little one of those genes, there's a very interesting pattern that I'm sure is obvious from the back of the room. That the HIV viremic individuals are expressing in their B-cells major categories of up regulated genes that interestingly are associated with interferon-stimulated genes and genes associated with B-cell terminal differentiation. Essentially showing at the gene expression level what we're seeing subtly, functionally in the B-cells themselves.

Now among these up regulated genes were genes that were associated with cell surface receptors. These are a few of them. We picked out one that was of interest and that is CD95 or FAS. Now FAS is a receptor that's referred to as a death receptor, because when you interact FAS with a FAS ligand, you induce apoptosis or cell death, activation induced cell death of these cells. So we thought that we would pursue this a little bit further. Again we get back to these cytofluorometric analysis and again for the uninitiated in the group I just want to point out the quadrants that are of importance. On the upper

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panel we're looking at B-cells that we don't stimulate and we're comparing 3 groups. HIV negative, HIV aviremic - namely patients who are treated, maybe 1 or 2 who have not been treated, but are aviremic - namely long-term nonprogressives, but fundamentally treated individuals, and then in the far right HIV viremic. And we're looking at what we call a nexant staining, which is a marker of cells undergoing apoptotic cell death. And we're going to look at the cells that are CD21 negative. So the only quadrant that you really want to concentrate on is the lower right quadrant in each group. And if you look at the negative in the aviremic, there are very few cells that are undergoing apoptosis, but look at the lower right under the HIV viremic and you see here a much greater number of cells that are spontaneously undergoing apoptosis.

If you add the ligand to that receptor or FAS ligand and go to the bottom panel, that effect is much more intensified as you see again in the lower right hand quadrant of each of the 3 study groups. So we wanted to pursue this and find out whether or not this viremic state is able to enact certain functions that are again very deteriorous. This is a correlation slide. On the horizontal access you see what we're measuring is apoptosis. In the vertical access is the expression of this death receptor. It's highly correlated with the expression of this receptor. If you look at the relationship between the level of HIV viremia, again associated

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with apoptosis or cell death, again another highly correlated phenomenon. So in summary for the B-cell relationship with immune activation, there were a large number of B-cell genes up regulated in the activated state. The majority of them are associated with pushing B-cells to terminal differentiation. Interestingly, changes in expression of a TNS super family of receptors, such as FAS or the death receptor for the cell are associated as you might expect with B-cell death by apoptosis. Now in NK cells for some years now have attracted the attention of immunologists because they are part of the innate immune system and in some situations definitively shown and in many hypothetically thought are major host defense mechanisms for a number of abnormal states. Now NK cells being innate don't have memory, but as I'm going to show you in a minute, they have some receptors that were first noted on primitive organisms, but now are shown to have analogs in humans. An NK cell will not destroy a normal cell. If it did, you would have a perpetual state of autoimmunity. And so there needs to be some control, but what NK cells do eliminate are abnormal or non self cells that are either virally infected, tumor transformed or are allogeneic as what we see in a transplant.

Now how does this system self regulate? It self regulates by a balance of activating an inhibitory receptors. And again for the aficionados in the audience I apologize for making it so simple, but I want to make sure everybody

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understand because this is a very interesting phenomenon that occurs. I'll get back in a moment to the fact that the inhibitory receptors are dominant. Which you want them to be, because if they're not dominant they're going to be eliminating a lot of very important good cells.

Now the next 2 slides that I'm going to show you I show you for a concept, not that I want anybody to try and read every single one of these receptors. These are the NK cell activating receptors, the business end of the killing. We don't know the ligands for all of them, but we do know the ligands for some. They're referred to as NKP46, p30, p44, etc. Irrelevant to what I'm going to tell you unless you really want to get to the fine distinctions, which are really not important for the point that I'm trying to make. There are also inhibitory receptors that go by different names and their receptors are fundamentally the self-molecules of HLA as shown on this slide.

Now what about that balance of control? Under a normal state the NK cell in green will recognize I say target cell, but it's a normal cell that has an MHC class 1 and so it triggers the inhibitory function and so inhibition is dominant. The cell shown in gray would have an activating receptor, but it's rendered functionless in the normal state. However, when MHC class 1 is either down modulated or non-present, as is the case in many tumor transformations in a number of viral disease

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or when it's non recognized because it's allergenic, then the inhibitory effect of the NK is obviated and the activating effect tells the cell to kill the target cell.

