

SEARCHLIGHT

NEWS FROM AIDS RESEARCH ALLIANCE

A National Leader in Fast-Track AIDS Research

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What's News—

Clinical Research

Our Best Shot?

Scientists agree that the only way to end the pandemic, especially in Africa and countries without a sophisticated health care infrastructure, is to find a safe and efficient AIDS vaccine. What are the challenges that the scientific community is facing in development of an effective vaccine?

(Articles on pages 3 and 13)

Virion-Based Vaccine

Whole virion-based vaccines have worked for a number of viruses, including retroviruses. And yet the effort devoted to the development of such a vaccine for HIV has been minimal. Researchers at UCLA, Kathie Grovit-Ferbas and Judith Currier, explain why they began to reexamine the development possibilities of a whole inactivated vaccine for HIV.

(Article on page 5)

Prostratin Update

An outline of the progress that has been made since the last issue of "Searchlight" to move forward the development of prostratin as an anti-HIV drug, eliminating the viral reservoirs.

(Article on page 7)

Microbicide: Scientific and Ethical Challenges

With recent advances in treatment of HIV, being HIV positive is not an immediate death sentence. Fear, the strong motivation for people to change their behavior has been diminished. Many of today's young people are no longer terrified of HIV. These factors may have led to an increase in the practice of unsafe sex and new transmission. While waiting for an AIDS vaccine, development of a successful microbicide to prevent the transmission of STD's, including HIV, could slow down the spread of infection, especially in poor countries.

(Article on page 10)

A Boosted Hope— AIDS Vaccine Research

By Marjan Hezareh, Ph.D.

The human immunodeficiency virus (HIV) continues to spread around the world, insinuating itself into communities previously little troubled by the epidemic. Already almost 20 million people have died of AIDS, over 30 million are currently living with HIV and 16,000 new infections occur daily. HIV chemotherapy has continued to allow substantial improvements in quality of life and life expectancy for HIV-infected individuals. However, these therapies are out of reach for most HIV-infected patients around the world. Even when drugs are available they are limited by their toxicity, complex regimens and elevated cost. Therefore, the biomedical community is redirecting efforts towards development of an effective AIDS vaccine. In this issue of *Searchlight*, we review some of the challenges facing the scientific community in designing and developing a safe and effective vaccine against HIV.

The term immunity is derived from the Latin word immunitas, referring to the exemption from a variety of civil duties offered to Roman senators during their tenures in office.

The term immunity is derived from the Latin word *immunitas*, referring to the exemption from a variety of civil duties offered to Roman senators during their tenures in office. Historically, immunity meant protection from disease—specifically infectious disease. The modern definition of immunity is a reaction to foreign substances, including microbes, viruses, and macromolecules such as proteins and polysaccharides. The *concept* of immunity existed long before, as suggested by the ancient Chinese custom of making children inhale powders made from the crusts of skin

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ARA envisions a future in which HIV and its effects are eliminated from infected individuals, and a vaccine preventing new cases eradicates the virus.

ARA's mission is to find and accelerate the development of effective treatments for HIV and its complications. We do this by conducting cutting-edge research and clinical trials in order to improve the longevity and quality of life for all people with immune deficiency.

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Message from the Executive Director

One should never direct people toward happiness, because it too is an idol of the market place. One should direct them toward mutual affection. A beast gnawing on its prey can be happy too, but only human beings can feel affection for each other, and this is the highest achievement they can aspire to . . . When we have enough loaves of bread to crush under our heels, when we have enough milk to choke us, we still won't be in the least happy. But if we share things we don't have enough of, we can be happy today!

-Alexander Solzhenitsyn, Cancer Ward

I am writing to you less than two weeks after the national tragedy that befell the United States on September 11, 2001. We all know stories of those who were lost or who lost friends or loved ones that day. Doubtless, some of you lost someone you loved. The generous response from the public has been both overwhelming and inspiring. Between the Red Cross and Hollywood's *America: A Tribute to Heroes* benefit for the United Way, over \$400,000,000 has been raised for relief efforts. Americans are also giving blood, sending clothes, and doing whatever they can do. All of us want some way to show that we are in common cause against the misinformed hatred that takes scores of lives and threatens freedoms that we only recently took for granted.

We at AIDS ReSearch Alliance stand squarely in solidarity with our friends and everyone else in the nation who lost family and loved ones.

As supporters of AIDS ReSearch Alliance, I need to speak frankly with you about what might be called "collateral damage" of the September 11 assault. The subject is not easy to raise in the current climate, albeit a climate of immense generosity and good will. In short, we must not let any act of terror succeed in causing us to lose focus on other battles that we simply don't have the luxury of time to postpone. The fight against HIV/AIDS is only one such fight. You have been partners in our progress. You have not lost vigilance, nor have you concluded that AIDS is no longer a big problem, or even one that has become "manageable". We appreciate that fact.

While we cannot ignore recent tragic events, neither can we afford to pause in what must be a relentless effort to address the global HIV pandemic—and the attendant 8,000 deaths from AIDS worldwide every day, with nearly twice as many daily infections. (The hardest hit? Children who either die from HIV/AIDS or are orphaned.)

With your help, AIDS ReSearch Alliance will continue to focus on our mission: finding more effective, more affordable, and less toxic therapies for HIV, with an outright cure remaining our ultimate goal. We would be capitulating to acts of senseless violence were we to do any less, or were we to avoid asking the friends who have sustained us for help in meeting our obligations.

