The long and winding road
to an HIV vaccine

Pam Skinner
University of Minnesota
skinn002@umn.edu

Overview of Seminar

• Why do we care?
• HIV pathogenesis
• HIV Vaccines and new approaches
• My work

Please feel free to ask questions during the seminar or afterwards
AIDS has killed more than 30 million people since it was first recognized in 1981, making it one of the most destructive epidemics in recorded history.

Global summary of the AIDS epidemic | 2011

<table>
<thead>
<tr>
<th>Number of people living with HIV</th>
<th>Total</th>
<th>34.0 million [31.4–35.9 million]</th>
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<tbody>
<tr>
<td></td>
<td>Adults</td>
<td>30.7 million [28.2–32.3 million]</td>
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<td>Women</td>
<td>16.7 million [15.4–17.6 million]</td>
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<td>Children (&lt;15 years)</td>
<td>3.3 million [3.1–3.8 million]</td>
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<table>
<thead>
<tr>
<th>People newly infected with HIV in 2011</th>
<th>Total</th>
<th>2.5 million [2.2–2.8 million]</th>
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<tbody>
<tr>
<td></td>
<td>Adults</td>
<td>2.2 million [1.9–2.4 million]</td>
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<tr>
<td></td>
<td>Children (&lt;15 years)</td>
<td>330 000 [280 000–390 000]</td>
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<tr>
<th>AIDS deaths in 2011</th>
<th>Total</th>
<th>1.7 million [1.5–1.9 million]</th>
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<tbody>
<tr>
<td></td>
<td>Adults</td>
<td>1.5 million [1.3–1.7 million]</td>
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<tr>
<td></td>
<td>Children (&lt;15 years)</td>
<td>230 000 [200 000–270 000]</td>
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Most new HIV infections occur in women via heterosexual sex.
**HIV in USA**

- ~1 in 5 adults and adolescents infected with HIV are unaware of their HIV status
- Most in MSM
- Disproportionately affecting Black Americans
- HIV 3rd leading cause of death among Black women (5th all women) ages 25-44
Statistics: Women and HIV/AIDS

In the United States

- Women account for one in four new HIV diagnoses and deaths caused by AIDS.
- The proportion of AIDS diagnoses reported among women has more than tripled since 1985.
- The vast majority of women diagnosed with HIV contracted the virus through heterosexual sex.
- African Americans constituted 64 percent of women diagnosed with HIV/AIDS in 2009.
- African Americans and Hispanics represent 26 percent of all women in the U.S. but they account for 82 percent of AIDS cases among women.
- African-American women have an HIV prevalence rate nearly 15 times that of white women.

Statistics: Women and HIV/AIDS

Worldwide

• Women constitute more than half of all people living with HIV/AIDS.
• For women in their reproductive years (15–49), HIV/AIDS is the leading cause of death.
• Women are at least twice as likely to acquire HIV from men during sexual intercourse than vice versa.
• Among young people aged 15-24, the HIV prevalence rate for young women is twice that of young men.
• A study in South Africa recently suggested that nearly one in seven cases of young women acquiring HIV could have been prevented if the women had not been subjected to intimate partner violence.
• In 2011, 57 percent of pregnant women living with HIV in low- and middle-income countries received effective drug regimens to prevent new HIV infections among children.
• In sub-Saharan Africa, women constitute 58 percent of all people living with HIV/AIDS.


We need increased understanding of HIV pathogenesis and antiviral host immunity in order to:

• Develop effective vaccines to stop the spread of HIV
• Develop better treatments for HIV infected individuals
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Can HIV be transmitted by oral sex?

HIV Transmission

- Vaginal, anal, or oral sex
- Contaminated syringes
- Blood transfusions
- Mother to child
  - Before or during child birth (25% untreated)
  - Breast feeding
What is the most efficient available technology to reduce the sexual transmission of HIV and other sexually transmitted infections?

HIV Transmission

The male latex condom is the most efficient available technology to reduce the sexual transmission of HIV and other sexually transmitted infections (UNAIDS/UNFPA/WHO, 2004)
HIV infection

- Two to four weeks after infection with HIV most people develop an illness similar to infectious mononucleosis.