Now over the past year we've been very interested in that. And we recently published a paper that I'm going to summarize here, but then I'm going to get a little more detailed about it about what we've been doing over the past several months. In this paper, Dominica Meviliu in our group looked at the differences between individuals who were viremic, i.e. activated, versus people who had a normal system, but were infected and controlled on antiretroviral therapy. And the sum total of what was in that paper is that we found an interesting dichotomy because we were trying to find out why NK cells don't work well in HIV infected viremic individuals. Something that we observed and many others have observed a long time ago. There was an interesting reversal where the activating receptors were down modulated and the inhibitor receptors either stayed the same or maybe had some increase. In that paper we made another observation that I'm going to tell you about in the next minute or so. And that is, there was the appearance or enrichment in viremic patients of an abnormal phenotype of NK cells, namely CD56 negative and CD16 positive. That is an abnormal phenotype, because most of the cells are CD56 positive. And again, to avoid any confusion just focus on the circled group in the middle. The viremic individuals, their

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NK cells were phenotypically quite abnormal. If you look at it as it's shown in this slide, there were a highly significant difference in the numbers of CD56 negative cells among the viremic group in the middle versus the healthy uninfected donor and the aviremic patients on the right. Now, again, I apologize that this is a difficult slide to see from the back so let me just give you the concept. If you look at inhibitory receptors, and in this we're looking at healthy donors on the far left of a box, the 56 positive cells of an infected patient and the 56 negative cells. And there's either maintenance or a slight increase. So the inhibition of the function appears to be stable. However, when you look at the activating receptors that are responsible for the killing, these receptors, not all of them but some of the important ones are down modulated. Again, in the viremic patient. So it's extraordinary the obvious and subtle effects that an aberrant state in an infected vs. uninfected people show and manifest.

Just to close the picture a bit on NK cells. There's a decreased production in patients who are viremic, in granzyme and perforin, which are two important enzymes for those of you not familiar with it in the capability of a cytotoxic cell to kill its target. In addition there's a decrease in important cytokines associated with immune competence, such as TNF alpha and interferon gamma.

Now we took this a little bit further to talk a look at

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what we saw in some respects in the B-cell pool. Namely the expression of this death apoptotic receptor and FAS as you can see obviously in the red column of the viremic individual is clearly over expressed, whereas the aviremic individual looks very much like the normal individual. Namely a recurrent theme of avireemics shutting off activation and reverting back in many respects to normal phenotype and function. And in fact, if you looked at the percent of the CD56<sup>+</sup> that actually upon exposure to a FAS ligand, they die as you might expect at a much greater rate than do the cells from the aviremic individuals.

So very quickly summarizing this part of it, HIV viremic patients have a dichotomy of expression of their receptors, activating down, inhibitory maintained or slightly up. The dichotomy is reflected in a decrease in cytotoxic function. I did not have a chance to show you all that data, but there's a very good correlation with cytotoxic function, and then I mentioned to you but didn't show you the data a the decrease in the production of important mediators like granzyme, perforin, TNF and interferon gamma.

There's an association with an enrichment of an abnormal phenotype of cells and the decreased function are concentrated within this abnormal phenotype and again these NK cells express increased levels of a state associated with an increase apoptosis.

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Now having looked in these ex vivo experiments, we wanted to talk a bit of a closer look by getting algometric envelope and looking at if you trigger the cell can you really follow some of these events and mimic an abnormal immune activation state in vitro? So we looked at the direct effects of envelope on immune cell function. This slide summarizes a lot of work over a number of years. And again, let me try and simplify for you and just march through it with you. We all know now from elegant work from other labs that the HIV envelope binds to a primary receptor CD4 and then with conformational change to a coreceptor depending on the virus it could be CCR5 or CXCR4, and in this situation events of intracellular signal transduction are affected. The first observation we made several years ago that when you do this with envelope you trigger the mobilization of intracellular calcium. Others have shown this in other situations also. But we pursued it a bit further and we stimulated the cell not enough to make it proliferate, not enough to make it express the classical activation markers, but enough for us to measure and monitor some interesting intracellular phenomenon. On the left we saw the phosphorelation of a number of substrates, such as focal adhesion kinase and PI2 and others that are associated with a state of permissiveness for virus replication. As if the envelope is setting up its target cell by activating it in a way that it will be permissive for virus replication. We did

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gene expression analysis with micro arrays and wouldn't you know the genes that light up are the genes that we showed before are associated with permissiveness for increased virus replication. One of these I want to spend a minute on, and that is what we call NFAT, the nuclear factor of activation of T-cells.