The mood in America even before the events of September 11 was one of scaling back, and making do with less. As the words of Alexander Solzhenitsyn suggest, however, sharing what we have can in its own way be enriching and bring all of us closer as a global community.

Be well,

Irl Barefield,
Executive Director

AIDS Vaccine Research—

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lesions of patients recovering from smallpox. Edward Jenner's successful vaccination against smallpox demonstrated for the first time that the immune system can be manipulated under controlled conditions. He noticed that milkmaids who had recovered from cowpox never contracted smallpox. He established that cowpox virus cross-reacts immunologically with smallpox virus and could be used to protect against smallpox. This led to the widespread acceptance of vaccination for inducing immunity to infectious disease.

...an effective vaccine may require stimulation of all major immune response mechanisms including humoral (antibody response), cellular (cytotoxic T cells and helper T cell), and local (mucosal response at the site of the infection).

Unfortunately, there is no population that has been cured from AIDS. Therefore, no immune parameters have been identified that correlate with protection from disease. Recent studies on exposed seronegative sex workers in Nairobi provided important insights into the mechanisms the body uses to maintain immunity, but they failed to identify definitive markers to guide vaccine development. Although immune correlates for protection against HIV remain unknown, there is evidence that both cell-mediated immunity and long term B-cell memory are crucial in immune protection. Furthermore, transmission of HIV occurs through multiple routes.

Therefore, an effective vaccine may require stimulation of all major immune response mechanisms including humoral (antibody response), cellular (cytotoxic T cells and helper T cell), and local (mucosal response at the site of the infection). Other vaccines such as the childhood vaccine for the prevention of pertussis have been successfully developed without precise knowledge of correlates of immunity. Development of a vaccine without a definitive marker of protection is possible, but remains one of the more challenging aspects in AIDS vaccine development.

The genetic diversity of HIV poses another difficulty. HIV has multiple sub-types or *clades*, around the world, and even in an individual mutations occurs very quickly and with a high frequency. Efforts to develop a universally-effective vaccine against all strains and clades of virus have been impeded by the unusual diversity of the HIV virus. Other vaccines have been designed that successfully protect humans against pathogens where strain variation occurs, such as pneumococcus, influenza, and polio. But the strain variation of these viruses is relatively limited compared to HIV.

Efforts to develop a universally-effective vaccine against all strains and clades of virus have been impeded by the unusual diversity of the HIV virus.

Further barriers to vaccine development include the fact that there is no ideal animal model for HIV/AIDS. Vaccine studies for

SIV on monkeys have provided important insights into HIV immunopathogenesis, but there are differences between HIV and SIV in their genomic organization that could affect vaccine efficacy. Although the animal models are not perfect they help determine the mechanisms of protection and may help us to make decisions about proceeding with human trials. Furthermore, we should keep in mind that other vaccines, including measles, mumps, and rubella, have been successfully developed where animal models were not available or less than ideal. The true test of a vaccine candidate will be determined in large human clinical trials, not in animal models.

Recent advances in immunology have led to the design and development of new and promising vaccine strategies.

Recent advances in immunology have led to the design and development of new and promising vaccine strategies. As summarized below, many of these strategies are being tried to develop an effective HIV vaccine. Some scientists are calling for a return to traditional methods of vaccine development such as live attenuated vaccine, which have greater risk but may have a greater chance of success. Others are pushing for the use of high-tech methods because of the unique features of HIV. Either way, human clinical trials are the crucial link between laboratory research and an effective vaccine. It takes lots of deter-

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AIDS Vaccine Research—

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mination and a series of vaccines and human clinical trials to develop a product capable of affecting

the epidemic. Although the hurdles to development of an effective HIV vaccine are high, we

should be heartened by the renewed interest in vaccine development.

Current AIDS Vaccine Candidates

Several types of vaccines are currently in development, either in human clinical trials or in experiments with primates. The following list summarizes different concepts currently in use for development of an AIDS vaccine.

Recombinant Subunit Vaccines: In this strategy, individual viral components are used to generate a protective immune response. This is the basis of AIDSVAX™ B/B, the first vaccine currently in Phase III clinical trials (*see Trials Chart page 9*). This vaccine contains synthetic copies of gp120 (HIV envelope) protein.

DNA Vaccines (or “naked DNA” or “nucleic acid”): contains an HIV immunogen under the control of an eukaryotic enhancer/promoter signal that confers an appropriate expression of the viral protein. Once introduced into muscle, cell surrounding the site of injection, internalize the plasmid and transport the DNA into nucleus, where appropriate expression of immunogene will occur. This is the basis of Merck’s “naked DNA” vaccine currently being tested in Phase I clinical trial.

Viral or Bacterial Vector-Based Vaccines: Recombinant vector-based vaccines contain replication-defective forms of non-pathogenic virus (e.g. Poxvirus, Modified Vaccinia Ankara) or bacteria (e.g. Salmonella typhosa) to carry one or more HIV genes. This is the basis of Aventis’s vaccine candidate, in which a harmless canary poxvirus has been used as a vector.

Live-Attenuated Vaccines: This concept is used to protect humans against broad range of infectious disease such as polio and measles. Monkeys infected with live-

attenuated SIV (*nef*-deleted Simian Immunodeficiency Virus) were not infected upon subsequent challenge with SIV or *nef*-deleted SIV. However, it was found that significant pathology occurred in monkeys after exposure to live-attenuated SIV. Furthermore, *nef*-mutated virus has been isolated in some patients infected with HIV. This approach may prove to be effective, but many safety concerns should be addressed before testing such a vaccine in human clinical trials.

