Winter 2005

Discovery

The Newsletter of the Institute of Human Virology

FROM LABORATORY TO CLINIC

Blocking HIV Replication From Within Ourselves

Dr. Alfredo Garzino-Demo's interest in factors that limit HIV infection dates back to his NIH days, when he was a young post-doctoral fellow in Dr. Robert Gallo's lab. There, together with Drs. Gallo (then lab chief, now IHV director), Fiorenza Cocchi and Anthony DeVico, Garzino-Demo contributed to the discovery of RANTES, MIP-1⁄ , and MIP-1€, all part of a family of proteins called beta-chemokines produced from cells of the immune system called CD8+ lymphocytes.

"I knew this was going to be important," Garzino-Demo recalls. "I was just grateful to be a part of that scientific team. It's the kind of finding that every postdoc hopes to 'stumble upon'."

The field of HIV research went to warp speed based on that discovery. Within months, thanks to observations by other laboratories that the chemokine report catalyzed, it became clear that beta-chemokines inhibit HIV by blocking a crucial port of entry, CCR5. So important is the role of this receptor that people who lack it due to mutation are highly resistant to HIV infection.

DR. ALFREDO GARZINO-DEMO, *HIV researcher*

The "factor" team, as those who work on beta-chemokines and other proteins that inhibit HIV are called at the IHV, wondered: Are beta-chemokines important in humans, protecting the body from infection or slowing the pace of AIDS?

An early report from Dr. Gallo and collaborator Dr. Daniel Zagury had shown strong evidence for a crucial role of beta-chemokines in HIV infection. Hemophiliacs who received blood transfusions prior to the development of HIV screening and who therefore received HIV-contaminated blood -- but despite this exposure did not get infected with the virus -- had blood cells that released very high levels of beta chemokines. Dr. Garzino-Demo was intrigued. He formed a collaboration with Dr. Margolick at Johns

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Message From The Director

ROBERT C. GALLO, M.D.
Director of the Institute

On the eve of World AIDS Day, we are witnessing the maturation of history's greatest and most unpredictable global epidemic, AIDS, and its cause, the human immunodeficiency virus (HIV). The impact of this pandemic is staggering: More than 25 million are dead, approximately 50 million are now infected, and an untreated infected person has an almost 100% chance of death. Unlike most widespread fatal epidemics, HIV is not going to go away, not even temporarily.

In the 20 years since we learned that HIV was the cause of AIDS, we have seen the fear surrounding a "mysterious" new disease dissipate, patient activists and scientists come to understand one another, and more and better scientific education become available for everyone -- not just those interested in science. Yet the need to work collaboratively remains paramount. Lives will depend on it.

To ensure our future success, I am advocating for the creation of Centers of Excellence in Virology throughout the industrialized countries. Each Center, supported by a budget so as not to have the delays caused by the necessity of pursuing financial support, would be linked to one or more facilities in a developing nation. Countries would be held responsible for finding the cause of new epidemics and their resolution.

Today, AIDS is no longer a death sentence. [The HIV blood test of 1984 protected our blood supply by early 1985, thereby blunting one major means of the spread of HIV. The availability of drug treatment for infected persons resulted in AIDS becoming a more chronic disease by 1995.] But drug resis-

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inside:

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Hopkins, who is responsible for a large and well organized HIV cohort called MACS.

Dr. Garzino-Demo measured beta-chemokine production from cells of individuals of the MACS cohort and found that patients who had full-blown AIDS produced very low levels of these proteins. Further, and perhaps most surprisingly, he found that MACS individuals who were HIV negative but had a history of frequent unprotected exposure to HIV produced levels of chemokines that were sky-high, compared to seronegative individuals that were not part of the cohort. "That is very suggestive of a protective role of beta-chemokines," says Dr. Garzino-Demo. His particular viewpoint in this study was to treat cells so that they would behave as in an immune response, something that is called "antigen stimulation."