- This illness can include a sore throat, fever, enlargement of glands, headache, sore neck, muscle and joint aches, skin rash and nausea and vomiting.

http://www.hiv.is/en/page/living_with_hiv_the_skin_and_hiv
CD4 T cells depleted

**HIV-**

**HIV+**

Figure 2: Endoscopic and histological analysis of the GI tract. Endoscopic photographs from subjects 1441, 600, and 1519 (B) were obtained by passing the endoscope past the anus and into the terminal ileum. Immunohistochemical staining for CD4 was performed by removing sections from the lymphoid tissues with the transverse colon and rectum using a disposable biopsie forceps. Staining for CD4 (+E and EG).
Most HIV replication occurs in lymphoid tissues during chronic infection


- Most CD4+ T-cells are located in secondary lymphoid tissues
- % CD4+ T cells productively infected is 5-10 times greater in LT than PBMC

**Time course of HIV infection**

(Nature Reviews | Immunology)
Acquired Immune Deficiency Syndrome (AIDS)

- AIDS is clinically diagnosed when the following criteria are met:
  - CD4+ T-cell count below 200/ul (normal is ~1200)
  - Two or more opportunistic infections associated with HIV:
    - Kaposi’s sarcoma, PCP, Candidiasis, CMV, HSV, and many others

- Patients may develop AIDS wasting syndrome
  - Loss of 10% or more of body mass due to decreased appetite, altered metabolism, decreased nutrient absorption

- Patients may develop AIDS dementia complex
  - Cognitive and motor problems, memory loss due to opportunistic infections, direct effects of HIV on the brain, toxic effects of drug treatments
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Types of vaccines

• Live attenuated HIV (too risky)
• Inactivated HIV (too risky)
• HIV Subunit, e.g. gp120 Env protein
• HIV DNA
• Recombinant vector containing HIV DNA, e.g. AAV
Past HIV Vaccine trials

None of these worked
Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand

Supachai Reks-Ngam, M.D., Phaninee Phitsutthum, M.D., D.T.M.H., Soracha Nitayaphan, M.D., Ph.D., Jurantit Kawkwangwai, Ph.D., Joseph Chiu, M.D., Robert Paris, M.D., Nakorn Premrini, M.D., Chawetsang Namwat, M.D., Mark de Souza, Ph.D.; Elizabeth Adams, M.D., Michael Benenson, M.D., Sanjay Gunaratna, M.D., Jan Tartaglia, Ph.D., John G. McNeil, M.D., Donald P. Francis, M.D., D.Sc., Donald Stabile, Ph.D., Deborah L. Bix, M.D., Suparni Chongsritratana, M.D., Chirasak Khamsomboonang, M.D., Prasit Thongharoen, M.D., Ph.D., Marline L. Robb, M.D., Nelson L. Michael, M.D., Ph.D., Prayura Kunaros, M.D., and Jerome H. Kim, M.D., for the MOPH-TAVEO Investigators

ALVAC-HIV (vCP1521) is a recombinant canarypox vaccine developed by Virogenetics Corporation (Troy, NY) and manufactured by sanofi pasteur (Marcy-Étoile, France). The recombinant canarypox was genetically engineered to express HIV-1 Gag and Pro (subtype B LAI strain) and CRF01 AE (subtype E) HIV-1 gp120 (92TH003) linked to the transmembrane anchoring portion of gp41 (LAI). ALVAC Placebo (sanofi pasteur) was a sterile, lyophilized product consisting of virus stabilizer and freeze-drying medium reconstituted in 1 ml of 0.4% sodium chloride.

AIDSVAX B/E (Global Solutions for Infectious Diseases, South San Francisco, CA), is a bivalent HIV gp120 envelope glycoprotein vaccine containing a subtype E envelope from the HIV-1 strain A244 (CM244) and a subtype B envelope from the HIV-1 MN produced in Chinese hamster ovary cell lines. The envelope glycoproteins, 300 μg of each, originally manufactured by Genentech, Inc., and further developed by VaxGen, Inc., are co-formulated with 600 μg of alum adjuvant. AIDSVAX placebo (VaxGen, Inc.) was 600 μg alum adjuvant.

For First Time, AIDS Vaccine Shows Some Success

By DONALD G. MCNEIL &
Published: September 24, 2009

A new AIDS vaccine tested on more than 10,000 volunteers in Thailand has protected a significant majority against infection, the first time any vaccine against the disease has ever partly succeeded in a clinical trial.

Thai Public Health Minister, Witthaya Kaepradita, right, speaks with U.S. Ambassador to Thailand, Eric John, during the press conference to announce the results of the world’s largest HIV vaccine trial ever conducted.

Scientists said they were delighted but puzzled by the result. The vaccine—a combination of two genetically engineered vaccines, neither of which had worked before in humans—protected too few people to be declared an unqualified success. And the researchers do not know why it worked.

“I don’t want to use a word like ‘breakthrough,’ but I don’t think there’s any doubt that this is a very important result,” said Dr. Anthony S. Fauci, director of the National Institute of Allergy and Infectious Diseases, which is one of the trial’s backers.