Whole-Inactivated Virus: This concept is used to protect humans against broad range of infectious disease such as polio, influenza, and rabies. In this approach virus is inactivated using different methods including chemicals, irradiation or heat. Inactivation of virus insures that every virus particle is killed while they maintain their primary structure necessary to stimulate HIV-specific immune response. A vaccine candidate being developed at UCLA by Dr. Katie Grovit-Ferbas (*see article page 5*) and at NCI by Dr. Larry Arthur based on killed, whole-inactivated HIV virus.

Virus-Like Particle Vaccines: These vaccines are made of pseudovirion that contain non-infectious portions of HIV viral particle but lack most or all of HIV genetic material. The advantage of this approach lies on the fact that HIV proteins are presented in their natural forms to the immune system. However, it is very difficult to prove that no infectious particles exist in the vaccine preparation.

Peptide Vaccines: Consist of chemically-synthesized pieces of HIV protein (peptide) known to stimulate HIV-specific immunity.



PHOTO BY ARMOND BAGDASARIAN

ARA Welcomes New Director, Scientific Communications & Searchlight Editor

ARA is pleased to announce that Marjan Hezareh, Ph.D. has joined its staff as Director of Scientific Communications. As Director, Marjan is responsible for assisting in the development and design of clinical trials and serving as Searchlight’s new editor. Marjan brings a strong scientific background to ARA, including a Bachelor of Science, Biochemistry and Chemistry from the University of Neuchâtel in Switzerland, a Ph.D. in Biochemistry from the University of Geneva, post-doctoral work at the University of California, San Diego and research work at the Scripps Research Institute in La Jolla, California.

Revisiting Virion-Based Vaccines

by Kathie Grovit-Ferbas, Ph.D. and Judith Currier, M.D.

Why do we need a vaccine?

Modalities to induce HIV specific immunity are urgently sought by those working to develop an effective preventive vaccine, as well as by those working to improve our therapeutic armamentarium. While combination antiretroviral therapy has proven to be enormously successful in reducing HIV-related morbidity and mortality, the currently available agents are neither capable of eradicating virus nor of restoring HIV-specific immunity to levels that allow for permanent discontinuation of treatment.

Moreover, we still have an incomplete understanding of the immune system, and therefore still have questions as to which immune responses we should be targeting for vaccine development. For instance, it appears that despite evidence for partial restoration of pathogen specific immunity (CMV, TB), the restoration of HIV-specific immunity is incomplete. Existing highly active antiretroviral therapy (HAART) has resulted in modest increases in CD4 cell function but has not resulted in the restoration of strong or persistent HIV-1-specific CD4⁺ T cell proliferative responses.^{2,3;11;22} Evidence suggests that restoration of HIV-specific CTL responses may be critical to the long-term immunologic control of infection²⁰, but what about the role of antibody responses¹⁶?

It is clear that these questions really can only be answered in the context of ongoing and future vaccine trials. Therefore, even vaccine trials which fail to demon-

strate protection may provide important information regarding correlates of immunity that will allow for the design of improved vaccine candidates.

The argument for virion-based vaccines

Sound arguments exist for testing a number of disparate vaccine strategies. The majority of the vaccine effort thus far has focused on recombinant subunit, DNA, and various vector-based vaccines. But the lack of overwhelming success to date suggests that multiple components or a complex virion structure might be required for protection. This is not an unreasonable hypothesis given that inactivated virion-based vaccines have worked successfully for a number of viruses, including retroviruses.^{10;23} And yet the total amount of research devoted to developing such a vaccine for HIV has been minimal.

In general, three concerns are cited for not exploring virion-based vaccines: 1) a belief in the inability to retain gp120 on virions, 2) the failure of early inactivated SIV vaccine preparations, and 3) safety concerns surrounding whole virion preparations as vaccines. We reasoned, however, that each of these concerns could be addressed by systematic studies. For instance, we and others^{9;15;17;18} have shown that retention of viral gp120 is strain specific, and that viral envelope can remain associated with the virion at high levels on many relevant isolates. Therefore it is possible to choose viral strains to use for vaccine development that will not

result in loss of the outer envelope which is critical for induction of protective antibody responses.

The second concern relates to early work on a killed SIV vaccine where virus-specific protection from infection was confounded by the subsequent observation that much, if not all, of the protection was conferred by a xenoantigenic immune response.¹ In simple terms, the monkeys in this study made powerful immune responses against human proteins that were incorporated into the virions while the vaccine was growing in human cells in the laboratory rather than against viral specific proteins. Before these groundbreaking studies were performed we did not even realize that cellular proteins were incorporated into virions at such high levels.¹

We now know that cross-species or xenogenic reactivity can easily be addressed by utilizing simian cells for virus expansion if the vaccine is going to be tested in non-human primates, and by using human cells for human studies. The more difficult question which remains, however, is whether allogeneic immune responses (the same types of responses which lead to rejection after organ transplantation) in humans that might occur with any virion-based vaccine will be harmful. But this cannot be answered at the theoretical level, and should not be an *a priori* reason to abandon virion-based vaccine strategies. Instead, this question should be incorporated into the design and testing schemes of vaccine candidates.

