DEAR READER

On December 5, 2002, The Center for AIDS hosted the second Basic Science Workshop on HIV. The meeting was cosponsored by the Center for AIDS Research (CFAR) unit at Baylor College of Medicine and held at The Woodlands Resort and Conference Center just outside of Houston. Approximately 40 people attended the day-long meeting, which featured a series of slide presentations, an activism roundtable discussion, and a keynote address by Sandra Bridges, PhD, from the National Institutes of Health. The theme of the meeting was novel therapeutic interventions.

The purpose of the Basic Science Workshop is to bring together researchers from Houston and around the nation to present their work, to discuss new ideas with their peers, and possibly to forge new collaborations. So why would a small, nonprofit, community-based organization like The Center for AIDS host a meeting of bench researchers? The reason is, quite simply, that only in research will we find a cure for HIV/AIDS. Almost everything that we know about HIV, from its shape to its lifecycle to its genetic composition, has been the result of basic science research. The therapies currently used to treat HIV disease have their origins in basic science exploration. Furthermore, HIV research has contributed a great deal to what we know about the human immune system and even some other diseases such as cancer. The Center for AIDS is committed to the search for a cure and recognizes that basic science research will continue to play a pivotal role in that search. By inviting researchers to present, to question, to ponder, and to interact meaningfully, The Center for AIDS works with the basic science community as a partner.

This issue of RI*TA!* covers the 2002 Basic Science Workshop in an effort to share this unique event with readers. Each presentation summary offers a glimpse at an exciting, cutting-edge area of HIV therapeutics or vaccine research. The stories of the science of HIV research seem so different, as if worlds apart. Yet there truly is only one story, one that we will know by heart—like the story of penicillin and the birth of antibiotics—when a cure for HIV/AIDS is found.

Many people continue to believe that a cure will come from a pharmaceutical company. Though such companies invest their fair share in research and development, a cure for HIV is just as likely to be discovered in a small laboratory at some academic, private, or government research institution. In truth, the pieces of this puzzle may come together from several areas. Nevertheless, we must advocate for and encourage all basic science research efforts. This is a challenge that The Center for AIDS enthusiastically accepts.

Finally, you may have heard that the Founding Director of The Center for AIDS, Joel Martinez, has stepped down as director and will pursue advocacy efforts as the organization's Director of Advocacy. I have agreed to serve as Interim Director, in addition to my work as editor, while the Board of Directors finalizes succession planning. It is an honor to run The Center for AIDS, and I hope I can do even half as good a job as Joel has done over the past 9 years. Also, I would like to welcome Jennifer Newcomb Fernandez, PhD, as a new contributor to The Center for AIDS publications. Jennifer is an accomplished writer and editor, as well as a member of the American Medical Writers Association. She will be a great addition to The Center for AIDS team.

Very truly yours,
The Center for AIDS:
Hope & Remembrance Project

Thomas Gegeny, MS, ELS
Interim Director & Editor

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The Second Basic Science Workshop

Sponsored by
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Objectives:

- To feature the work of prominent national and local bench researchers investigating the basic science of HIV infection.
- To promote dialogue and collaboration among Texas-based researchers and researchers in other parts of the country.
- To feature research that may translate to novel approaches to HIV therapeutics.
- To identify the implications for advocacy and activism in the work presented.

Presenters at the 2002 Basic Science Workshop (left to right): Michael Barry, PhD; Frederick Siegal, MD; Ronald Collman, MD; Sandra Bridges, PhD; John Shiver, PhD; Richard Sutton, MD, PhD; and Qizhi (Cathy) Yao, MD, PhD.

"As people who are directly and indirectly affected by this epidemic, we are very thankful for the work that you are doing. Many of us would not be here but for the work that you are doing."

— L. Joel Martinez, founder of The Center for AIDS, in his closing remarks at the 2002 Basic Science Workshop

HIV-1 Env-chemokine receptor interactions in primary human macrophages: entry and beyond

Ronald G. Collman, MD
University of Pennsylvania School of Medicine and
Penn Center for AIDS Research

Abstract

While HIV has subverted the **chemokine** receptors CCR5 and CXCR4 for its own use as an entry co-receptor, their normal functions are to **transduce** signals in response to extracellular **ligands**. Our lab is interested in understanding how HIV-1 glycoprotein 120 (gp120) may activate intracellular signals through these receptors in **primary** human **macrophages**, and how these responses may contribute to **pathogenesis**. Our studies demonstrate HIV-1 gp120 elicits several different types of signals in macrophages through CXCR4 and CCR5, including calcium elevations, ionic channel activation, non-receptor protein tyrosine kinase activation, and activation of MAP kinases. Receptor activation is triggered by both monomeric gp120 and whole HIV virus. Furthermore, gp120 elicits a number of functional responses in macrophages, such as secretion of chemokines and other soluble products, and we demonstrate that specific pathways linked to the chemokine receptors are responsible. These studies help illuminate the pathways through which chemokine receptors are coupled in primary macrophages, and provide a mechanistic basis for effects that HIV has on macrophage function. These signaling responses may play a role in the pathogenesis of organ dysfunction such as HIV **encephalopathy** and lymphocytic interstitial **pneumonitis** where macrophages are the principal infected cell type and inappropriate immune activation plays a central role.

Presentation Summary

Ronald Collman, MD, was the first speaker of the day. Collman's research focuses on interactions between the viral envelope and the receptor/co-receptor complex on the surface of the cell, specifically in macrophages. Though not as extensively studied as CD4 **lymphocytes**, macrophages play a critical role in HIV pathology. HIV-1 strains that infect macrophages (macrophage-tropic isolates) are responsible for person-to-person transmission and are the predominant virus type during the clinical latency phase. In addition, macrophages are the main infected cells in organs such as the brain and lungs, and appear to be responsible for inflammation and injury in these sites, which lead to neurological dysfunction and AIDS Dementia Complex (ADC), or pulmonary inflammation and Lymphoid Interstitial Pneumonitis (LIP).

Collman began his presentation by reviewing the structure of HIV and the specific interactions between the viral envelope and the cellular receptor complex, which comprises CD4 plus a chemokine receptor. While the CD4 receptor is required for infection by all naturally occurring HIV-1 strains, specific cell surface molecules called chemokine receptors also are necessary. He went on to discuss how HIV strains are defined on the basis of their **tropism**. Macrophage-tropic (M-tropic) strains infect primary macrophages and primary lymphocytes and use the chemokine receptor CCR5 as a co-receptor. T cell-tropic (T-tropic) strains infect primary lymphocytes but not macrophages, and use CXCR4 as the co-receptor.

Dual-tropic strains infect all 3 types of cells and use both co-receptors as entry pathways. In addition, some strains of HIV-1 can use a variety of other chemokine receptors in studies *in vitro*, however it does not appear that they have major relevance to HIV infection *in vivo* and pathogenesis. The use of CCR5 by M-tropic strains, CXCR4 by T-tropic strains, and both receptors by dual-tropic strains had suggested a simple paradigm for the cellular determinants of tropism whereby CCR5 would be expressed on macrophages, CXCR4 on T cells, and both types of co-receptors on primary lymphocytes. Collman's group has questioned to what extent this paradigm holds true for macrophages derived from *in vivo* samples, as well as the consequences of these interactions in macrophages.

To test the validity of this paradigm, Collman's lab performed a series of experiments using CCR5-deficient macrophages from individuals **homozygous** for the CCR5Δ32 polymorphism. This genetic polymorphism leads to an absence of CCR5 expression on cells from these individuals. M-tropic and T-tropic strains were unable to replicate in these cells. Surprisingly, 89.6, a dual-tropic strain, was able to replicate and blockade of the CXCR4 co-receptor prevented infection, demonstrating that macrophages have functional CXCR4 co-receptors that can be used for viral entry. This result was not specific to 89.6 and was observed with DH12, another dual-tropic strain. Furthermore, some strains that use CXCR4 only, such as UG021, could also infect macrophages through the CXCR4 co-receptor. Cell-cell fusion experiments confirmed that 89.6 uses both CCR5 and CXCR4 co-receptors on macrophages, while UGO21 uses macrophage CXCR4 exclusively. Collman explained that the co-receptor determinants of tropism are more complicated than originally believed, which led him and his colleagues to formulate a revised paradigm of tropism (see Figure 1). Macrophages express both CCR5 and CXCR4 and can be infected by M-tropic and dual-tropic strains, but not T-tropic strains. Some strains of HIV use both receptors *in vivo*, others use CCR5 or CXCR4 exclusively, and others may predominantly use one co-receptor in macrophages and another co-receptor in lymphocytes. It appears that use of co-receptors on target cells differs depending on the virus and that factors other than co-receptor expression determine tropism.

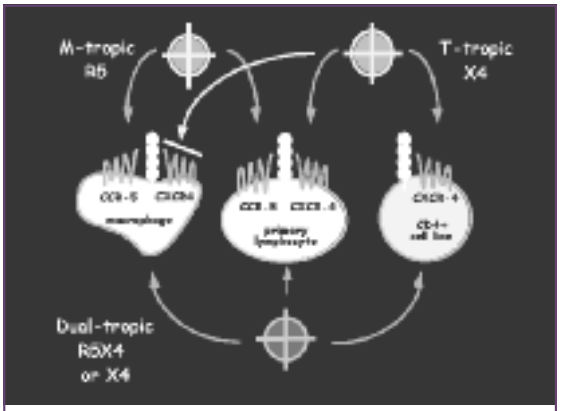

Figure 1. A model for the cellular determinants of HIV-1 tropism. Primary macrophages and primary lymphocytes express both CCR5 and CXCR4 (in addition to CD4), while T cell lines express only CXCR4. Macrophage (M)-tropic HIV-1 strains infect macrophages and lymphocytes via CCR5. T cell line (T)-tropic HIV-1 strains infect lymphocytes and T cell lines via CXCR4 but cannot use macrophage CXCR4 for entry. Dual-tropic strains infect all 3 cell types, either by using CCR5 and CXCR4 on macrophages and T cell lines, respectively, or through an ability to use CXCR4 on all target cell types.