“After more than 20 years now, vaccine trials have essentially been failures,” he went on. “Now it’s like we’re going down an unlit path, and a door has been opened. We can start asking some very important questions.”

Results of the trial of the vaccine, known as RV 144, were released at 2 a.m. Eastern time Thursday in Thailand by the partners that ran the trial, by far the largest of all AIDS vaccines: the United States Army, the Thai Ministry of Public Health, Dr. Fauci’s institute, and the patent-holders in the two parts of the vaccine, Sanofi-Pasteur and Global Solutions for Infectious Diseases.

Col. Jerome H. Kim, a physician who is manager of the Army’s HIV vaccine program, said half the 16,402 volunteers were given six doses of two vaccines in 2006 and half were given placebos. They then got regular tests for the AIDS virus for three years. Of those who got placebos, 74 became infected, while only 31 of those who got the vaccines did.

Although the difference was small, Dr. Kim said it was statistically significant and meant the vaccine was 31.2 percent effective.
Vaccines to date have not worked well

- Virus mutates to evade antibody response
- Not protective across clades
- Recombinant viral and DNA vaccines that target CTL have not shown much promise - short lived
- Pre-immunity to vaccine vectors
- Viral reservoirs
New Approaches

• Neutralizing antibodies
  – Immunotherapy
  – Vaccination
• Stimulating DC to present HIV epitopes
• Heterologous prime boost --CTL
• Chronic antigen presentation –CTL
• CCR5 targeting-block infection
• Targeting HIV reservoirs

PD1-based DNA vaccine amplifies HIV-1 GAG-specific CD8+ T cells in mice

Jingying Zhou,1 Allen K.L. Cheung,1 Zhiwu Tan,1 Haibo Wang,1 Wenbo Yu,1 Yanhua Du,1 Yuansi Kang,1 Xiao fen Lu,1 Li Liu,1 Kwok-Yung Yuen,1,2 and Zhiwei Chen1,2

1AIDS Institute and Department of Microbiology and 2Research Center for Infection and Immunity, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China.

Viral vector-based vaccines that induce protective CD8+ T cell immunity can prevent or control pathogenic SIV infections, but issues of preexisting immunity and safety have impeded their implementation in HIV-1. Here, we report the development of what we believe to be a novel antigen-targeting DNA vaccine strategy that exploits the binding of programmed death-1 (PD1) to its ligands expressed on dendritic cells (DCs) by fusing soluble PD1 with HIV-1 GAG p24 antigen. As compared with non-DC-targeting vaccines, intramuscular immunization via electroporation (EP) of the fusion DNA in mice elicited consistently high frequencies of GAG-specific, broadly reactive, polyfunctional, long-lived, and cytotoxic CD8+ T cells and robust anti-GAG antibody titers. Vaccination conferred remarkable protection against mucosal challenge with vaccinia GAG viruses. Soluble PD1-based vaccination potentiated CD8+ T cell responses by enhancing antigen binding and uptake in DCs and activation in the draining lymph node. It also increased IL-12-producing DCs and engaged antigen cross-presentation when compared with anti-DEC205 antibody-mediated DC targeting. The high frequency of durable and protective GAG-specific CD8+ T cell immunity induced by soluble PD1-based vaccines suggests that PD1-based DNA vaccines could potentially be used against HIV-1 and other pathogens.

Coerce B cells to develop broadly neutralizing antibodies similar to natural development.
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HIV and Prion diseases

HIV
- HIV SIV CTL
- Vaccines
- Novel immunotherapy

Prion diseases
- Genomic fingerprint
- Novel diagnostic tools
- Novel therapy

CTL response to SIV
Too little too late
CD8 T cells important for control of HIV/SIV infection

- Appearance of HIV specific CD8 T cells coincides with decline in HIV viremia during primary HIV
- Removal of CD8 T cells leads to increased SIV viremia
- Long term non-progressors maintain strong HIV CTL responses
- Inverse correlation of HIV CD8 T cells and viral load
- CTL escape mutants associated with progressive disease
Mamu-A*01 Tetramers detect SIV-specific CD8 T cells

In situ MHC-class I tetramer staining to label virus-specific CD8 T cells

Matt Reynolds
In situ tetramer staining

- Fresh tissue sections
  - Draining LN
  - Non-draining LNs
  - Spleen
  - Ileum
  - Vagina
  - Cervix

MamuA*01/gag(CM9)
MamuA*01/tat(SL8)
MamuA*01/flp (neg control)

70% of response
Analyze stained tissue sections with confocal microscope

SIV-Specific CTL Too Late

13 dpi vagina

28 dpi vagina

20 dpi Lymph node

Tetramer CD8
Conclusion

SIV disseminates systemically long before the development of the adaptive CTL response—too little too late.
Hypothesis

Effective HIV vaccine requires the induction of a robust sustained virus-specific CTL response at portal of viral entry and in lymphoid tissues—enough, on time.

