Global Diseases: Voices from the Vanguard
University of Georgia

Hookworm
“The Great Infection of Mankind”

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Professor & Chair
Dept. Microbiology Immunology & Tropical Medicine
The George Washington University

Principal Scientist
Human Hookworm Vaccine Initiative
Sabin Vaccine Institute
The Rockefeller University

2003 Roderick Maciejewski
2001 Paul Nurse
2000 Paul Greengard
1999 Gunter Blobel
1984 R. Bruce Merrifield
1981 Torsten Wiesel
1975 David Baltimore
1974 Albert Claude
1974 Christian de Duve
1974 George E. Palade
1972 Stanford Moore
1972 William H. Stein
1972 Gerald M. Edelman
1967 H. Keffer Hartline
1966 Peyton Rous
1958 Joshua Lederberg
1958 Edward L. Tatum
1953 Fritz Lipmann
1946 John H. Northrop
1946 Wendell M. Stanley
1944 Herbert S. Gasser
1930 Karl Landsteiner
1912 Alexis Carrel
Repairing The World: *Tikkun Olam*

- **Concept from The Kabbalah**
  - God left a corner of the world unfinished after 6 days
  - Poverty and Disease
- **Rabbi Isaac Luria (1534-72)**
  - Kabbalist
  - Communicated with the souls of *tzaddikim*
  - "See" people’s sins
  - Dressed in white on *Shabbat*
- **Post-Holocaust**
  - Elie Wiesel
  - Shlomo Bardin
  - Emil Fackenheim
  - Mario Cuomo
  - *Pursuit of Compassion, Peace, and Social Justice*
As it was when I first saw it, so it is now, one of the most evil of infections. Not with dramatic pathology as are filariasis or schistosomiasis, but with damage silent and insidious. Now that malaria is being pushed back hookworm remains the great infection of mankind. In my view it outranks all other worm infections of man combined...in its production, frequently unrealized of human misery, debility, and inefficiency in the tropics.
The Soil-Transmitted Helminths

Disease          | No. Infected |
-----------------|-------------|
Ascariasis       | 1,221,000,000|
Trichuriasis     | 795,000,000  |
Hookworm         | 740,000,000  |

Village of Paquila, Guatemala
Highest Worm Burdens in School-aged Children

Mean Worm Burden

Mean Age (years)

0  10  20  30  40

Mean Age (years)
Soil-transmitted Helminths and Children

Haiti

Paraguay
Soil-Transmitted Helminth Infections and Child Health

Ascariasis Post-treatment
With Albendazole or Mebendazole
Soil-transmitted Helminths and Schistosomes Impair Physical & Intellectual Development

![Graph showing weight percentiles over age (months) with Thiabenazole treatment for 2 days.]

**Title:** Parasitology Today

- Helminth Infection and Educational Achievement
- Dilations in mosquito ovarioles
- Recurrent leishmaniasis and immunosuppression
- CD8+ T cell-coccidia interactions
<table>
<thead>
<tr>
<th>Disease Condition</th>
<th>Disease Burden</th>
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<tbody>
<tr>
<td>HIV-AIDS</td>
<td>84.5 million</td>
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<tr>
<td>Helminth Infections</td>
<td>49.9 million</td>
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<tr>
<td>(Hookworm = 22.1 million)</td>
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<tr>
<td>Malaria</td>
<td>46.5 million</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>34.7 million</td>
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</tbody>
</table>

The “Gang of Four”
Resolution 54.19

Goal of attaining a minimum target of regular administration of anthelmintic chemotherapy (BZA + PZQ) to at least 75% and up to 100% of all school-age children at risk of morbidity by 2010

Source: www.who.int/wormcontrol
1. Mebendazole Cure Rates = 21%

2. Hookworm infected patients reacquire hookworm to pre-treatment levels within 4-12 months following anthelmintic chemoRx

3. Efficacy of BZAs diminishes with increasing use
Hookworm-Blood Loss

Adult worms injure their host by causing intestinal blood loss:

30 to 200 μL blood per day per hookworm
Loss of Host Iron and Protein
40 Hookworms = 1.2 ml Blood daily = 0.6 mg Fe