The second part of Collman's presentation concentrated on inappropriate macrophage activation in HIV pathogenesis. Since the normal function of the chemokine receptors used by HIV-1 for entry is to activate cells in response to various stimuli, he hypothesized that the virus itself (or its envelope glycoprotein gp120 that mediates attachment to cells) might mimic natural activation signals and be responsible for inflammation and injury in organs like brain and lung where macrophages are infected and also activated. The researchers examined what effects gp120 has on intracellular signals in macrophages and how these changes relate to HIV pathogenesis. Specifically, Collman and his group investigated the effects of gp120 on ion channel activation, intracellular Ca^{++} levels, protein kinase activation, and the functional consequences of these changes on the target macrophage. Their data revealed that CCR5-binding ("R5") gp120 and CXCR4-binding ("X4") gp120 trigger a multitude of ion channel responses, including activation of Cl^- channels, Ca^{++} channels (in conjunction with the

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release of Ca^{++} from intracellular stores), non-selective **cation** channels, and Ca^{++}-activated K^+ channels. These ionic changes are mediated by the specific chemokine co-receptor, rather than the CD4 receptor, as shown by experiments using CXCR4 antagonists and CCR5-deficient macrophages. Whether the opening of these channels is necessary for viral entry has not been determined;

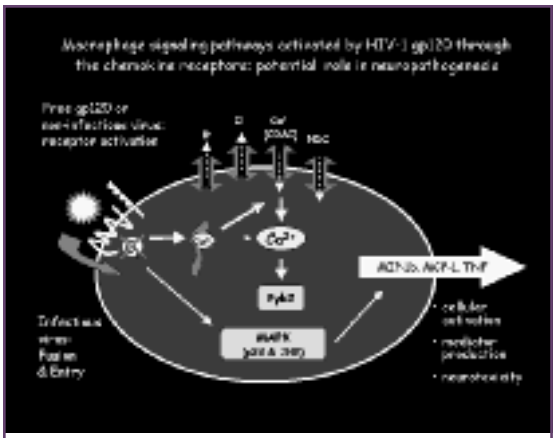

Figure 2. Intracellular signaling pathways that are activated by HIV-1 gp120 through the chemokine receptors. In addition to mediating entry and infection, interaction of gp120 with CCR5 and CXCR4 can induce functional changes in the cell. Several ion channels are triggered, intracellular calcium is elevated, and protein kinase pathways including Pyk2 and the MAP kinases are activated. Triggering of these pathways leads to activation of macrophages and secretion of soluble products, including products that can injure neurons.

these experiments are difficult to interpret since pharmacological blockade of these channels would have a dramatic effect on cell function, regardless of **Env** binding.

To explore further how interactions between the virus and these receptors might elicit cellular changes that contribute to HIV pathogenesis, his laboratory proceeded to run a series of experiments looking at protein kinases, enzymes important in relaying cellular signals. Exposure to R5 gp120 and X4 gp120 resulted in activation of proline-rich tyrosine kinase 2 (Pyk2), a non-receptor tyrosine kinase related to focal adhesion kinase. They showed that activation of Pyk2 is Ca^{++}-dependent and is mediated by CCR5 and CXCR4; binding only to CD4 is not sufficient for activation. Collman's laboratory also investigated effects of gp120 on the mitogen-activated protein (MAP) kinase family of signaling proteins. MAP kinases exert their effects through upregulation of transcription factors and function downstream of Pyk2 in several cell types. Both R5 gp120 and X4 gp120 activate the MAP kinases p38 and c-Jun amino terminal kinase/stress-activated protein kinase (JNK/SAPK), though it is unclear whether a third member of the MAPK family, extracellular regulated kinase (ERK 1/2), is activated. Collman's group has also observed that chemokines such as macrophage-inflammatory protein-1β (MIP-1β) and macrophage chemoattractant protein-1 (MCP-1) are secreted by macrophages in response to gp120, and that secretion of these inflammatory factors is dependent on MAP kinase activation. Exposure to gp120 also resulted in upregulation of tumor necrosis factor-α (TNF-α) mRNA expression that was partially inhibited by a MAP kinase antagonist. Though the exact mechanisms regulating macrophage secretion have not yet been elucidated, these data demonstrate that these intracellular cascades, specifically MAP kinase activation, are involved and necessary (see Figure 2).

Nevertheless, the question remains as to how these cellular changes relate to the pathogenesis of HIV. In ADC, infected macrophages, increased macrophage activation, reactive gliosis (activated **astrocytes**) and **apoptotic** neuronal death are manifest. Much work has been performed examining the mechanisms of AIDS dementia, though the majority of this work has concentrated on the neurotoxic factors secreted by macrophages in response to HIV. Little work has examined the mechanisms within macrophages that are responsible for the production of these toxic factors. Using a neuronal cell line, NT2.N, Collman's lab showed that neurons die in response to **supernatant** from macrophages exposed to gp120. Cell death was significantly decreased by treating the macrophages with a MAP kinase inhibitor before being exposed to gp120. Thus, the pathways for MAP kinase activation seem to be responsible for the specific products secreted by macrophages and the resulting neurotoxicity.

Collman concluded his presentation by describing how these pathways could contribute to HIV encephalopathy by causing continued inflammation, astrocytosis, activation of other macrophages, and recruitment of uninfected and infected cells into the brain (see Figure 3). He reminds us that the best correlate of clinical dementia is not the amount of HIV in the brain, but rather the amount of macrophage/**microglia** activation. This activation may be responsible for astrocyte activation and subsequent production of neurotoxins. Collman admits that many questions remain. His laboratory continues to examine the relationship between the intracellular changes in macrophages exposed to HIV and the resulting pathogenesis.

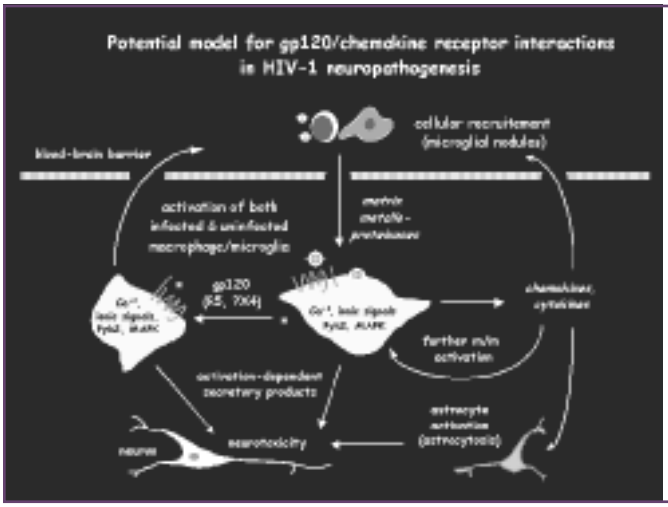

Figure 3. A model for how HIV-1 signaling through CCR5 and CXCR4 on macrophages (and the closely related microglial cells) in the brain can contribute to the pathogenesis of AIDS Dementia Complex (ADC). The viral glycoprotein gp120 (either as a free protein or on the surface of virus particles) can trigger activation of both infected and uninfected macrophages. This leads to secretion of mediators that attract inflammatory cells (such as lymphocytes and more macrophages from blood), that can activate astrocytes, and that may injure neurons directly.

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Interferon-producing plasmacytoid dendritic cells and the pathogenesis of AIDS

Frederick Siegal, MD
St. Vincent Catholic Medical Center

Abstract

Interferon-α (IFN-α) generation by peripheral blood mononuclear cells (PBMC) responding *in vitro* to HSV was first found to be deficient in patients with severe ulcerative herpes simplex virus (HSV) infections early in the AIDS epidemic. Such deficits were soon found to be associated with all opportunistic infections (OI). Further studies during the natural history of HIV infection indicated that OI did not occur so long as IFN generation remained relatively intact. OI occurred only when both IFN-α generation and CD4 T cell counts were sufficiently depressed. The IFN-α response to HSV was **innate**, not **adaptive**. Evidence that the IFN-α response to HSV was derived from a rare and previously undefined cell type prompted studies eventually revealing that the IFN-producing cells were identical to the "enigmatic plasmacytoid T cells" described by Lennert in lymphoid tissues in 1958. The normal functions of these cells appear to be diverse, but one such function involves initiation of the **Th-1** pathway in response to certain microbial antigens. The IFN-producing cells are now known as plasmacytoid **dendritic cells** (pDCs), because they differentiate following appropriate stimulation, into type-2 dendritic cells. During therapy for HIV infection, pDCs recover somewhat more rapidly than CD4 T cells to levels associated with resistance to OI, and their renewed response appears closely associated with clinically apparent immune reconstitution. Increased pDCs have been associated with nonprogressor status. In HIV infection and in certain other clinical states, PBMC IFN-α generation and pDCs numbers correlate closely, suggesting that numerical depletion of circulating pDCs is an important component of the immune deficiency of AIDS. Losses of pDCs during HIV progression and repletion during antiretroviral therapy could be involved in both the progressive loss and reconstitution of the Th-1 pathway.