Antigen stimulation is so crucial to the field of vaccine research that Dr. Garzino-Demo performed an in-depth study of how important beta-chemokines are in the immune memory to a vaccine. His latest results, obtained with his "right arm" associate, Dr. Lingling Sun, show that beta-chemokines are perhaps the best marker of memory response to a vaccine compared to several other classical markers. "And that is a double-whammy," he explains. "With the production of beta-chemokines you have, at the same time, a very good marker of immune response, which the field of vaccine research has long awaited for, and a potent HIV-blocker, that could provide that extra protection that you need for HIV vaccination.

Dr. Garzino-Demo hopes to expand his studies of memory response to other vaccines, aspiring to pin down chemokines as one of the components of a correlate of protection. With Dr. Lydia Temoshok he is evaluating beta-chemokines in the context of research linking improved immune responses to better coping responses to HIV infection. With Dr. David Oldach, he is exploring a shared interest in recent findings that infection by a flavivirus, GBV-C, can slow disease progression in HIV-positive patients. In this case, chemokines also have been named as possible "culprits."

Another class of proteins, called € defensins, have also recently attracted the attention of Dr. Garzino-Demo for their possible role in protective immunity against HIV. These proteins are found in high concentrations in cells lining the mouth (oral mucosal cells).

"What we discovered was that € defensins inhibit HIV infection, but by some novel, unexpected mechanism, appearing to involve signaling across cell membranes and within the cells," he says. With external collaborators, Garzino-Demo plans to examine € defensins in sections of oral tissue from HIV-positive and negative patient populations. "Since € defensins are natural proteins that can inhibit HIV, this has important implications for developing a topical microbicide (e.g., a prophylactic, vaginal cream), especially for countries where other forms of protection from transmission are not made available," he says.

Message from the director, *continued from page 1*

tance by HIV is a rapidly increasing problem and, because of the need for life-long therapy, so is drug toxicity. In addition, new forms of HIV are arising through genetic mixing of different strains. This means that now, more than ever, studies of the basic biology of HIV are needed to develop new approaches to therapy.

Bringing drugs to developing countries is another urgent need, but it is one that has to be handled with enormous care lest we foster new epidemics from multi-drug resistant mutants of HIV. President Bush's PEPFAR (President's Emergency Plan for AIDS Relief) is a major step in the right direction. Over five years, the United States will provide countries, primarily Haiti, Viet Nam, and Guyana, with $15 billion to combat AIDS.

The war against AIDS must be fought locally as well as globally. The AIDS problem in many urban areas of the U.S., including Baltimore, rivals that of sub-Saharan Africa, and women are increasingly affected. It would be exciting to see the President create a PEPFAR for Inner City USA, which could have many positive effects on diseases that go far beyond HIV/AIDS because of the improvements in health education and the health system infrastructure that it would bring.

The end of HIV/AIDS will come from a successful preventive vaccine. But no scientific challenge has proven more elusive, so much so that serious writers have recently called for an end to vaccine research, arguing that the money could be better invested elsewhere such as for more education. I am of a contrary view: I believe we need a crash program for a vaccine. [Some of us have advocated for this since 1988, and the idea is now taking hold, due to the Gates Foundation and the National Institutes of Health, among others.] Although the development of an HIV vaccine is very difficult for several reasons, there is good news. An in-depth understanding of how HIV enters our cells is emerging, and this is information that I believe is vital for the development of an effective vaccine.

Also, relations and collaborations between medical scientists in the U.S. and developing countries, especially in Africa, have never been better. I have witnessed the severity of the AIDS epidemic in China and Russia, and am heartened to see both countries embracing U.S. collaboration and that both countries have a formidable wealth of human resources with scientific skills. I dream that a three nation linkage of the U.S., China and Russia driven by scientists outside of government might not only bring this epidemic to an end but may also herald a new age for scientific research.