Live Attenuated SIVs are the Best Model

- Most animals Protected
  - Most (94%) show >3 log suppression of viral load
- Sterile protection observed in 50% challenged animals
- What are correlates of protection?


Koff et al. NATURE IMMUNOLOGY VOLUME 7 NUMBER 1 JANUARY 2006
CD8 T cells likely play an important role in protection induced by live-attenuated SIV vaccines

- Temporary depletion of CD8 T cells leads to increase in viral loads

Schmitz et. al, J. Virology, July 2005, p. 8131–8141

SIVΔnef vaccination SIVmac251 challenge
Mamu A*01 animals

5 wks no significant protection 20 Weeks protection 50% sterilizing
Vagina and Cervix

Tetramer+ cells detected mainly in the lamina propria

Lymphoid Aggregates in Vagina and Cervix

Clusters of tetramer+CD8+CD3+, CD8+CD3+, and CD8-CD3+ in the lamina propria and submucosa

Associated with no, a few, to many B cells
Localization of tetramer+ cells mainly in T cell zone (CD3+ or/and CD8+).
Tetramer+ cells localize with CD3+ cells mostly in marginal zone, and red pulp of the spleen.

**Ileum**

#174.96 (140 dpi dHef+0dpc SIV A01 genotype) ileum section x14

CD3/CD20/CM9

Peyer's patch
Abundance of SIV-specific CD8 T cells

- SIV-specific CD8 T cells present in all tissues at time of challenge
- No expansion of SIV-specific CD8 post-challenge

Ki67 expression in SIV-specific CD8 T cells

- Populations of tet+Ki67+ maintained in lymphoid and genital tissues
- Relatively high ongoing levels in genital tissues
- Ongoing local replication contributes to maintenance of populations of CTL in genital tissues
Conclusions

Protection induced by SIVΔnef:

• CTL present in genital and lymphoid tissues at time of challenge “Enough on time”
• Does not require a change in the location or abundance of SIV-specific CTL
• Change in CTL quality? Change in antibodies?

Mechanism of Protection

SIV-specific CD8 $T_{EM}$ cells present in tissues at time of challenge cooperate with antibodies to kill most challenge virus-producing cells at the portal of entry and maintain control of intermittent SIV-producing cells throughout the body as they appear.
CTL and follicular reservoirs of HIV

– immunotherapy to treat HIV
Lymph Nodes

HIV-producing cells are concentrated inside B cell follicles

Lymphoid follicles are a reservoir for HIV

Folkvord, et al., AIDS Res and Human Retroviruses 21 (5), 2005
HIV-specific CTL excluded from B cell follicles

Same true during SIV infection?

Yes
Successful HIV vaccine must prevent seeding of follicles with HIV or enable clearance of reservoir

Immunotherapy for existing HIV

We hypothesize that introduction of CXCR5 into virus-specific CTL will enable them to home to follicles and suppress HIV and SIV replication.
No CTL
HIV/SIV similar inside and outside of follicle

CTL Present
HIV/SIV concentrates inside of lymphoid follicles

CXCR5-CTL immunotherapy
Deliver CTL to follicles
HIV/SIV is eliminated

Acute HIV/SIV

Chronic HIV/SIV

CXR5 CTL therapy

CXCR5 immunotherapy may clear HIV reservoir in B cell follicles and lead to a functional cure

CXCR5 transduction of SIV-specific CTL

A. cmv puf rre EF1a CXCR5 Ires GFP

B. CXCR5 Transduction of SIV-specific CTL

C. Todd Reinhart gave us rhCXCR5 gene; Scott McIvor gave us lentivirus vectors
CXCR5-GFP transduced rhesus macaque CD8 T cells

Future

• Expand CXCR5-transduced SIV-specific CTL
• Inject into autologous SIV infected macaque
• Determine whether cells migrate to follicles and virus levels are reduced
• Cure HIV
Acknowledgments

University of Minnesota
Ashley Haase
Qingsheng Li
Lijie Duan
Cavan Reilly
Scott McIvor

University of California Davis
Chris Miller
Txell Genesca

University of Wisconsin
WNPRC
David Watkins (now in Florida)
Matt Reynolds
Eva Rakasz
Nancy Wilson

Harvard NEPRC
Paul Johnson

U Pittsburgh
Todd Reinhart