Presentation Summary

Frederick Siegal, a physician at the St. Vincent Catholic Medical Center in New York City, began his presentation by discussing his first encounter with AIDS in 1980 when he and colleagues began seeing young homosexual males with severe HSV-induced ulcers; prolonged HSV infection of this type only occurs in severely immunocompromised people. Collaborating with Carlos Lopez and Patricia Fitzgerald-Bocarsly, Siegal began investigating herpes-specific immune responses in patients with AIDS. The group initially studied natural killing of HSV-infected cells, but quickly focused their efforts on identifying a specific type of peripheral blood mononuclear cell (PBMC) that possessed the innate ability to generate IFN-α in response to viral infection. The researchers referred to these elusive cells as "natural interferon producing cells" or "NIPCs." Interferons are proteins rapidly generated in virus-infected cells and they suppress infection by interfering with viral replication in neighboring cells. The researchers believed that identifying and understanding these NIPCs could provide insight into viral-induced immune responses.

Work in Siegal's and Bocarsly's laboratories demonstrated that HSV selectively stimulates NIPCs to generate IFN-α. The HSV response provided an excellent marker for studying these unusual cells. Siegal's group used herpes-infected target

cells as triggers for IFN-α production in PBMCs from healthy volunteers and patients with AIDS. Preliminary studies in cells from patients with AIDS revealed a relationship between the inability to make IFN-α and the likelihood of experiencing an OI. When patients who had an OI were prospectively studied, marked deficits in CD4 T cells counts and IFN-α production were observed. The researchers also analyzed cells from patients who had not yet experienced an OI, and discovered that these patients did not get an OI within 4 months of follow-up unless their CD4 T cell count dropped to fewer than 250 cells/mm^3 and IFN-α production decreased to less than 300 IU/mL, a value subsequently determined to be a critical level for preventing an OI. Patients with deficits in both CD4 T cell counts and IFN-α production tended to get an OI or die within 24 to 30 months, while patients with a deficit in just one of these factors were unlikely to experience an OI or die during the ensuing 36 months. In patients with AIDS, failure to generate IFN-α appeared to be just as important as failure to produce enough CD4 T cells when predicting clinical outcome.

Questioning whether the relationship between IFN-α production and susceptibility to intracellular pathogens and OIs was specific to diseases involving retroviruses, the researchers examined IFN-α production in patients with other immune-compromised conditions. Defects in IFN-α production occur in hairy cell leukemia (HCL), spindle cell thymoma with immunodeficiency, and in some cases of **idiopathic** CD4 T cell **lymphocytopenia** (ICL). Data collected from patients with HCL provided some insight into the origin of these cells. Untreated patients had very low levels of IFN-α production; however, when patients were treated with chlorodeoxyadenosine, normal levels of IFN-α were detected one year later. This time frame coincided with complete remission in the bone marrow, suggesting that the bone marrow could be the origin of NIPCs.

Convinced that the NIPCs were a crucial factor in the clinical outcome of patients with AIDS or other immunodeficiencies, Siegal and colleagues pursued identifying the specific cell type responsible for IFN-α production. Preliminary experiments showed that NIPCs were not natural killer or T cells. Study of these cells was complicated because NIPCs were present in only a small fraction of PBMC and were difficult to keep alive. Several laboratories were trying to enrich and isolate these cells. These efforts were only partially successful; data were difficult to interpret because of the large numbers of contaminating cells. At the same time, a future collaborator of Siegal's, Yong-Jun Liu, had developed a technique to separate a cell type he believed to be precursors of dendritic cells and recognized that these cells were similar to a cell described in the 1950s by Lennert, a German pathologist. Lennert had observed that there were plasmacytoid cells in deep cortical tissue of secondary lymphoid tissues that tended to cluster in areas with extensive apoptosis. Liu and his group also witnessed these cells (once purified to nearly 99%) undergo rapid apoptotic death that was prevented if cells were cultured with interleukin-3 (IL-3), a critical survival factor. When cultured in the presence of CD40 ligand and IL-3, these cells made an astonishing morphological transformation from smooth plasma-like cells to extremely complex dendritic cells, which the researchers referred to as "type 2 dendritic cells" or "DC2s." Moreover, they discovered that DC2s tended to promote **Th-2** immune responses *in vitro* when co-cultured with T cells. Liu and his laboratory struggled to determine what factor these secretory cells were producing. During this time, Siegal attended a presentation given by Liu discussing this work and was struck by the similarities between Liu's precursor DC2s and his NIPCs. A series of experiments showed that these cells were, in fact, the same and they referred to these cells as "pDC2s". They now had identified the origin of the elusive cell responsible for IFN-α production and published these findings in *Science* in 1999.

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Siegal discussed a simplified model of how pDCs may play a role at the interface of innate and adaptive immunity (see Figure 1). These cells circulate in the blood until they encounter a microbial stimulus, such as a virus. This microbe acts as a trigger for the subsequent immune response. Using toll-like receptors (TLR) and other receptors, pDCs may pick up these antigens through pattern recognition. Following migration to T-cell areas of lymphoid tissues, these cells produce IFN-α, which causes immature T cells to express IL-12 receptors. These T cells then receive an IL-12 signal from DC1s; however others believe that pDCs are also capable of generating this signal. This entire process fosters the Th-1 pathway. When cultured with IL-3, CD40-ligand, and virus, these cells can differentiate into mature DC2s, which foster the Th-2 pathway.

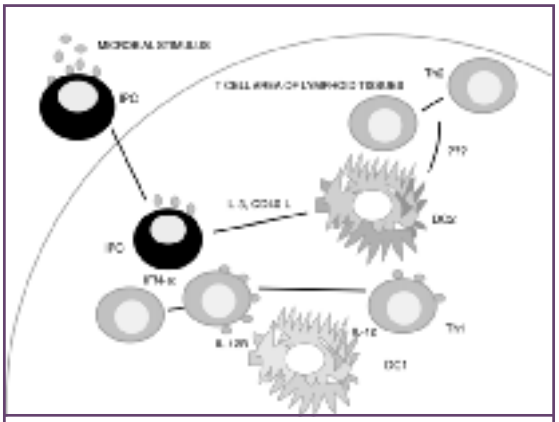

Figure 1. A model of how interferon-producing cells (IPCs or pDCs) may play a role at the interface of innate and adaptive immunity

Questions still remained about how pDC2s and IFN-α production affect HIV pathogenesis. As previously discussed, IFN-α production is substantially decreased in patients with AIDS, which the researchers discovered was caused by both decreased production per cell as well as a decreased number of pDCs. When Siegal's group examined IFN-α production in patients with AIDS over the last decade, they noticed an increase in IFN-α production that coincided with the introduction of AZT therapy; however, that increase subsequently waned. Antiretroviral therapy appeared capable of causing immune reconstitution of CD4 T cell counts and IFN-α production, which is reflected in the clinical outcomes of these patients. When patients are treated with antiretroviral therapy and made **aviremic**, CD4 T cell counts slowly recover in approximately 10 months. However, IFN-α production returns much faster, typically in about 4 months. Siegal's data demonstrated that when there is immune reconstitution of either IFN-α or CD4 T cells (usually with viral suppression), AIDS patients no longer experience OIs. However, when immune reconstitution does not occur, approximately half the patients experience an OI and die.

Siegal proposed a plausible model to illustrate the hypothetical role of pDCs in the loss of Th-1 immunity in HIV infection and how these events lead to HIV pathogenesis (see Figure 2). HIV is capable of infecting pDCs, as well as triggering pDCs to generate IFN-α. Once infected, pDCs traffic HIV into the T-cell areas of lymphoid tissues, where they generate IFN-α, upregulate IL-12 receptors, and produce HIV. Neighboring CD4 T cells are subsequently infected. As a result, the Th-1 immune response is completely abrogated and the specific Th-1 response to HIV is selectively eliminated. Moreover, as HIV infection progresses, pDCs are depleted, and IFN-α production becomes inefficient as viremia increases. This may explain why immature, naïve T cells do not become part of the Th-1 pathway. In this situation, the HIV-specific immune response is eliminated, as well as other specific Th-1 immune responses.

Recent work by Soumelis, Levy, Liu, and others independently confirms that IFN-α production is an important predictor of clinical outcome in AIDS.

For example, data collected from HIV-positive nonprogressors show that some of these patients have an abundance of circulating IFN-α-producing cells, which may explain why their disease has not progressed. Siegal and colleagues recently published data showing that there is a selective decrease in the number of pDCs as a person ages, though each cell continues to generate the same amount of IFN-α. People with an innate ability to make higher than normal levels of IFN-α, such as the young, may be more intrinsically resistant to primary HIV infection. Many questions remain about the specific mechanisms of pDCs *in vivo*. Whether pDCs differentiate into DCs *in vivo*, or if these cells produce as much IFN-α *in vivo* as they do in cell culture, remains unclear.