In the coming years, we need to develop new models for intercontinental collaboration, foster increased scientific study, and improve health education. We must intensify our search for a vaccine. With support from the public and private sector, the war against AIDS can be won in Baltimore and in countries around the world. Let's renew our efforts – right here, right now.

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IGOR LUKASHEVICH
Assistant Professor, IHV

Is a Vaccine in Sight for Lethal Hemorrhagic Lassa Fever?

Russian-born Assistant Professor Igor Lukashevich of the IHV began his research on Lassa virus, a contagious and potentially lethal hemorrhage-inducing fever virus, decades ago in his mother country. He worked within a series of plexiglass glove boxes, far removed from the bubble-suit, negative pressure containment facilities required today for Lassa research (Biohazard Safety Level 4), helping spearhead his country's first research on Lassa. At the IHV, his current research interests focus on "two areas: the mechanisms of Lassa pathogenesis (cause and progression of Lassa fever) and development of a vaccine against this disease."

Once in the bloodstream, Lassa infects and multiplies within human macrophage cells in proximity to capillaries, causing functional alteration of platelets and vessels hence its reputation as a hemorrhagic virus. Lukashevich has used macrophage and endothelial (blood vessel) cell culture systems, as well as rhesus monkeys, to model and study Lassa pathogenesis. "Our data show that acute Lassa infection is associated with only weak inflammatory responses in tissues and initiates an extensive program of liver cell regeneration," says Lukashevich.

> "We are now using the highly immunogenic parts of the Lassa virus in combination with the replicative machinery of a non-lethal relative, the Mopeia virus, and have gotten exciting results with three animal models."
>
> - IGOR LUKASHEVICH

Alterations in liver function and liver cell proliferation are observed, along with small increases in levels of certain inflammatory molecules, a phenomenon whose magnitude is proportional to the numbers of virus particles in the blood. "We don't yet know what these liver changes mean," he says. "One idea that's emerging, however, is that Lassa may work as a suppressor of inflammation."

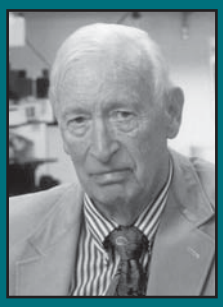
MARIA SALVATO
Professor, IHV

Collaborating with Maria Salvato of the IHV, Lukashevich began his vaccine work using a related virus, lymphocytic choriomenigitis virus (LCMV), which can cause a Lassa-like illness in monkeys, as a model for human Lassa infection. He discovered that if he first inoculated monkeys with a less aggressive strain of LCMV, by stomach administration, he could protect them from a later, lethal challenge dose of the Lassa-like LCMV strain. Armed with this knowledge and a $2 million dollar NIH grant, Lukashevich and Salvato are evaluating a conceptually similar, yet novel, genetically-manipulated Lassa vaccine in various animal models.

"We are now using the highly immunogenic (immune stimulating) parts of the Lassa virus in combination with the replicative machinery of a non-lethal relative, the Mopeia virus," says Lukashevich, "and have gotten exciting results with three animal models." Essentially, his vaccine seems very well tolerated across a wide range of doses, and has been one hundred percent protective against more than 3,000 lethal challenge doses of Lassa in a guinea pig model. "We also learned," Lukashevich states, "that even when our vaccine is given simultaneously with the Lassa challenge, there is protection,"—an unusual outcome, suggestive of a novel protective mechanism.

With further research into the protective mechanisms, and additional testing, Lukashevich is highly optimistic that they will ultimately "go forward to develop a master vaccine stock" that will meet regulatory standards.

Paul Zamecnik honored



Dr. Paul Zamecnik was the recipient of this year's Lifetime Achievement Award, presented annually by the Institute of Human Virology at its International Meeting in Baltimore to honor senior scientists whose contributions have been significant within their field and to the IHV.