Finally there are the safety concerns. We know that there are

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Revisiting Virion-Based Vaccines—

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physical and chemical treatments which can be used in combination to theoretically inactivate virus to the recommended 15 logs.¹⁴ Furthermore, a virion-based vaccine does not have to be a replication competent virion. Rather, mutations can be made to yield a construct that is non-infectious but which still retains antigenic epitopes. This would result in a pseudovirion vaccine—that is, a vaccine which is nearly, but not completely a virion based vaccine.

And so, based on these ideas, we began to reexamine the possibility of developing a virion-based vaccine. Our prototype vaccine was a full-length replication competent molecular clone with a primary R5-tropic envelope. Using this virus as a basis for our studies, we demonstrated that relatively simple processes involving thermal and chemical inactivation could inactivate the virus at least 10 million fold (the highest titer we could grow the virus to in the laboratory).

We were also able to demonstrate retention of viral envelope and major envelope regions (known scientifically as *epitopes*) which appear to be important for neutralization during the natural history of infection. In fact, reactivity of monoclonal antibodies directed towards these envelope epitopes increased after treatment, suggesting greater exposure of the viral epitopes in the vaccine preparations. This was in direct contrast to treatment of free envelope under the same conditions.⁹

Most inactivated vaccines target antibody responses rather than cellular responses. But we

were curious to see whether this was true of our prototype vaccine so we set up a series of experiments to look at one particular cellular response: the induction of *interferon gamma*. Interferon gamma is a protective cytokine that is made by cells of the immune system in response to invading pathogens, and is viewed as a potential surrogate marker for cell mediated immune responses in HIV infection.⁸ Surprisingly, our *in vitro* studies also demonstrated that this preparation could stimulate interferon gamma from the blood of HIV infected persons. Taken together, these data gave us hope that this sort of vaccine strategy might be used for both prevention and treatment of HIV disease.

Because of the safety considerations inherent in any whole killed vaccine approach, we took the information we garnered from our prototype vaccine, and used it to design an envelope containing heat-treated pseudovirion. In principle, the use of pseudovirions would provide a safe and complex antigen source. Pre-clinical testing of these vaccine candidates is currently underway.

The significant declines in opportunistic infections and HIV-related mortality are evidence that combination antiretroviral therapy improves immune function. And yet, despite changes in the phenotype and number of T cells the functional aspects of T-cell immune reconstitution remain incomplete. Recent reports suggest that transient withdrawal of antiretroviral therapy in HIV-infected patients may re-expose

the repaired immune system to critical HIV antigens.^{4-7;12;13;19;21} If exposure to virus is needed to produce protective HIV immunity, then we hypothesize that a vaccine with exposed antigenic epitopes may be more efficient and safer than allowing viral rebound to occur. Based on this hypothesis and on our preliminary data, we are in the process of conducting the specific pre-clinical tests of this vaccine candidate that are required prior to filing an investigational new drug application with the FDA.

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PROSTRATIN UPDATE

The phorbol ester, prostratin, used in Western Samoa as an ethnobotanical treatment for viral hepatitis, was isolated at NCI in 1992. Prostratin up-regulates expression of viral products from latently infected cells such as U1 and ACH-2 cell lines. It also inhibits the replication of HIV-1 and viral spread. Prostratin represents a distinct subclass of Protein Kinase C (PKC) activators based on the fact that it is non-tumor promoting. The lack of tumor promotion of prostratin coupled with its ability to up-regulate latent HIV-1 provirus expression are important features that could be exploited as an effective therapy to eliminate latent reservoirs for patients on Highly Activated Antiretroviral Therapy (HAART). More information on prostratin was detailed in the last issue of "Searchlight" available at www.aidsresearch.org. Considering the critical issue of latent virus in HIV chemotherapy, it is vital to enter prostratin into clinical trials as soon as possible.

Since the last issue of "Searchlight", the following progress has been made to move forward the development of prostratin as an anti-HIV drug, eliminating the viral reservoirs:

- Dr. Jerome Zack (UCLA) has obtained funding from amFAR to investigate the effects of prostratin on HIV latent reservoir in SCID-hu (Thy/liv) mice.
- Dr. Karen Copeland (University of Ottawa) is looking at the effects of prostratin on the activation of latently infected CD8+ cells.
- A grant application has been submitted to Glaxo-Smith-Kline to perform *in vivo* toxicology studies in rat and dogs for prostratin.
- A grant proposal has been submitted by Dr. Aftab Ansari's group at (Emory University) to study the pharmacokinetic of administration of prostratin to Rhesus macaque.

C L I N I C A L T R I A L S

STUDY	DESCRIPTION	STATUS
<p>Pre-clinical & basic research AIDS RESEARCH ALLIANCE</p>	<p>AIDS Research Alliance is engaged in a number of ongoing preclinical and basic research projects not ready for human clinical trials. For one example, see the box on page 7 outlining important progress made to date in our research into <i>prostratin</i>.</p>	<p>Ongoing</p>
<p>Serostim™ SERONO LABORATORIES</p>	<p>A study testing the safety and effectiveness of a growth hormone in treating HIV-associated lipodystrophy.</p>	<p>Currently enrolling</p>
<p>Hydroxy-chloroquine <i>(in combination with hydroxyurea and didanosine)</i> AIDS RESEARCH ALLIANCE</p>	<p>An open-label, Phase I/II study of the safety and antiviral efficacy of hydroxy-chloroquine in combination with hydroxyurea and Videx® (ddl or didanosine) in HIV-1 infected patients.</p>	<p>Currently enrolling</p>
<p>APV-30002 GLAXOSMITHKLINE</p>	<p>A Randomized, Open-Label, Two Arm Trial to Compare the Safety & Antiviral Efficacy of GW433908/Ritonavir QD to Nelfinavir BID when Used in Combination with abacavir and lamivudine BID for 48 weeks in Antiretroviral therapy naïve HIV-1 infected subjects.</p>	<p>Enrollment complete; study ongoing</p>
<p>Tipranavir™ PHARMACIA & UPJOHN</p>	<p>An open-label, randomized study comparing combination therapy (tipranavir and ritonavir vs. saquinavir and ritonavir) used with two nucleoside reverse transcriptase inhibitors in single protease inhibitor-experienced HIV-1 patients.</p>	<p>Enrollment complete; study ongoing</p>