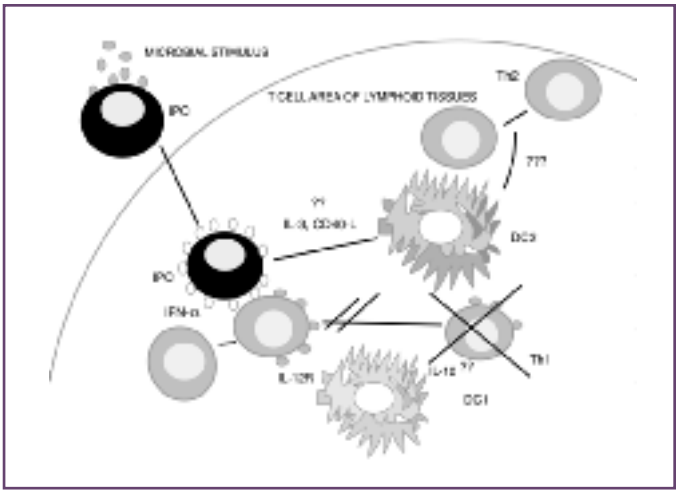

Figure 2. A model of the hypothetical role of interferon-producing cells (IPCs or pDCs) in the loss of Th-1 immunity in HIV infection and how these events lead to HIV pathogenesis

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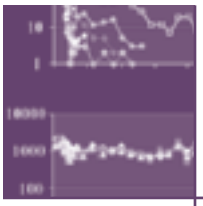

A non-replicating adenoviral vector as a potential HIV vaccine

John Shiver, PhD
Merck & Co., Inc.

Abstract

HIV-specific T cell immune responses will play an important role in any HIV vaccine paradigm. Studies in rhesus monkeys have shown that significant and persistent virus-specific T cell responses can be elicited with vaccines incorporating viral genetic sequences and that these responses are primarily mediated by CD8 T cells. Benefits such as stable CD4 levels and viral control have resulted. Two vaccine candidates developed by Merck and Co., Inc., including a non-replicating adenoviral vector, have been studied in animals and are now being studied in Phase I clinical trials in humans. Important considerations include cross-**clade** reactivity (effectiveness in diverse HIV-infected populations), tolerability, and durability of response. Ongoing studies are looking at responses in both uninfected and infected individuals. Optimal vaccine combinations as well as the development and testing of vaccines with multiple genetic targets are part of future plans investigating this vaccine strategy.

Presentation Summary

The first speaker in the afternoon session was John Shiver, PhD, a researcher with Merck and Co., Inc. His presentation, "A non-replicating adenoviral vector as a potential HIV vaccine," began with an outline of the clinical and immunologic goals of the Merck vaccine research program. Clinical goals include decreasing the likelihood of persistent virus infection and establishing a clinically significant lower viral load subsequent to infection. Immunologic goals include eliciting HIV-1-specific CD8 (cytotoxic or CTL) and CD4 (helper) T cell immune responses and directing a broad response against multiple viral determinants in the infected host. Shiver's group has focused on the cellular immune response for several reasons. First, research has generally shown that neutralizing antibody responses are weak and virus type specific. Second, the work of several groups demonstrates that control of initial viremia following infection in humans correlates with the detection of anti-HIV CTL responses, but not with antibodies. Finally, experimental data from studies in rhesus monkeys infected with Simian Immunodeficiency Virus (SIV) indicate that viral control is a function of the antiviral CD8 T cell response.

Merck is currently investigating 2 vaccine candidates that encode **codon**-optimized HIV-1 **gag**, **pol**, and **nef** genes based on consensus sequences. The first candidate is a **plasmid** DNA vaccine encoding full-length p55 gag (no pol) that is delivered in saline intramuscularly with or without **adjuvant**—alum or a "CRL1005" polymer. A 5-mg dose that uses the CRL1005 adjuvant consistently showed the best results, with CD8 T cell responses in particular, in rhesus monkeys immunized at 0, 4, and 8 weeks. The second vaccine candidate uses a non-replicating or "replication-defective" **adenovirus** type-5 vector (Ad5) containing an optimized gag sequence. In several studies, a dose of 10^{11} virus particles (vp) or 24 μg of adenovirus protein, which appears to be the upper limit of tolerability in the animals stud-

ied, elicited strong anti-gag responses in blood samples from animals vaccinated at 0 and 24 weeks. In addition, CD8 T cell responses (interferon-γ production) were much stronger than CD4 responses. The Merck group also has looked at a "prime/boost" strategy using these vaccines. The best T cell responses were found when the DNA/adjuvant vaccine was used first as a primer, followed by the adenovirus vaccine later on as a boost. One additional consideration is pre-immunity to Ad5, which can lower T cell responses when the Ad5 vaccine is used; Ad5 immunity is fairly common in humans.

In another group of studies, the researchers have used several vaccine groups in rhesus monkeys infected with a chimeric HIV-SIV virus, known as SHIV. All vaccines encoded the same codon-optimized SIV p55 gag gene. Envelope (env) was specifically excluded to separate the contribution of neutralizing antibodies, even for priming, versus the challenge virus. In each animal, a challenge with SHIV occurred 3 months after the last immunization. In the animals given DNA vaccines, a transient loss of CD4 cells occurred (lymphopenia) that was not seen in animals given adenoviral vector vaccines. Viral control was best in the animals given the Ad5 and DNA+CRL1005 vaccines. In addition, animals given the Ad5 vaccine have shown stable CD4 T cell levels and viral control out to 2 years after SHIV challenge (see Figure).

In humans, one important consideration for making an effective vaccine is genetic variability of HIV. Epidemiologic comparison studies show that the viral genes gag, nef, and pol are the most conserved across the various viral clades found in different parts of the world. Therefore these genes represent major targets of T cell immune responses in HIV-infected humans. In addition, there seem to be substantial cross-clade T cell responses for gag and nef in humans.

The second part of Shiver's presentation was an update on Merck's vaccine clinical program looking at vaccine candidates in Phase I study in HIV-infected and uninfected human subjects. At this meeting, he was able to present data on the HIV-1 gag-expressing DNA vaccine with just saline (no adjuvant) and on the HIV-1 gag-expressing Ad5 vaccine in uninfected humans. For the DNA vaccine group (n=109), injections of placebo (n=24), 1 mg of vaccine (n=42), or 5 mg of vaccine (n=43) were administered at 0, 4, 8, and 26 weeks. The vaccine was generally well tolerated with some injection site tenderness and a few complaints of headache and muscle aches. The best responses were seen in the group given the 5-mg dose of vaccine, but even at week 30, fewer than half (42.1%) of the subjects in that group had gag-specific T cell responses. In the Ad5 vaccine studies, uninfected volunteers were given doses at 0, 4, and 26 weeks with placebo

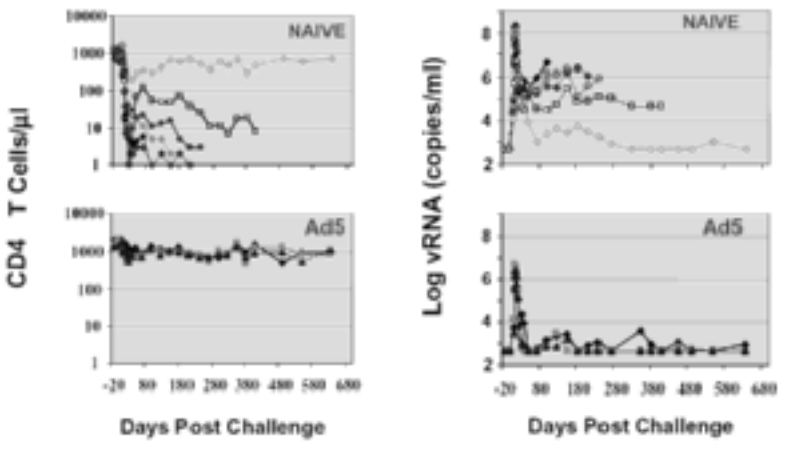

Figure. SHIV challenge of Ad5-SIV gag immunized rhesus monkeys: Durability of viremia

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(n=22) or different concentrations of viral particles: 10^8 (n=17), 10^9 (n=16), 10^{10} (n=24), and 10^{11} (n=26). The vaccine was well tolerated overall, but adverse events (mild or moderate injection site pain, fever less than 102°F often with malaise or chills lasting about 24 hours) occurred at higher doses, with fewer symptoms after subsequent injections (rechallenge). In general, about two-thirds of the vaccinated subjects had significant anti-gag T cell responses. Overall, this preliminary Phase I clinical data from uninfected human subjects show that the Ad5 vaccine is more immunogenic (67% responders across all doses) than the DNA vaccine and that cross-clade anti-gag responses can be induced using these vaccines.

Shiver concluded by pointing to the next steps in Merck's vaccine research program. First, the researchers are working to complete the current clinical studies to allow selection of the best vaccine combination for continued trials. Second, work is underway to introduce additional vaccine components, such as pol and nef. Third, the clinical trials will be expanded internationally and vaccine evaluations will be continued in HIV-infected subjects.

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Generation of multivalent genome-wide T cell responses in HLA-A*0201 transgenic mice by an HIV-1 expression library immunization (ELI) vaccine

Rana A. K. Singh, PhD, and Michael A. Barry, PhD
Baylor College of Medicine and Rice University

Abstract

HIV-1 is a fundamentally difficult target for vaccines because of its high mutation rate and its repertoire of immune evasion strategies. To address these difficulties, a **multivalent** genetic vaccine or "live genetic vaccine" was recently developed against HIV-1 using the expression library immunization (ELI) approach. In this HIV-1 vaccine, all **open reading frames** of **HTLV**-IIIb are expressed as protein fragments to retain all viral T cell **epitopes**, but destroy protein toxicity, inactivate immune escape functions, and reveal subdominant epitopes. In addition, each **antigen** fragment is fused to the **ubiquitin** protein to increase antigen expression and target these antigens to the **proteasome** to enhance cytotoxic T lymphocyte (CTL) responses. This multivalent vaccine also has the advantage of being incapable of generating infectious HIV-1 virus because of the segregation of the HIV genome into 32 separate **plasmids**. In this work, we demonstrate the ability of this genetic vaccine to provoke robust **HLA**-A*0201-restricted T cell responses in MHC class I humanized mice against **gag**, **pol**, **env**, and **nef** after a single round of immunization. In addition, this HTLV-IIIb-derived vaccine demonstrated cross-**clade**, envelope-specific, HLA-restricted CD8 responses against clades A, D, and E. HLA-restricted CD8 responses were generated against all 32 open reading frames encoded by the multi-plasmid genetic vaccine demonstrating that a broad repertoire of human relevant CD8 responses are provoked by this vaccine. This work supports this approach to generate multivalent T cell responses to control the highly mutable and immuno-evasive HIV-1 virus.