Few scientists make a contribution of the monumental importance made by Dr. Zamecnik. Fewer still make two. But in a 60-year career characterized by sheer scientific originality and brilliance, Zamecnik first provided the tools for deciphering the genetic code and then later was the first to conceive of the successful use of anti-sense DNA for the highly selective inhibition of gene expression.

It was Zamecnik and his team who first defined the biochemistry of amino acid activation and their assembly into proteins. This provided the first hard evidence for the existence of transfer RNA, the key piece in the puzzle of how genetic information in DNA is translated into the specific sequence of amino acids that gives each protein its distinct molecular identity. His work provided a totally new picture of protein synthesis.

His next major discovery was the conceptualization and demonstration that anti-sense DNA, in which short chains of DNA, chemically synthesized to be complementary to selected RNA targets in the cell, are used to selectively inactivate the expression of specific genes. This groundbreaking work has spawned the development of a new field of research, which in turn has led to a new approach to investigating new drugs using anti-sense DNA to block or stop the replication of viruses. Clinical trials of anti-sense DNA drugs for AIDS, cancer and infectious disease are conducted now in laboratories around the world because of Zamecnik's discoveries, some of which are in his own laboratory where he continues as a major player, publishing new work regularly.

Bob Gallo says of Paul: "He is that unusual combination of brilliance and dedication, coupled with exceptional warmth and generosity. He never tires of sharing his most precious commodities – his ideas."

Zamecnik also has been honored with the Albert and Mary Lasker Award, as well as the National Medal of Science.

The 2005 Annual International Meeting of the Institute of Human Virology will be held Aug. 29-Sept. 2. For more information, contact 410-706-8614.

Dr. Richard Klausner speaks at First Annual Stewart and Marlene Greenebaum Lecture

At the First Annual Stewart and Marlene Greenebaum Lecture hosted by the Institute of Human Virology, Dr. Richard Klausner, executive director of the Bill and Melinda Gates Foundation's Global Health Program, spoke on the "Grand Challenges in Global Health."

The Bill and Melinda Gates Foundation is focused on reducing global health inequities by accelerating the development, deployment and sustainability of health interventions that will save lives and dramatically reduce the disease burden in developing countries. Fourteen thousand people become infected with HIV every day, the majority in sub-Saharan Africa. Stopping the transmission of HIV is the foundation's top global health priority.

Founded in 1996 by Dr. Robert C. Gallo, co-discoverer of the virus that causes AIDS, the Institute of Human Virology is the first center of its kind with broad expertise in AIDS research, treatment and prevention. The IHV has created a leading vaccine candidate that is slated for human testing in 2005; treats approximately 4,000 patients locally and has international collaborations underway at 109 sites in 36 countries. Baltimore ranks third nationally for HIV infection.

Dr. Klausner, former director of the National Cancer Institute, led one of the world's largest research and health agencies creating successful national and international programs aimed at applying science and technology to improving the public health. With Dr. Anthony Fauci, he oversaw the creation and development of the NCI's Vaccine Research Center. Dr. Klausner is the author of more than 280 scientific articles and several books and is a member of the National Academy of Sciences and the Institute of Medicine. He has won numerous honors and awards for his work.

Stewart Greenebaum is a founding member of the IHV's Board of Directors and is committed to improving the human condition. Greenebaum and his wife, Marlene, have received many humanitarian awards for their contributions. Together, they have founded charitable organizations with worldwide reach, made countless monetary contributions and devoted hours of service through leadership and volunteerism. Greenebaum was the recipient this fall of the IHV's first-ever Humanitarian Award.

STEWART GREENEBAUM, DR. RICHARD KLAUSNER AND DR. ROBERT C. GALLO

Spotlight: IHV Scientific Advisory Board Member Scott Hammer

SCOTT HAMMER
IHV Scientific Advisory Board Member

Scientific Advisory Board member Scott Hammer, Chief of the Division of Infectious Diseases, Harold C. Neu Professor of Medicine, and Professor of Epidemiology at Columbia University Medical Center, brings with him a wealth of experience in treating HIV infected patients and pursuing HIV clinical research. Among his many roles, Hammer serves as site principal investigator for two National Institutes of Health-sponsored HIV/AIDS projects: the AIDS Clinical Trials Group (ACTG), which tests safety and efficacy of antiretroviral therapies for HIV-positive patients, and the HIV Vaccine Trials Network (HVTN), which develops and tests preventive vaccines.