STUDY	DESCRIPTION	STATUS
<p style="text-align: center;">Remune™ + HAART</p> <p style="text-align: center;">AGORON PHARMACEUTICALS</p>	<p>A randomized, double-blind, adjuvant-controlled, multicenter study to compare the virologic and immunologic effect of Highly Active Antiretroviral Therapy (HAART) plus REMUNE™ versus HAART plus Incomplete Freund's Adjuvant (IFA) on antiretroviral-naïve patients infected with HIV-1.</p>	<p style="text-align: center;">Enrollment complete; study ongoing</p>
<p style="text-align: center;">Zerit® (Stavudine)</p> <p style="text-align: center;">BRISTOL-MYERS SQUIBB</p>	<p>Evaluation of the safety and antiviral activity of stavudine <u>extended release</u> formulation as compared to stavudine <u>immediate release</u> formulation, each as part of potent antiretroviral combination therapy.</p>	<p style="text-align: center;">Enrollment complete; study ongoing</p>
<p style="text-align: center;">PMPA Prodrug</p> <p style="text-align: center;">GILEAD SCIENCES</p>	<p>A Phase II, randomized, double-blind, placebo-controlled study of the safety and antiviral activity of the addition of PMPA Prodrug to combination antiretroviral regimens in treatment-experienced HIV-infected patients.</p>	<p style="text-align: center;">Enrollment complete, study ongoing</p>
<p style="text-align: center;">AIDSVAX™ B/B</p> <p style="text-align: center;">VAXGEN, INC.</p>	<p>A double-blinded, placebo-controlled, Phase III trial to evaluate the efficacy of the AIDSVAX™ B/B vaccine in adults at risk of sexuality transmitted HIV-1 infection.</p>	<p style="text-align: center;">Enrollment complete, study ongoing</p>

For information about enrolling in any of our studies, contact Corie Castro at 310/358-2429. Transportation to our clinical research facility is available upon request. For priority notification of new/enrolling clinical trials, sign-up for our Priority Notification Program.

Lube Job—An Introduction to Microbicides

By Michael Slattery

Introduction

As the HIV epidemic continues to spread around the world and as we all wait for an effective vaccine to prevent HIV, the prevention field (with some notable exceptions) has had limited options and continues to focus on the use of condoms during sex in order to prevent HIV transmission. Although the terms may have changed from “Safer Sex” to “Harm Reduction”, the message taken home by many people is that they must reduce risky sexual activity by reducing sexual activity and/or consistently using condoms. Early in the epidemic there were some notable successes in preventing transmission of HIV and other STD’s as well. The primary motivation for those changes, as with many health related behavior changes, was fear of infection, disability, disease and death.

So it is not surprising that, with some of the recent advances in treatments for HIV, the Safer Sex message is increasingly being forgotten and behavioral relapses into riskier behavior are now evident. Recent reports from several cities of syphilis outbreaks among men who have sex with men (MSM) are indeed troubling.

Given the delays in vaccine development, the upswing in new HIV infections in certain populations, and some cultural and social aspects of the epidemic, (especially in Africa, Asia, and South America), which make condom use unacceptable, there has been a welcome resurgence of interest in alternative methods to prevent STD transmission.

Definition

Microbicides (literally: microbe killer) are envisioned as components of gels, creams, foams, lubricants or some other mechanism for delivering a topical agent which would prevent STD transmission¹. The agent in the lubricant would be targeted hopefully at multiple STD’s including HIV. Ideally, the lubricant would be used by both women and men during sexual relations, preferably with the concurrent use of a condom as well. This prevention method could be controlled by women and therefore may allow them the opportunity to protect themselves in cultures where the status of women doesn’t allow for a strong negotiating position, for example, demanding condom use from their partners.

There is some disagreement on whether or not microbicides would need to be contraceptive as well. Some fear that women would not use a “stand alone” microbicide for fear of the potential stigma associated with use of STD targeted microbicides but would feel comfortable buying a contraceptive that “by the way” also reduces STD risk. On the other hand, in some countries, a contraceptive-containing preparation might be less attractive for social or religious reasons.

How would microbicides work?

There are several mechanisms by which a successful microbicide could work. It could block infection by creating a barrier, it could kill or inactivate microbes or virus-

es or it could prevent viral replication in infected cells. For each of these actions the microbicide could be specific (like antibodies, fusion inhibitors, and antiretroviral medications), or more general (such as detergents, surfactants and pH stabilizers).

Microbicides are unlikely to be 100% effective in preventing transmission of HIV. However, a microbicide with a low level of anti-HIV activity that prevents, for example 30% of infections, but which might be used over 60% of the time would actually prevent more infections than if the more effective condoms were used in only 20% of all sexual acts.