Presentation Summary

Michael Barry, PhD, opened his talk by acknowledging that his work focuses on developing new technologies and is not primarily immunologic or virologic in nature. Barry's lab works in gene therapy, including the use of gene therapy in vaccines, a strategy known as genetic immunization. The process basically involves putting antigens into plasmids and injecting them into animals or humans (with a syringe or a gene gun) to elicit immune responses (see Figure 1). This strategy appears capable of eliciting both cellular and **humoral** immune responses. An advantage of genetic vaccines is simplicity of the system overall to deliver antigens intracellularly, causing CTL responses. Also, DNA functions as a stable vaccine and is not tied to the biology of the pathogen. In other words, there is no risk of infection, yet there is high-level antigen expression; problem pathogen antigens (such as those involved in

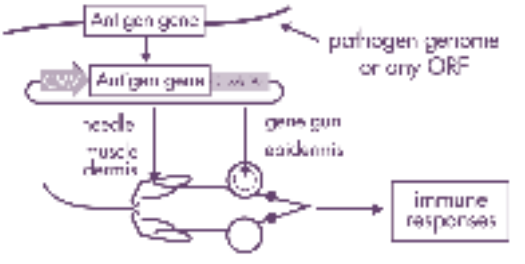

Figure 1. Genetic Immunization

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immune evasion) can be removed, or other genes added; and antigens can be easily manipulated using recombinant DNA technologies that are now well established.

One challenge to using live-attenuated vaccine for HIV is that the vaccine uses viral proteins to elicit immune responses. However, the virus has evolved to evade the immune system, which vaccines themselves are intended to activate. Therefore, Barry believes that any potential vaccine would become biased *against* the immune response. So, how can an effective vaccine be created to target a virus that evades the very immune system that the vaccine activates? To overcome this dilemma, the best possible antigens would be needed to elicit the best possible immune response. A useful approach may involve the use of an expression library, whereby the genome of a pathogen is fragmented and the pieces of DNA captured to create a pathogen library that represents most of the T cell epitopes that exist for that pathogen. The library can be broken into sublibraries and used in animal immunization studies to select for ideal antigens to use in vaccines. Such libraries can be created through a random sheering process or through a directed process with fragments created deliberately at specific points.

The strategy of using expression libraries as vaccines has worked with several types of bacterial pathogens. Considering HIV's relatively small genome, Barry's group has been looking at using an entire expression library as a "genomic vaccine" representing all or many of HIV's antigens. The question is whether such an expression library immunization (ELI) vaccine could deliver multivalent epitopes to drive immune responses to HIV. Barry and his colleagues have been studying such a vaccine, which uses 32 plasmids and is derived from HTLV-IIIb (see Figure 2). The vaccine antigens have been fused to ubiquitin, which has been shown to enhance MHC presentation and CTL responses. The group's work in mice has demonstrated that the ELI vaccine was able to elicit CTL responses against subdominant epitopes of gag, whereas a protein vaccine of gag plus **adjuvant** was able to include only one such response against the main gag epitope. Such broadened responses against one viral protein target may reveal new ways of effectively targeting CTL responses against "hidden" or subdominant epitopes of HIV.

In a Center for AIDS Research (CFAR) collaboration at Baylor College of Medicine, the vaccine was studied in rhesus macaques and, in at least some animals, seemed to generate multivalent T cell responses that apparently correlated with better control of viral load after challenge with a hybrid simian-human immunodeficiency virus (SHIV). Macroaggregated **albumin** (shown to target lung tissue) was used as an adjuvant to help elicit systemic as well as mucosal responses. Since the ELI vaccine that was used generates random epitope fragments, **codon** optimization may produce more robust CTL responses.

Studies in mice indicate that the presence of multiple antigens (32) does not appear to interfere with immune responses. Also, the immune responses appear to be HLA-restricted. In a study evaluating the relative contribution of each of the 32 plasmids to the vaccine's **immunogenicity**, 32 cell lines were created expressing HLA-A*0201 and one of the library members. When interferon-γ production was measured in these cell lines after 6 hours of *in vitro* stimulation, every library member appeared to generate a response, thus suggesting a multivalent response. In another study, HLA **transgenic** mice were immunized with a gag-pol plasmid and an env plasmid. Next, T cell responses were measured against the panel of 32 cell lines. Fairly strong

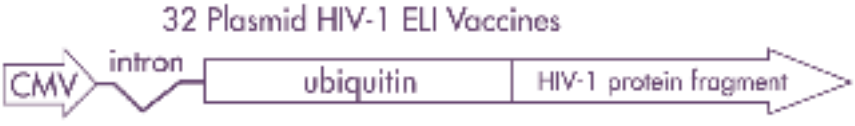

Figure 2. Vaccine candidate

responses were seen in the genomic areas coding for the immunodominant epitopes gag, pol, and env. In contrast, when the ELI (whole library) vaccine was used, strong responses were still seen in the gag, pol, and env regions, but also in adjacent regions, further suggesting a multivalent response (see Figure 3). Ongoing research is looking at whether such responses can be elicited across clades. Preliminary research thus far suggests some cross-clade CD8 T cell responses.

Next steps include codon optimization of the libraries, investigation of other potential vectors, and the use of gene delivery vectors (like engineered adenoviruses with specific **ligands** attached) that can target immunologically relevant cells. The use of targeted vectors would decrease the amount of particles needed and the immunization of cells that do not play immunological roles.

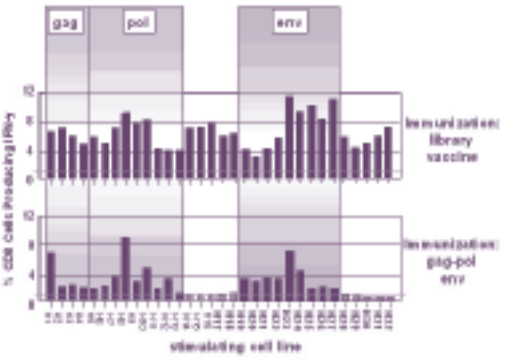

Figure 3. Immune responses generated with whole-library vaccine versus epitope-targeted vaccine

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Enhancement of mucosal immune responses by chimeric influenza HA/SHIV virus-like particles

Qizhi (Cathy) Yao, MD, PhD
Baylor College of Medicine

Abstract

To enhance **mucosal** immune responses using simian-human immunodeficiency virus-like particles (SHIV VLPs) as a mucosal HIV vaccine, we have produced phenotypically mixed, **chimeric** influenza HA/SHIV 89.6 VLPs and used them to immunize C57B/6J mice **intranasally**. Systemic and mucosal antibody responses, as well as cytotoxic T cell (CTL) responses, were compared in groups immunized with SHIV 89.6 VLPs or HA/SHIV 89.6 VLPs. Intranasal immunizations were given using VLPs either with or without the addition of the mucosal **adjuvant** cholera toxin. Total serum IgG, IgG1 and IgG2a, and IgA in saliva, vaginal lavage, lung wash, and fecal extracts were evaluated by enzyme-linked immunosorbent assay (ELISA). The level of serum IgG production to HIV **Env** was highest in the group immunized with chimeric HA/SHIV 89.6 VLPs. Similarly, mucosal IgA production was also enhanced in the mucosal HA/SHIV 89.6 VLP-immunized group. Analysis of the IgG1/IgG2a ratio indicated that a **Th1**-oriented immune response resulted from these VLP immunizations. High levels of serum IgG and mucosal IgA against influenza virus were also detected in mice immunized with HA/SHIV VLPs. HA/SHIV 89.6 VLP-immunized mice also showed significantly higher CTL responses than those observed in SHIV 89.6 VLP-immunized mice. Furthermore, a Major Histocompatibility Complex (MHC)-class-I-restricted T cell activation ELISPOT assay showed elevated interferon-γ, interleukin-2, and interleukin-12 production in HA/SHIV 89.6 VLP-immunized mice, indicating that phenotypically mixed HA/SHIV 89.6 VLPs can enhance both humoral and cellular immune responses at multiple mucosal sites. Therefore, chimeric HA-containing VLPs represent a potential approach for mucosal immunization for prevention of HIV infection.

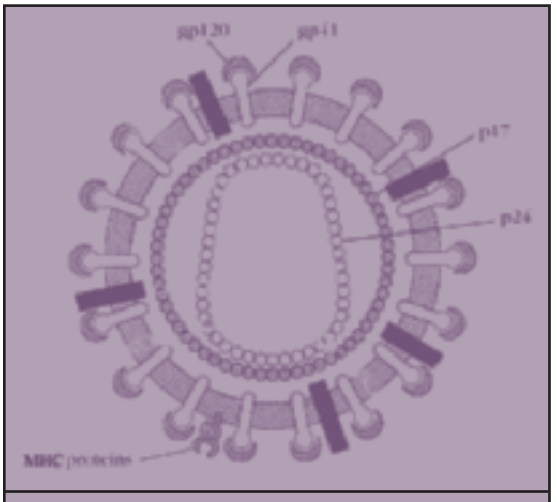

Figure. Virus-like particle resembling HIV.