NFAT is a very important transcription factor. And again trying to take a lot of data and schematically diagramming it for you, when you interact envelope with a target cell, NFAT is not only activated it translocates to the nucleus and very interestingly binds to an NF kappa B binding site, not only on the promoter of the HIV long term and repeat, but also on promoter regions of different cytokines, again providing an interesting subtle state of permissiveness with activation that you don't generally measure by looking at cell surface activation markers, thereby creating a permissive state for replication.

Again we took it a step further and by gel shift analysis we found that interestingly enough smart virus, this happens with envelope, but not with other ligand signals. Because if you look at the far left at an untreated versus an envelope treated cell for which you have an extract, you see an up regulation of activated NFAT. If you take the ligand for CXCR4 named the SDF1, you don't do it. If you take MIP 1 beta the ligand for CCR5 you barely do it. If you just cross link

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CD4 without touching the coreceptor you don't do it, but when you put the ligand with the appropriate envelope, you markedly activate NFAT saying that it requires coordinate signaling through the CD4 and the coreceptor. This is really interesting because what it essentially might mean, now we haven't definitively proven this but we're certainly pursuing it, is that HIV is evolved in a certain way to trigger an activation effect that a bunch of ligands individually won't do, but when you have the coordinate signaling that we've known now for some time occurs with the receptor and a coreceptor, you do get this state.

Now the obvious question is, is there any difference between R5 and X4 viruses? So we looked at gene expression there because the original paper from several years ago indicated that calcium flux was induced by an R5 but not by an X4 virus. That's of some interest because we know that the transmitting virus is almost exclusively an R5 virus and R5 viruses tend to activate cells more. So we did gene expression looking at envelopes prepared by Jim Arthers and Claudia Checoler in our lab that were R5 versus X4 and we found a striking difference. The R5 is in blue and the X4, so I've bar graphed diagrammed the relative expression of genes a clear dichotomy when you're looking at transcription factors, protein modification and cell cycle genes. Now whether this has anything to do with the interesting difference in the ability

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of an R5 versus an X4 to initially infect somebody I don't know, but this is interesting enough that we're going to pursue it a bit.

So the conclusion from this is that HIV envelope modulates gene expression, interestingly of genes that are associated with virus replication as well as the secretion of cytokines that are intimately connected with immune activation and replication competence. The induction of this gene expression requires coordinate signal, which makes it relatively, not completely, but relatively specific for what the envelope can do. There's a difference between X4 and R5 as I just showed you a moment ago. And the conclusion we can make is that the envelope induced cell signaling might well play an important self-propagation role in maintaining this necessary state of aberrant immune activation associated with HIV pathogenesis.

So let me summarize all of this for you now in a few schematic slides. HIV enters the body and infects cells of the immune system schematically diagrammed here. This is followed by an appropriate activation of the immune system, which under normal circumstances if it weren't this very special virus would eliminate the antigen and everything return to the normal state as I showed you on one of those schematic first slides. But that's not what happens, because at the same time as you target and eliminate one way or the other a number of HIV

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specific cells, the virus itself can actually directly activate the immune system or the cytokines associated with normal immunological responsiveness to an invading microbe, instead of helping with the elimination, actually expands into a state of aberrant immune activation which has the negative feedback effect of making the virus replicate more efficiently. Then a bad cascade of events occur. You get immune cell depletion. You get immune cell dysfunction. You get normal if it weren't a perpetually replicating virus lymphocyte turnover, which becomes aberrant because the turning over lymphocytes are exactly what the virus is looking for a target. So the normal response becomes a risk associated phenomenon for the immune system.