The Case for Microbicides I: STD’s

In a recent report by the US Surgeon General², STD’s including HIV infect 12 million persons a year in the US. Five of the ten most commonly reported infectious diseases in the US are STD’s. More than 65 million people in the US are currently living with an incurable STD. Nearly two thirds of all STD’s occur in people younger than 25. In fact STD’s have a disproportionate impact on women, adolescents, and racial ethnic and sexual minorities.

But there is no one single STD epidemic; rather, there are multiple epidemics each with its’ own characteristics³. Some STD’s have been declining: syphilis has been declining since the early 90’s and with recent substantial decreases among African Americans it is now at an all-time low. CDC officials are now actually hoping to eliminate syphilis from

Sexually Transmitted Disease	New Cases Per Year in the United States
Chlamydia	3 million
Gonorrhea	650,000
Syphilis	70,000
Genital Herpes	1 million
Human Papillomavirus	5.5 million
Hepatitis B	120,000
Trichomoniasis	5 million

the US. But other STD's, such as gonorrhea and chlamydia and HPV (the virus that causes genital warts), have been on the increase.

Despite the large number of STD's, public awareness of the problem is not widespread. Many STD's do not have symptoms, especially in women. Yet, STD's can have devastating effects, *especially* in women and especially during pregnancy. Untreated, STD's in women can lead to Pelvic Inflammatory Disease (PID), which is one of the most common causes of infertility and ectopic pregnancy (which can be fatal). Human *papillomavirus*, believed to be the most common STD among young sexually active people, causes genital warts and some types of this infection are linked to increased rates of cervical cancer.

The Case for Microbicides II: HIV

According to the recent US Surgeon Generals report, to date 775,000 AIDS cases have been reported. Currently in the US, there are an estimated additional 900,000 HIV infected persons (without an AIDS diagnosis), and 40,000 new HIV infections occur each year.

Worldwide, the toll of HIV infection is enormous. Thirty-six million people world wide are liv-

ing with HIV/AIDS, 22 million have already died and new infections world wide are estimated at 15,000 a day! The cost to meet recent goals established by the UN to reduce HIV transmission worldwide, and increasing access to care, has been estimated at 9.2 billion dollars in 2005⁴.

Scientific Challenges

The scientific challenges to the development of a microbicide are many. The compound needs to be non-toxic to vaginal, penile and rectal tissue. It should be stable, not have the potential to cause birth defects (non-mutagenic), should not disrupt normal bacterial flora and pH, should not be absorbed systemically, and should be acceptable in terms of appearance, color, taste and consistency. It should be available without prescription, be compatible with barrier methods of contraception (eg, condoms) and the less it costs the better. This is obviously a tall order, and we do not even have answers to many basic scientific questions.

Rectal Use of a Microbicide—The MSM issues

Any approved microbicide will probably be used by MSM for rectal use^{5,6}. Such "off label" use has been prevalent historically, as

was the case with Nonoxynol-9 (N9)-containing products. But as a recent study of N9 showed, such use without proper testing and research could prove deleterious. Compared to the vaginal lining the rectal lining is very thin. Blood and cellular targets (for HIV) are closer to the surface, and the fact that the rectum is not closed at one end leads to questions of how much lubricant is needed, as well as how much is too much. In addition, both the pH and normal bacterial occupants of the vagina and rectum are quite different.

Ethical Challenges

Challenges to the development of a microbicide formulation are not only scientific. There are extensive ethical hurdles. Any clinical trial, especially large phase III trials, would have volunteers who, in the course of their normal sexual activity, expose themselves to STD's and thus might potentially become infected. All studies would therefore need to include extensive risk reduction counseling similar to that used in the Phase III trial of the HIV vaccine candidate AIDSVAX B/B[®]. Study participants would be encouraged to use condoms with each sexual act. This conservative approach increases the number of volunteers needed to show a difference between those using a condom with a placebo microbicide, versus those using a condom and the active microbicide.

Pharmaceutical Company Hurdles

Most of the work being done on microbicide development has been done at university centers and small biotech firms. So far, no large pharmaceutical companies

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involved. Part of the reluctance on the part of big pharmaceutical companies is their low estimate of the potential market for such products. This situation is exacerbated by tensions between big pharmaceutical companies and the developing world over such issues as patent protection, and the fact that poor men and women in developing countries are not a commercially “desirable” primary market. This problem persists despite pledges from several organizations (including the WHO) to buy microbicides for use in the developed world.

Allocated Resources

Fortunately, though later than one would have hoped, several government agencies have recently increased their commitments to funding research into microbicides. Notably, there has been a Topical Microbicide Program supported by NIAID⁷ with similar groups in the CDC⁸. The 2000

Conference on Microbicides held in Washington, DC brought together many of the groups working in the field. Recently the NIH hosted a meeting looking at the critical issues involved in designing microbicides and testing them for rectal use, and also published a Request for Proposals (RFP) to get research sites involved in planning such studies. Private foundations and research groups have begun to get involved, eg., the Population Council and Rockefeller Foundation have entered a collaboration called the Microbicide Basic Science Network.

Drugs in Development

There currently are over 60 candidate microbicides in early preclinical and early phase I studies around the world. In a follow-up article, and as the field moves forward, we will publish an article specifically about the “microbicide pipeline”. Since our space here is limited, for a full list of drugs in

development refer to the *Alliance for Microbicide Development* website at www.microbicide.org (see “Clinical Trials Information Center” and “Products Database” pages). The Alliance for Microbicide Development, directed by Dr. Polly Harrison⁹ is a coalition of 120 small biotech companies, academic researchers, and advocacy groups. Numerous additional relevant resources can be found at the same site on their “links” page. Hopefully, the renewed interest in the development of microbicides, combined with additional government and private resources, will serve to move this important field decisively forward in the near future.