Presentation Summary

Cathy Yao, MD, PhD, began her presentation by explaining that mucosal tissues are major sites of HIV entry and initial infection, and hypothesizing that mucosal immunization could induce remote mucosal site IgA production. In addition, mucosal administration of live, attenuated simian immunodeficiency virus (SIV) or HIV viruses presents safety concerns. Instead, virus-like particles (VLPs) are an attractive approach for developing effective HIV vaccine candidates since these particles can induce both humoral and cellular immune responses, and they can be administered repeatedly. These particles contain Env anchored to the viral envelope, thus retaining native conformation, and although they neither replicate nor contain the HIV genome, they do

closely resemble intact HIV virions (see Figure). Finally, VLPs can induce **neutralizing antibody** and CTL responses.

Yao's lab has been looking most recently at SHIV VLPs that incorporate a human influenza virus component. Yao noted that other VLPs (Rotavirus, Norwalk virus, Papillomavirus, etc.) are capable of stimulating mucosal immune responses and that intranasal immunization with SIV VLPs and SHIV VLPs can induce both systemic and mucosal immune responses. Also, mucosal immunization with formalin-inactivated influenza virus can induce strong protective responses against virus challenge in CD4 T-cell-deficient mice. The VLPs produced in Yao's lab contain truncated envelope protein rather than full-length Env because the truncated version results in better incorporation into VLPs.

Several series of experiments in mice have led Yao and her colleagues to the following conclusions:

- A successfully produced, phenotypically mixed, influenza HA/SHIV 89.6 VLP can be created by using a baculovirus expression system;

- Intranasal immunization of HA-containing SHIV VLPs elicits augmented humoral and cellular immune responses in both systemic and mucosal compartments;

- The common mucosal immunization system was activated since IgA was produced at multiple mucosal surfaces;

- HA-containing SHIV VLPs enhance Th1-type **cytokine** production (interferon-γ, interleukin-2, and interleukin-12) in both systemic and mucosal sites;

- The adjuvant activity of HA was higher than mucosal adjuvant cholera toxin in inducing neutralizing antibodies; and

- Chimeric HA/SHIV VLPs could also induce neutralizing antibodies against HIV 89.6 in CD4 T-cell-deficient mice.

Some studies have shown that **intraperitoneal** immunization may prime peritoneal B cell precursors for IgA production. These studies suggest that a combination of mucosal and systemic immunization may optimize the mucosal immune response. To test this hypothesis, Yao's group began further studies 1) to compare both humoral and cellular immune responses induced by intranasal or combined intraperitoneal/intranasal SIV VLP immunizations and 2) to confirm the adjuvantic property of phenotypically mixed HA/SIV VLPs other than HA/SHIV 89.6 VLPs. They found that the combination of intraperitoneal and intranasal immunization with SIV VLPs were able to enhance mucosal IgA production as well as CTL responses (as measured by interferon-γ levels). They also found that HA-containing HA/SIV VLPs showed higher immune responses than SIV VLPs and both humoral and cellular immune responses observed in HA/SIV VLP immunized groups were equal to or greater than in those groups immunized with the mucosal adjuvant cholera toxin.

Yao's group is next looking into why the influenza virus component HA enhances the immune responses in immune-deficient mice, as well as whether other immune cells (such as B cells) are also affected. Studies on VLPs, and the best ways to administer them, may lead to the development of safe and effective HIV vaccine candidates.

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Development of a mouse model for HIV/AIDS

Richard E. Sutton, MD, PhD, Ayse K. Coskun, MD, and Van Nguyen
Baylor College of Medicine

Abstract

A small animal model would be very valuable for HIV/AIDS vaccine testing, investigating HIV pathophysiology, and exploring anti-HIV therapeutics. Unfortunately, HIV does not replicate in mouse cells. Provision of mouse cells with human CD4, CCR5 and cyclin T1 (cycT1) has uncovered a block to HIV assembly or release. Since mouse-human cell fusions allow viral replication, mouse cells lack at least one critical factor that permits completion of the viral life cycle. To identify this factor(s) we are employing 2 similar genetic approaches. Each cell line of a panel of **monochromosomal** mouse-human somatic cell hybrids was individually **transduced** with an HIV vector encoding both cycT1 and blasticidin resistance (HIV-CIB). Each was then **transfected** with vesicular stomatitis virus (VSV) G protein and measurable virus was recovered from only the hybrid-containing chromosome 2. This was verified with an M-**tropic** envelope and was shown to be specific to HIV. In addition, the amount of p24 release from that hybrid was substantially greater than that from the parent. A second cell line expressing chromosome 2 had a similar phenotype. CycT1 has been introduced into one chromosome 2 line to monitor the spread of HIV. In a related but separate approach, an entire collection of ~500 mouse-human microcell hybrids was transduced with HIV-CIB and broken down into manageable pools. Virus was similarly recovered as above from a few of the pools. Those pools were then broken down to clones and several cell **clones** have been identified that allow virus release. Revertants that no longer have the human chromosome are now being tested for loss of phenotype. Clones will then be tested for ability to support both HIV replication and **Gag** processing. Human chromosomal content of the clones of greatest interest will be determined by STS content analysis. Results from the 2 approaches are expected to be in agreement and may provide direction for an expression cloning approach.

Presentation Summary

The final presentation of the afternoon, given by Richard E. Sutton, MD, PhD, was about the development of a mouse model for HIV/AIDS. Sutton first outlined some of the reasons why a small-animal model of HIV disease would be beneficial. He admitted that while such a model might not be as useful in therapeutics development, there could be considerable benefit for vaccine testing and **pathogenesis** research. Also, the animals used in the non-human primate model are expensive and scarce, thus preventing some research from moving forward more quickly. A mouse model of HIV/AIDS would allow the application of **transgenesis** techniques and the initial testing of many candidate vaccines that have not yet been tested because of a shortage of rhesus monkeys. In addition, mouse studies have already resulted in some discoveries about HIV, including the identification of co-receptors, cyclin T1 (a necessary co-factor for **Tat** and **TAR** during **transcriptional** elongation), and host factors required for viral assembly and release.

Mouse cells have been modified to express CD4, a co-receptor, and cyclin T1, and the processes of HIV entry, integration, and transcription can be reproduced in mice. However, virus release from cells has not been achieved. HIV-infected mouse cells exhibit gag precursors (p55) and little **capsid**. Fusion of mouse with human cells causes a marked increase in viral production, suggesting mouse cells lack certain factors that are needed to release virus. For instance, viral protease may require a co-factor found in human cells or the Gag protein may be incorrectly **ubiquitinated** in mouse cells. As Sutton noted, the possible explanations abound for why this occurs, and there are 2 approaches to solving the problem: biochemical and genetic. Sutton's lab has chosen the latter.

The group used mouse-human somatic cell hybrids to screen a panel of mouse cell lines each containing a single human chromosome. The hybrids containing human chromosome 2 produced markedly higher levels of HIV than the other monochromosomal hybrids. Other tests show that this characteristic carried on chromosome 2 appears to be specifically associated with HIV release. Also, the cells containing chromosome 2 had levels of p24 and p17 comparable to human cells, and **supernatant** from the cell cultures expressing chromosome 2 contained viruses that were able to infect cells at a similar frequency as supernatant from infected human cells. Mouse cells without human chromosome 2 did not show these effects.

Another approach used by Sutton's group involves a collection of microcell hybrids where each clone was derived from a mouse melanoma cell line and contains a small amount of a single human chromosome. An HIV-based vector encoding cycT1 was introduced into the entire collection. The clones were separated into smaller pools and **media** from each clone was tested for viral infectivity of human cells. Certain cell clones produced very good HIV release. Ongoing preliminary analysis has shown that some of these clones contain part of chromosome 2. The lab will also look at whether reverting the phenotype in these clones will cause the cells to lose the ability to release infectious virus.

Sutton and his colleagues have also begun to employ expression-cloning strategies using an HIV **cDNA** expression vector. This strategy allows the introduction of a cDNA library into non-dividing cells and subsequent completion of complex functional selections. The group plans to use this technology for several investigations including the identification of unknown viral receptors as well as human host factors that are critical for HIV replication. Initial work has involved the introduction of the cDNA library into mouse cells containing cycT1. The cDNA and vector were rescued by transfecting the mouse cells with packaging vector and vesicular

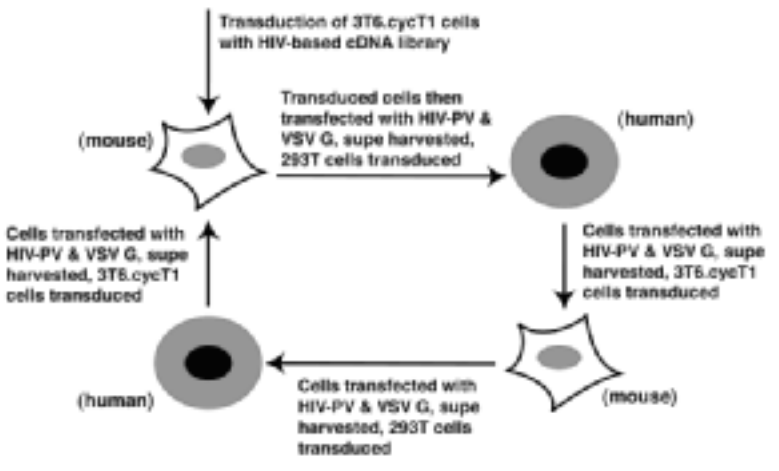

Figure. Scheme for recovery of host factors during HIV replication in the mouse

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stomatitis virus G (VSV G). The released virus was then amplified in human cells—only as an intermediate to increase the levels of virus, which were very low in the mouse cells. The virus was recovered from the human cells and reintroduced into mouse cells. This process was repeated several times to enrich for vectors encoding cDNA that allow completion of the viral lifecycle (as seen with the cell hybrids containing chromosome 2; see Figure). The amount of virus recovered increased after each round of this process, indicating the enrichment of some factor possibly responsible for improving virion release. However, cDNAs recovered at this point were considered artefactual and not relevant to the HIV life cycle. Therefore, the cDNA vector used in this set of experiments was not considered optimal and an improved vector is already being tested. Sutton admits that using the human cells as an intermediate step for amplification may not have been ideal, and future studies will look at ways to improve the selection strategy (without human cells). The group is considering a standard, albeit laborious, cDNA screening approach. If such a virus-release factor was isolated, one goal would be to create a quadruply-transgenic mouse to study HIV pathogenesis and vaccine development. Another objective would be to learn how the putative factor might increase particle infectivity. One application of this work may be the eventual identification of a novel therapeutic target.