You introduce into this antiretroviral therapy, which in fact simultaneously has 2 effects. It's directly antiretroviral so you start eliminating some of the virus, which then brings down the intensity of the immune activation. Because of that, lymphocyte turnover starts reverting back to the normal state and with it comes immune cell dysfunction reversal, immune cell depletion reversal and hence reconstitution in many of CD4 positive T-cell levels. This then decreases more so the immune activation and therefore what you then have is reverting back to a normal state where virus replication is minimal and ultimately essentially is well controlled which is the reason why of all the slides I've been

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showing you the only unfortunate situation is you don't eliminate the reservoir, which means that when you decrease or eliminate the antiretroviral suppression of replication you regenerate this terrible cycle of self propagation of an HIV replicative permissive state. As long as the virus is not replicating, the activation does not occur and does not self propagate. So this is an avenue now that is being pursued very actively by ourselves and a number of groups to try and see what type of a safe intervention might synergize in controlling, and a number of laboratories have already tried that. It's going to be very tricky business, but only by understanding this very interesting interaction between what should be a salutary effect of activating immune system that unfortunately translates into a common denominator deleterious effect. So this should be the subject of some very interesting experimentation in future years. I'd like to close by thanking the members of my laboratory for the performance of this work. On the far left and moving over to the right is Tau Chun who's done all the work on our reservoir studies, Audrey Kinter who presented some interesting work at this conference that I didn't get a chance to show today on T regulatory cells, CD25 positive cells. Audrey has done much of the work on the cytokines. Jim Arthurs and Claudi Chekola did the work with envelope. Angela Monaspina and Susan Moia did the work on B-cells. Dominico Mavillio and Sham Cotalil did the work on NK

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cells and Mark Diabol for a number of years now has been working on the relationship with antiretroviral therapy and many of the phenomenon that I mentioned to you. And finally other collaborators both in our lab, particularly Cliff Lane and his group and others who have collaborated over the years with us and finally, and very importantly, our large group of patients that have been so generous in allowing us to study them. Thank you.

**MALE SPEAKER:** We have time for one or two questions if people would like to come up to mic #3, just one or two brief questions.

**MALE SPEAKER:** Thomas Christens out of Copenhagen, Denmark. Do you believe that the CCR5 antagonists might prohibit the immune activation of HIV envelope?

**ANTHONY FAUCI, MD:** Yeah, that's an excellent question and that's one of the things that we're looking at and you're referring obviously you picked that up, thank you, that if you need the coordinate signaling that you don't get when you just cross link CD4 our thought is that if you do have an antagonist it will serve 2 functions. Not only would it block the entry and ultimate infection of a cell through the CCR5 receptor, but it might obviate the interesting side effect of the envelope itself activating it. So it's a very good point and we're going to be looking at that.

**MALE SPEAKER:** One quick last question?

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**MALE SPEAKER:** I'm Mark Mulatto with Act Up and Health Cap. I always love to hear you speak about immune activation Dr. Fauci. We've talked many years about this ourselves and the fact that we have control of our replication but we have not really controlled in inactivation. And you have always said to me you agree, we should be looking at ways to suppress the immune activation and yet I never see the trials. Where are the clinical trials of immunosuppressance, antiinflammatories, drugs that might be able to suppress this chronic immune activation? They never seem to occur.

**ANTHONY FAUCI, MD:** Good point, Mark. They are occurring. Some of them have been done; Jepi Pontelay has done some experiments with that. We did some experiments in animals. It's difficult because it can be dangerous. But having said that that there needs to be the very fine balance between blocking activation but not blocking it so much that you lead to a state of such immunosuppression or immunoinability to respond to the opportunistic infections that you get into trouble. There's an example of an individual that we saw in our clinic who was HIV infected and didn't realize it. Was on another service for an autoimmune phenomenon and was put on an activation blocker, mycophenelate and she presented with 4 opportunistic infections because they didn't' know she was HIV infected, so they obviously gave her more than she really needed. So your point is well taken. They should be done, but

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you've got to proceed with a great deal of caution because there's a very fine line between blocking the activation and shutting the virus off versus blocking it enough that you make a host very susceptible.

**MALE SPEAKER:** Thank you very much for coming out today and hearing Dr. Fauci's talk. I'd also like to invite people today to satellite on HIV treatment for drug users by the Thai Treatment Action Group in Room G at 6 o'clock tonight.

[END RECORDING]