Although his time is limited, Hammer sees patients and provides general consults as well as HIV/AIDS in- and outpatient care. He feels strongly that, especially with HIV/AIDS, "you need to keep your clinical skills alive, to provide a touchstone with patients undergoing therapy." One of his proudest academic achievements was the "bringing to fruition of two major antiretroviral trials and the subsequent new standards for HIV patient care that arose from them." Hammer derives great personal satisfaction from watching HIV's evolution from a lethal to a therapeutically manageable disease, and seeing patients "get their lives back."

Hammer has also appreciated the strides taken by the Institute of Human Virology, even though he is a relatively recent addition to the Board. Recruited via his ongoing contact with IHV staff also involved in ACTG and HVTN activities, Hammer feels that "the IHV has exceeded initial expectations, especially with the quality of its staff and their work over time."

He views his role as "helping the IHV focus on its research agenda and efficiently achieve its mission," particularly by providing "an outside, reasoned perspective." Hammer hopes his legacy to the IHV, albeit a quiet one, will be advice that "helps steer researchers in new directions and leads to new discoveries."

"I am happy to be part of one of the biggest positive aspects in virology and HIV work, namely, collaborating across institutions as well as across the nation," states Hammer. "It's a privilege being asked to help advise a major Institution and help foster greater coordination and collaboration."

C. DAVID PAUZA
IHV Assistant Director

C. David Pauza has been named assistant director of the IHV. A senior scientist in the Basic Science Division, Pauza is charged with assisting Dr. Gallo with scientific administrative matters resulting from the Institute's growth and collaborative endeavors. "He has already been acting in this capacity and will continue to greatly enhance and streamline the Institute's ability to meet the global demand for its expertise in research and international outreach," says Dr. Gallo.

Stewart Greenebaum Honored with IHV's First Humanitarian Award

Former IHV Board of Advisor chair Stewart Greenebaum, president of the Greenebaum and Rose Associates real estate company, was honored at this year's annual meeting as recipient of the IHV's first-ever Humanitarian Award. Greenebaum has been a longtime friend and supporter of the Institute. Attendees were touched by his acceptance speech, peppered with humor, humility and generosity. Some excerpts:

"I must tell you that to be the recipient of an award such as this from a group such as yours is not only humbling, but downright embarrassing. I must admit that when I was in high school, I was one of the most promising students in science. I was always promising that I could do better if given a chance! I remember my high school chemistry teacher describing me as someone who couldn't properly pour water out of a beaker even if the instructions were printed on the bottom!

"Indeed, I should be too embarrassed to accept this award tonight but for one consideration. Upon reflection, I discovered there is something I wanted awfully much to say to you and that I might never again have the opportunity to address so many brilliant scientists as are assembled here tonight. What I want to way is "thank you." Thank you on behalf of all of us who can't begin to fathom how your brilliant minds work, but nonetheless benefit every day from the work you do, the dreams you dream and the vision that guides you into uncharted territory.

"I have long felt that our society reveres and rewards the wrong people. We bestow wealth and celebrity on individuals who can sprint 80 yards for a touchdown or hit one out of the park with the bases loaded. We should be honoring all of the individuals who find ways to improve the human condition! People who can make the sick well and people who have the ability to ward off significant diseases all together. People whose efforts are not aimed at entertaining us but at bettering our lives in a thousand different ways. We should be honoring the scientists and the healers who quite literally stand between us and oblivion.