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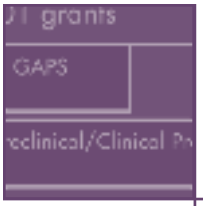

Basic science priorities for therapeutics research

Sandra Bridges, PhD
Division of AIDS, National Institute of Allergy and Infectious Diseases

Abstract

For more than 10 years, drug discovery efforts have concentrated on relatively few viral targets: reverse transcriptase (RT) and protease (PR). While viral load can be reduced by combining RT and PR inhibitors in regimens referred to as highly active antiretroviral therapy (HAART), recent studies suggest that many treatment failures occur because of the development of drug resistance and lack of adherence to complicated and often toxic regimens. Recently, new classes of agents directed at the virus binding and entry process have entered the development pipeline, with one such agent now approved for use in patients with HIV. Early data suggests, however, that the development of resistance will continue to be a problem as new agents are introduced into HAART regimens. With regard to the immune system, it has become clear that the damage caused by HIV infection is only partially reversed by HAART. Vaccination represents a major new immunologic approach to complement drug treatment. Thus, while advances continue to be made, there remains an urgent need for the identification of new host and viral targets, novel drugs and delivery systems, and immunologic approaches to address the dual problems of drug resistance and toxicity. To address this need, the Division of AIDS has established basic science priorities in the following areas of therapeutics research: host and viral targets, inhibitors, vaccines, **innate** immunity, viral reservoirs, and gene therapy.

Presentation Summary

Sandra Bridges, PhD, is the Chief of the Targeted Interventions Branch in the Basic Sciences Program at the Division of AIDS (DAIDS) at the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Department of Health and Human Services (DHHS). Part of her job as a program officer at DAIDS is to formulate the basic science priorities for HIV/AIDS therapeutics research and then to facilitate research in those areas. In her keynote address, Bridges outlined the most important questions of basic science research as identified in an earlier basic sciences program retreat in which she participated.

Are there important viral or host targets yet to be exploited? The most commonly used therapies target 2 viral enzymes, reverse transcriptase (RT) and protease (PR), and a great deal of research is being done on improving dosing, overcoming problems of viral resistance, reducing toxicities, etc. However, not enough emphasis is being placed on identifying

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new targets that, for instance, would not be affected by viral RT or PR resistance. Some of the targets that might be important include structural elements (such as the highly conserved zinc finger motif of the HIV **nucleocapsid** or the viral enzymes integrase and RNase H), regulatory and accessory proteins, processes (such as **transcription**, nuclear import and export of viral **nucleic acid**, and macromolecular interactions), and host targets (adhesion molecules, transcription factors, **apoptosis**, and signaling pathways). One important aspect of working on a new target for an anti-HIV drug is to validate the target *in vivo* using an animal model.

What are the best candidates for development as topical microbicides? Bridges noted that research in this area is still in its infancy. Such agents might be HIV-specific or nonspecific, and one challenge has been to identify chemical classes of potential **microbicides**. Nonhuman primates are being used to study topical microbicides for the prevention of sexual transmission, but such a model has not yet been validated (ie, there is no "gold standard" to match any success against) and the development of other animal models may prove useful. Also, some experimental drugs that are not suitable for systemic use because of toxicity or unfavorable physical characteristics (eg, solubility) may be applicable as topical microbicides.

Can innate immunity be manipulated to benefit HIV-infected individuals? The identification of molecules that affect innate immunity remains a challenge. Bridges pointed out that it might be better to find agents that *stimulate* innate immune cell expansion and function *in vivo* rather than simply to administer already identified molecules, such as cytokines, which may be insufficient to mimic the complex interactions that lead to responses *in vivo*. Fully characterizing innate immune responses in infected humans or animals remains to be accomplished, as does the development of nonhuman primate models to study potential interventions.

Can vaccines be useful in the treatment of HIV-infected individuals in the era of HAART? With therapeutic vaccine candidates, the route and schedule of administration, as well as the type of patient populations (for instance, acutely versus chronically infected patients) that can most benefit from such vaccines, have yet to be determined. In addition, there are safety issues to consider. Early attempts to study therapeutic vaccines did not yield promising results because viremia could not be controlled. However, according to Bridges, HAART offers a second chance for such vaccines to be studied, and this area of research is a high priority for the basic science program at DAIDS. The goal of this approach would not be to cure HIV, but to allow infected individuals to better control viremia, to build better immune responses, and to spend less time on HAART.

Can viral reservoirs be targeted therapeutically? Viral reservoirs continue to be an area of interest in HIV research. One challenge in this area is that new reservoirs in the human body continue to be discovered. Some reservoirs comprise cells that are transcriptionally silent, while others contain cells that allow restricted HIV replication. Ongoing work aims to identify molecules or agents that can "purge" reservoirs. Recently, Robert Siliciano's group has developed a complete set of reagents for quantitating reservoirs in nonhuman primates. This development will be helpful in assessing the actual effects of new therapeutic vaccines or pharmacologic agents on the reservoirs (eg, determining whether the reservoir has been reduced and by how much). Several preclinical and clinical studies

on viral reservoirs have been carried out or are in progress using agents such as interleukin-2, OKT3, interferon-γ, and prostratin.

What are the current obstacles to the clinical assessment of gene therapy strategies for HIV/AIDS? Now that several therapeutic targets have been identified for HIV, gene therapy in HIV research faces the same challenges that confront gene therapy for any disease. One such problem is low **transduction** frequency. Other challenges include establishing long-term and stable expression of **transgenes**, ensuring survival and expansion of transduced cells *in vivo*, and improving delivery of gene therapy.

In conclusion, Bridges reviewed the resources available to researchers through the Division of AIDS. In terms of funding, besides the unsolicited R01 grants, innovation grant programs in therapies (R21 grants) are now available. Another funding program is "Targeting Research Gaps." A third program deals with translational research, which brings mature therapeutic concepts from basic science into clinical study in small numbers of patients, usually with a commercial partner. Several vaccines and other therapeutic approaches are being tested through this program (see Figure). Other resources include the NIH AIDS Research and Reference Reagent Program, as well as the Inter-Institute Program between the National Cancer Institute (NCI) and NIAID. The Inter-Institute Program facilitates access to specialized contract resources such as pharmacology and toxicology, drug resynthesis, immunology, confirmatory *in vitro* testing, and testing in animal models including SCID-hu mice, nonhuman primates, etc.

For more information and related resources, visit ***www.niaid.nih.gov/daids/*** on the Web.

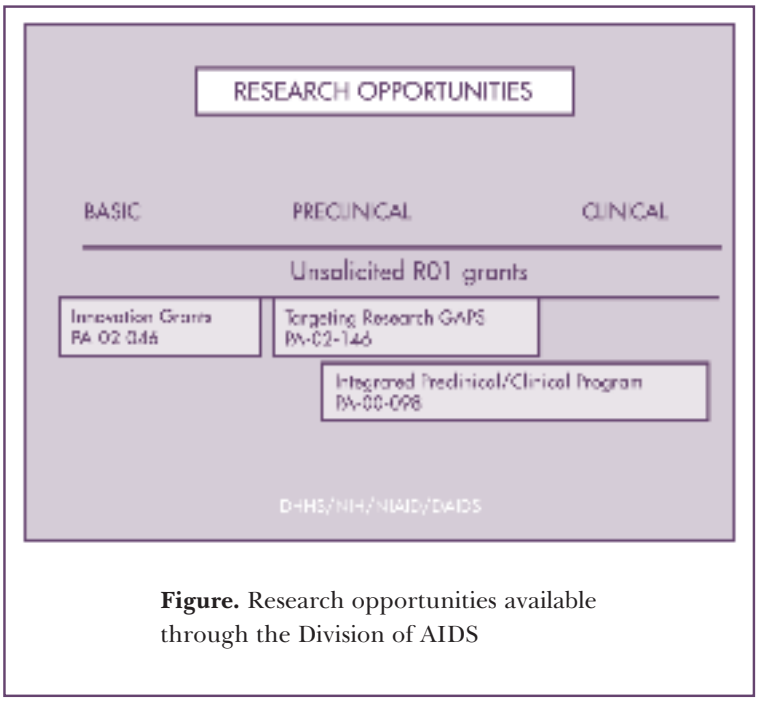

Figure. Research opportunities available through the Division of AIDS

Adaptive: when referring to immunity, the cellular response involving lymphocytes and the establishment of immune memory.

Adenovirus: a type of DNA-based virus.

Adjuvant: a substance that improves the immune response to an antigen (see below).