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“Microbicide development has gained size and speed over the past few years, but it’s still way too slow. That’s deeply distressing. From a public health standpoint, you could say it’s just plain negligent. Microbicides could make a real difference for the millions worldwide who can’t adequately protect themselves from these epidemic infections, and they could really make a difference in stemming the epidemics themselves. We need to speed up the clinical testing of products that look safe and have a reasonable chance of being effective, so we can say to the world, “Hey, these things can work; they can save lives.” This isn’t easy business, but lots of researchers and small struggling companies have been taking big risks for years with little help. Now we need policy-makers and scientific leaders and bureaucrats and funders to go and do likewise, not tomorrow, not next year, but NOW!” Polly Harrison, Director of the Alliance for Microbicide Development.

AIDS Vaccines in the New Millennium Meetings Updates

By Marjan Hezareh, Ph.D.

Researchers at both the *AIDS Vaccines in the New Millennium* conference in Keystone (28 March-3 April) and at the *AIDS Vaccine 2000* in Philadelphia (September 5-8) presented a broad range of studies on HIV vaccine candidates. The need for a vaccine has never been greater and important progress toward this goal was presented during these meetings. NIH has increased HIV-vaccine research more than six-fold since 1990, to an estimated \$356.6 million for fiscal year 2002. At NIAID (NIH institute for HIV vaccine research), an estimated \$450.7 million will be devoted to all vaccine research in year 2002, with 61 percent of that total (\$276.5 million) dedicated to HIV vaccine development. "The field of vaccine research owes great thanks to the largesse of the American people, the Administration and Congress for making these resource available," said Dr. Fauci in his keynote lecture in Philadelphia.

The data being discussed demonstrated an increasingly complex collection of vaccine candidates and approaches in development, which offer a new basis for new optimism. A better knowledge of envelope structure allowed researchers 1. to define new HIV antigen structures to induce useful immune response, and 2. to better understand how the immune system first recognizes and responds to HIV.

These advances helped to design new strategies that may provoke efficient antigen uptake and presentation leading to better T-cell activation. A growing number of studies were directed toward the role of mucosal immunity in HIV protection and the probability of including mucosal immunity as a component of vaccine-induced pro-

tection. Like the skin, the mucosal epithelia are barriers between the internal and external environments, and are therefore an important first line of defense, specifically in HIV infection that is transmitted sexually. Furthermore, the ongoing phase I and II trials, and diverse vaccine approaches including gp120 and canarypox vectored HIV-1 candidate vaccines (ALVAC) provided increasing clarity about the safety and immunogenicity thresholds required to take a vaccine from animal studies into human trials and Phase III evaluation of efficacy. Here we summarize some important data on HIV vaccine development presented at these conferences.

Protection with Adeno-Associated Virus Vectors

Philip Johnson from the *Children's Research Institute*, Columbus, Ohio, showed data from ongoing studies of an SIV vaccine based on vectors of recombinant Adeno-Associated Virus (rAAV). Wild type AAV is a replication defective parvovirus, which is non-pathogenic in humans and animals. In this study, they generated rAAV vectors (which lack any AAV gene) expressing SIV env, rev, gag and protease gene and used them to immunize intramuscularly 8 monkeys (single dose). They detected a strong CTL response and persistent neutralizing antibody titers over 14 months. After immunization, animals were challenged intravenously with a high dose of SIV-E660. In vaccinated animals, viral peak was reduced by about 1.3 log and viral load by 3 logs. After 6 months, vaccinated animals were still healthy, while 3 out of 8 control animals died. Based on these data, rAAV vectors stimulate robust, durable and effec-

tive immune responses against SIV. In addition, they demonstrated that immunization with DNA prime/rAAV boost stimulate better immune responses than rAAV alone. Dr. Johnson suggested that these data provide evidence necessary to move forward the clinical trial of cognate rAAV/HIV vaccine in human.

Protective immunity induced by whole recombinant Yeast-Based HIV Vaccine

Dr. Franzusoff from GlobeImmune, Inc., Denver, presented data on the development of a novel therapeutic vaccine based on using, whole, recombinant, non-pathogenic yeast (*Saccharomyces Cerevisiae*) as a vector. The optimal stimulation of CTLs requires presentation of antigens by Dendritic Cells (DCs). These cells are unique in their ability to process antigens into the MHC class I pathway for presentation to CTLs. In this study they engineered recombinant yeast expressing HIV-1HXB2 p55 gag protein (HIVAX-2). Mice (C57B1/6) were immunized subcutaneously with 20 million intact live or heat-killed yeast. The vaccination stimulated strong immune response in mice, measured by intracellular IFN γ staining, by CTL-mediated cell lysis and by T cell proliferation assays. To show protective immunity, they used HIV-gag dependent tumor model. Mice were protected against subsequent challenge with B16 melanoma cells stably expressing HIV-1HXB2 p55 gag. Therefore, the recombinant yeast-based HIV vaccine is a potent activator of cellular-immune responses and is currently being prepared by GlobeImmune, Inc. for use as a therapeutic HIV vaccine in a phase I clinical trial.