"So I want to say "thanks" to each and every one of you for the intense, often backbreaking work that you do every day, for fighting through the bureaucracies and paperwork that seems to stifle creative thought, for faithfully following the dictates of your limitless intellectual curiosity. To me, what you do is a dazzling mix of magic and the miraculous. I am so honored to have a front row seat. You are the people who will turn all these imaginings into reality in the years ahead. Your impact on the human condition will be immeasurable. And so on behalf of all those lives you will touch through your brilliance, your determination and your unique inspiration, I say one more time this evening: Thank you.

THE INSTITUTE OF HUMAN VIROLOGY (IHV) at the University of Maryland was established to create and develop a world-class center of excellence focusing on chronic diseases and virally linked cancers.The IHV is dedicated to discovery, research, treatment, and prevention of these diseases and cancers. Its unique structure seeks to connect cohesive, multidisciplinary reasearch and clinical programs so that new treatments are streamlined from discovery to patient. The IHV serves patients locally and the scientific community globally.

AIDS Vaccine Conference 2004 Offers Learnings, Progress

The AIDS Vaccine 2004 Conference was held in Lausanne, Switzerland, from August 30th to September 1st. Approximately 800 delegates from more than 50 countries gathered and more than 300 scientific papers were presented. Here, we summarize the major highlights of the conference, and the state of development in the fields of immunogens, cellular immunity, neutralizing antibodies and clinical trials.

Immunogens

A large number (N=90) of immunogens are currently being developed. Fifty-six percent of the immunogens are poxvirus (primarily MVA) and DNA constructs, 34 percent are recombinant proteins and virus like particles, and 10 percent are adjuvant molecules or immunogens targeting innate immunity. The main messages from the immunogens field were the need to: a) focus on fewer poxvirus and DNA constructs, b) develop a coordinated strategy for inserts design, and c) increase emphasis on development of immunogens/adjuvants targeting innate immunity.

Cellular Immunity

Compelling data were presented clearly indicating that IFN-g measurement is not enough to monitor T-cell responses. IL-2 measurement needs to be added for the evaluation of vaccine-induced T-cell responses. Interesting new data on the characterization of CD4 and CD8 T-cells have been obtained using a multidimensional approach evaluating the phenotype and function of effector and memory T cells. Data were also presented clearly demonstrating that the functional heterogeneity of virus-specific T cells observed in different models of virus infections is dictated by Ag/virus exposure, persistence and levels.

Neutralizing Antibodies

A series of approaches to induce neutralizing antibodies were also presented and included: a) structure-based envelope design, b) deletion variants of gp140, c) immunogenicity of coreceptor site, and d) envelope gp120 and gp140-CD4 complexes. A number of interesting papers were presented showing a correlation between high titers of maternal neutralizing antibodies and lack of HIV transmission to infants. Along the same line, several trials are underway to test HIVIG, monoclonal antibodies to limit perinatal transmission. Furthermore, passive administration of human neutralizing monoclonal antibodies was investigated in the context of structured treatment interruption. The results indicated transient control of viremia in a subset of patients. There was strong/broad consensus that more work is needed to design, develop and test envelope immunogens that can elicit broad neutralization.

Clinical Trials

There are about 30 phase I/II trials ongoing. The results of nine phase I clinical trials were presented at the conference. Different poxvirus vectors (MVA and NYVAC), protein subunits, lipopeptides, DNA vectors alone or in combination with MVA vaccines, and Adenovirus 5 vectors were investigated in these clinical trials. The conclusions from these trials were: a) all the vaccines stimulated T-cell responses and only one antibody responses, b) promising immunogenicity data (50% T-cell responses) with NYVAC, c) good immunogenicity data (60%-90% T-cell responses) with lipopeptides (both CD4 and CD8 T-cell responses) and protein subunits (only CD4 T-cell responses), d) poor immunogenicity data (<20%) of the DNA/MVA combination in the IAVI006 trial, and e) Adenovirus 5 vector is so far the most promising vaccine candidate.

-- Giuseppe PANTALEO

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