Albumin: a common protein found in many animal tissues (eg, blood, muscle, etc.)

Antigen: a substance (usually a protein or carbohydrate, such as from an invading bacterium or virus) that stimulates an immune response.

Apoptotic (apoptosis): referring to genetically "programmed" cell death, a natural process in which DNA-damaged or otherwise unwanted cells are eliminated.

Astrocyte: a star-shaped cell found in the brain that protects and maintains neurons.

Aviremic: without active viremia; having a low or "undetectable" viral load.

Capsid: the protein coating or shell of a virus, which surrounds its nucleic acid (see below).

Cation: an ion (charged particle) with a positive charge.

cDNA: abbreviation for "complementary DNA," which matches a given RNA that serves as a template for synthesis of the DNA in the presence of reverse transcriptase.

Chemokine: a type of cytokine (see below) that can direct the movement of white blood cells to sites of inflammation in the body.

Chimeric: having a mixed genetic composition; a genetic cross.

Clade: A subtype of HIV made up of a group of related HIV isolates classified according to their degree of genetic similarity (such as the percentage of identity within their envelope genes). There are currently 3 groups of HIV-1 isolates M, N, and O. Isolate M (major strains) consists of at least 10 clades, A through J. Group O (outer strains) may consist of a similar number of clades.

Clone: a line of genetically identical cells, usually created or expanded from a single parent cell.

Codon: a specific sequence of 3 nucleotides (nucleic acid building blocks) that is part of a genetic code and denotes a particular amino acid in a protein chain; the sequence may also start or stop protein synthesis.

Cytokine: proteins (such as interleukins, tumor necrosis factor, and interferons) that are secreted by immune cells.

Dendritic cell: an antigen-presenting cell with long, branching extensions or processes.

Encephalopathy: disease of the brain, especially involving structural alterations.

Env: an HIV gene that encodes the 2 major viral glycoproteins (gp120 and gp41), which are associated with the viral envelope and are involved in viral attachment.

Epitope: a specific area on the surface of an antigen (see above) that can cause an immune response and can bind with a specific antibody produced by the immune system.

Gag: an HIV gene that codes for the p55 core protein, which is the precursor of the HIV proteins p6, p7, p17, and p24. These form HIV's capsid (see above).

HLA: human leukocyte antigens, which are marker molecules on the surface of cells that identify cells as "self" and prevent the immune system from attacking them.

Homozygous: having 2 identical copies of a particular gene (eg, each coding for the same particular trait, like blue eyes).

HTLV: a type of retrovirus (related to HIV) known as "human T-cell lymphotropic virus."

Humoral: part of the immune response that involves antibodies secreted by B lymphocytes (see below) and circulating in body fluids such as blood or lymph.

Idiopathic: happening suddenly or from an unclear or unknown cause.

Immunogenicity: an ability to produce an immune response.

Innate: when referring to immunity, the local barriers to infection such as skin, stomach acid, mucous, enzymes in tears and saliva, etc.

Intranasal: administered or introduced in the nose.

Intraperitoneal: administered or introduced in the peritoneum (abdomen).

Ligand: a molecule that forms part of a complex.

Lymphocyte: type of immune cell that originates from stem cells and differentiates in lymphoid tissue (such as the thymus or bone marrow); lymphocytes comprise 20% to 30% of the white blood cells in human blood.

Lymphocytopenia: a decrease in the number of lymphocytes (see above) in the blood.

Macrophage: a cell of monocyte-origin that can be stationary or mobile in the body and protects against infection by engulfing (phagocytizing) foreign substances, dead cells, etc.

Microbicide: a substance that kills microbes such as bacteria and viruses.

Microglia: a cell of the central nervous system that helps maintain and protect neurons.

Media: a liquid nutrient environment used in cell cultures.

Monochromosomal: having only a single human chromosome.

Mucosal: involving the tissues that line body cavities, tracts, and passages (like the nose, genital tract, etc.) that function in immune protection, nutrient absorption, and secretion of mucus, enzymes, etc. The mucosa often induces or initiates immune responses against certain antigens and can be a site for administering some vaccines.

Multivalent: effective or active against more than one antigen (see above).

Nef: an HIV regulatory gene that plays a major role in viral pathogenesis; the Nef protein has numerous effects including protecting infected host cells from apoptosis (see above) and altering cell receptor expression and distribution.

Neutralizing antibody: a type of protein produced by B lymphocytes (see above) after stimulation by an antigen; such proteins bind to specific antigens in an immune response and usually counteract or inactivate antigens.

Nucleocapsid: the nucleic acid (see above) and protein coat (capsid) of a virus.

Nucleic acid: DNA or RNA (ie, genetic material made up of building blocks known as nucleotides).

Open reading frame: a reading frame in a genetic sequence that does not contain a signal to stop protein translation (see below) before creating a complete protein.

Pathogenesis: the beginning and development of a disease.

Plasmid: a ring of DNA that replicates on its own and is usually found in bacteria; plasmids can be used to transfect (see below) cells with desired genes.

Pneumonitis: a disease that causes inflammation of the lungs.

Pol: An HIV gene that encodes the viral enzymes protease, reverse transcriptase, and integrase.

Primary: originating in or taken from humans (when referring to cells).

Proteasome: a structure in cells where damaged or unneeded proteins are broken down.

Supernatant: a usually clear liquid left after material (like cells) has been precipitated or centrifuged.

TAR: transactivation response element of HIV RNA located at the 5' end of all viral transcripts.

Tat: An HIV regulatory gene that is believed to enhance virus replication.

Th-1: a collection of cytokines (see above) that lead specific immune fighting cells to target viruses at the intracellular level.

Th-2: a collection of cytokines (see above) that lead specific immune fighting cells to target bacteria at the extracellular level.

Transduce (transduction): referring to the transfer of genetic material from one organism to another by a genetic vector, such as a virus or plasmid (see above).

Transfect (transfection): referring to the introduction and incorporation of outside DNA (eg, from a virus) into a cell.

Transgene (transgenic, transgenesis): referring to an artificial process whereby a gene is taken from one organism and introduced into the genetic make-up of another organism.

Transcription: a cellular process that makes a messenger RNA molecule using a DNA molecule as a template.

Translation: a cellular process accomplished at special structures known as ribosomes that make a protein molecule from information contained in messenger RNA.

Tropism (tropic): affinity or specificity for a particular target or stimulus.

Ubiquitin: a cellular protein that marks other proteins in the cell for degradation.

One of the objectives of the Basic Science Workshop is to "identify from the work presented the implications for activism." In other words, how can advocates for people with HIV support the work of bench researchers? Although many are familiar with the work that activists do in HIV/AIDS-related social policy (eg, funding for state ADAP programs), advocates who work with bench researchers draw comparatively little public attention. Like the researchers whose work they support, basic science activists are a small and quiet lot. But without a vigorous basic science research agenda, progress against the virus—and especially the search for a possible cure—would stall.

So, what problems confront the laboratory scientists whose discoveries have brought us this far and whose efforts are essential to ending this epidemic? And how can activists help with the solutions?

1. A need exists to link bench researchers and community resources. Even in preclinical work, investigators sometimes require the help of human research subjects. This help is usually in the form of providing blood samples. But how does the basic science investigator who has no clinical practice find patients who are willing to help? In the United States, several organizations maintain close ties to both bench researchers and patients, such as Project Inform in San Francisco (***projinf.org***, 800.822.7422); Treatment Action Group in New York (***aidsinfonyc.org/tag***, 212.253.7922); Philadelphia Fight in Philadelphia (***www.fight.org***, 215.985.4448); and The Center for AIDS in Houston (***centerforaids.org***, 888.341.1788). These organizations can help link investigators with volunteers. The nascent AIDS Treatment Activists Coalition (ATAC) may also eventually be in a position to serve as a national clearinghouse for facilitating community participation in bench research (***atac-usa.org***).

2. Clinical investigators and laboratory investigators need opportunities to talk to one another. For basic scientists to appreciate fully the clinical implications of their work, they need feedback from clinicians. Yet there are relatively few meetings designed to facilitate direct communication between clinicians and bench researchers, and a paucity of clinicians, owing to the heavy demands on their time, attend basic science gatherings. However, 2 community organizations are providing national opportunities for clinicians and investigators to interact. One is the Immune Restoration Think Tank sponsored by Project Inform; the other is the Basic Science Workshop sponsored by The Center for AIDS. One-day meetings (perhaps on a Saturday) that include a clinical component may help to increase the number of clinicians who attend. Still, as one bench researcher put it, "Everybody is asked to do too much." The time constraints on both clinicians and bench researchers are significant and no amount of planning will ease them all.

3. A lack of ready access to primates is frustrating the work of bench researchers. At this year's Basic Science Workshop, as in the past, bench researchers again expressed their frustration with the lack of available primates. For self-evident ethical reasons, some basic research (eg, terminal research) cannot be conducted on human subjects and must instead be done in the animal model. Research in animals not only furthers the understanding of HIV's pathophysiology, but also allows inquiry into experimental vaccines and treatments. The shortage of macaques, as well as structural barriers to accessing those that are available, is hampering the work of some basic scientists. Despite its significance, very few activists are working on this issue. Bench research may be facilitated by greater community participation in the process of allocating animal resources to worthwhile projects and in exploring ways to increase the number of available primates. Such participation requires educational preparation and a significant commitment of time. Yet the absence of a variety of community voices in the call for primates may be slowing the speed of research.

Brenda Lein, from Project Inform in San Francisco, and Paul Simmons, RN, from Houston, facilitated the roundtable discussion at the 2002 Basic Science Workshop.

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