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AIDS Vaccines in the New Millennium—

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Merck's DNA and Adenovirus-Based Vaccine

Merck researchers have developed two types of vaccine; one based on "naked DNA" and the other on an adenovirus vector. At the Keystone meeting they presented pre-clinical studies that led them to move these 2 vaccines candidate into phase I trials. In his presentation, John Shiver, Merck's Director of Vaccine Research showed data from comparative studies of several HIV vaccine approaches. All vaccines contained SIVgag gene but no env gene. In this study rhesus macaques were immunized with one of the following vaccines: plasmid DNA in saline, alum or adjuvant (CRL10050 adenovirus type 5 (Ad5)-based viral vectors Modified Vaccinia Ankara (MVA)-based viral vector. Each vaccines were administered intramuscularly to 3 animals 3 or 4 times over 32 weeks. Immunization with the Ad5 based vector resulted in the highest immune response. Response to plasmid DNA in saline or alum was much lower than DNA in adjuvant (CRL1005), showing a clear advantage of adjuvanting DNA. CD4 responses were induced by all vaccines and were higher in the DNA group. The Ad5-based vaccine tends to induce higher CD8 responses. Three months after immunization animals were challenged with a high dose of SHIV89.6P, a very pathogenic lethal strain. At day 180 after challenge all vaccinated animals were alive (although infected), while 4 out of 6-control animals had died. In Ad5 groups, CD4 count remained high and viral load slowly brought under control and peak viremia was reduced by 1.5 log. This group showed the best clinical course fol-

lowed by adjuvanted DNA with CRL1005 and MVA-based vaccine. Vaccination with DNA in saline or alum resulted in a strong drop in CD4 cells count and the poor control of viremia.

Next Emilio Emini reported on pre-clinical evaluation of Merck's HIV vaccine candidates currently in clinical trials. In his presentation he addressed the issue of pre-existing immunity to adenovirus' which could make this a less immunogenic vector. About 10% of the US population have significant neutralizing antibodies to adenovirus and 40% of them shows lower level of antibody. Therefore they preexposed 6 monkeys to adenovirus and then immunized them with a high dose (1011 particles) of Ad5-gag (HIVgag). The presence of neutralizing antibodies reduced by several folds the T-cell responses but did not eliminate it. He also presented data on a DNA prime/Ad5 boost study. In this study animals were given first DNA/CRL1005 adjuvant and then boosted with a high dose (107 particles) of Ad5-gag (HIV gag). The vaccinated animals showed a 5-10 fold increase in the number of HIV-specific-T cells response after the boost. This is generally reflected by an increase in CD4 and CD8 T cell response, again CD8 response predominating after Ad5 boost. Thus the preexisting immunity could be overcome, especially if using DNA prime/Ad5 boost strategy. Vaccination with DNA in saline or alum was a less effective prime showing little T cell response. Emini suggested that a 109 particle dose, should still induce a good T-cell response even if neutralized by 99%. He also said that nef and pol would be added to Merck's vaccines in

addition to gag. This is based on data showing that HIV-infected people with good immune responses to these proteins maintain lower viral load. A DNA gag trial began last year in uninfected people and a trial in HIV-infected people started in March 2001.

Importance of mucosal CTL to prevent mucosally-transmitted SIV/HIV

In his study, Jay Berzofsky from the *National Cancer Institute*, Bethesda, compared mucosal versus systemic immunization in macaque. They demonstrated that mucosal, but not systemic immunization, protected macaques against an SIV challenge. In this study animals were immunized intrarectally (ir), or subcutaneously (s.c.) with peptide HIV vaccine including SIV gag and pol epitopes. After immunization, animals were challenged with SHIV-ku and monitored for 200 days. All animals showed similar viral peaks after challenge. However, following this peak, the viral loads dropped to undetectable levels in ir-immunized animals and they maintained a high CD4 count up to 200 days post-infection. In contrast the sc-immunized animals had high viral loads and a low CD4 cell-counts. All immunized animals showed some degree of protection compared with control animals. Two hundred days post-infection, animals were sacrificed and HIV level in colon and jejunum was monitored. Little or no virus was seen in the gut tissue of ir-immunized macaques, while 10-100 times more virus has been found in control and sc-immunized animals. The ir-immunized animals displayed a higher level of HIV-specific CTLs in their colon than sc-immunized animals. These data is in agreement with Berzofsky's study in mice. In this study they showed a clear relationship between mucosal vaccina-

tion, high level of mucosal CTLs and protection against subsequent mucosal challenge. Berzofsky's study is the first showing a correlation between mucosal immunization and improved protection in primates.

Future Directions

Why there are relatively few studies on mucosal immunity? This is mainly because analysis of mucosal immune activity requires an invasive procedure, while systemic immune activities can be easily measured from blood samples. Several groups are developing techniques to use homing markers as a way of measuring mucosal response in blood. Homing markers are cell surface molecules that indicates destinations of cells in blood stream. Therefore, it is possible to monitor cells bearing these markers from blood samples rather than from mucosa. One example of such a marker is alpha4beta7, the latter is an integrin that appears on all cells trafficking to the gut. More research has been focused on understanding the early events in HIV infection, such as the role of dendritic cells in the mucosa. It appear that these cells carry HIV to lymph nodes and from there virus spread to other sites. More effort has been focused on understanding the mechanism of action of DC-SIGN, the receptor that carries HIV to lymph nodes, and to find a way to inhibit its action. Although a vaccine that prevents or delays HIV infection remains an elusive goal, more good news is coming our way.

"Perseverance is a great element of success. If you only knock long enough and loud enough at the gate, you are sure to wake up somebody."

— Henry Longfellow



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