Necator americanus  Gut attachment  Hookworm Blood Loss
Maternal-Child Consequences of Hookworm Disease
Iron Deficiency Anemia and Protein Malnutrition

37% of Iron Deficiency Anemia in Brazil (Brooker et al, 2006)
35% of Iron Deficiency Anemia in Zanzibar (Stoltzfus et al, 1997)
22-73% of Severe Anemia Africa
Hookworm = Malaria as a Cause of Anemia

Consequences of Hookworm Disease:

Child growth retardation
Child Intellectual & cognitive impairments

Adverse maternal-fetal outcomes
Increased maternal mortality
Low birthweight
Increased infant mortality

Hookworm and Malaria

Geographic Overlap

Anemia Co-Morbidity

Brooker S et al. Unpublished data
Life Cycle of *Necator americanus* (Hookworm)

**Diagram:**
- Eggs pass out in feces
- Larvae hatch and develop in soil
- Larvae enter lung capillaries
- Larvae enter alveolar spaces
- Larvae migrate up trachea, are swallowed
- Adults mature in small intestine
- Pathology: Normal vs. Anemia
- Filariform larvae "quest" on blades of grass
- Larvae enter bloodstream, reach heart

**References:**
Feasibility of Anti-Hookworm Vaccine Development

Lines of Evidence with L3 (third-stage infective larvae)

• Success vaccinating dogs against canine hookworm infections (*Ancylostoma caninum*) with L3

• Trickle doses of live L3 or live L3 attenuated by ionizing radiation (X-rays, gamma-rays, Ultraviolet irradiation)

• Vaccine protection mediated by L3 secreted antigens

Hotez et al. *Int J Parasitol* 2003; 33: 1245-58
The Human Hookworm Vaccine Initiative

Early Strategy

To reproduce the effect of attenuated L3 vaccines by substituting a genetically-engineered recombinant L3 antigen

Hookworm L3 Secreted Antigen: Discovery and Selection

1) The Major L3 Secreted Proteins
- 45 kDa Ancylostoma secreted protein 1 (ASP-1)
- 22 kDa Ancylostoma secreted protein 2 (ASP-2)
- 62 kDa Astacin Metalloprotease 1 (MTP-1)

2) Released by L3 in response to Host Serum

3) Released during Early Host Entry

**ASP**s = Single and double PR-1 domain Proteins

<table>
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<tr>
<th>ASP-1</th>
<th>SP</th>
<th>PR1</th>
<th>MT</th>
<th>PR1</th>
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<tr>
<td>ASP-2</td>
<td>SP</td>
<td>PR1</td>
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<td></td>
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<tr>
<td>21-22 kDa</td>
<td>c</td>
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</table>

PR-1 Protein
Pathogenesis
Related Protein
Superfamily
Invertebrates
Vertebrates
Plants
IMMUNE RECOGNITION of RECOMBINANT L3 SECRETED ANTIGENS : ASP-2

Epidemiological evidence pointing to ASP-2

Cross-sectional studies
Brazil (Minas Gerais)
China (Hainan)

Anti-ASP-2 antibody responses associated with 62% risk of Acquiring Heavy Hookworm Infection

Relationship with ASP-2 was unique and not associated with other antigens

Bethony et al. Antibodies against a secreted protein from hookworm larvae reduce the intensity of hookworm infection in humans and vaccinated laboratory animals. FASEB J. 2005 19(12):1743-5.
**Na-ASP-2**: 3 layer αβα sandwich
Pathogenesis Related-1 Protein

**Possible Functions**
- Protease
- Chemokine Mimic
- Ligand Binding Domain

Immunolocalization of ASP-2:
Secreted protein from the Glandular Esophagus

PARASITOLOGICAL EVALUATION of ASP-2 VACCINATED DOGS (*Ancylostoma caninum*)

32% worm burden reduction (P<0.05)

55% reduction in host blood loss (P<0.05)


*Parasitological Evaluation of ASP-2 Vaccinated Hamsters (Ancylostoma ceylanicum)*

32% worm burden reduction (P<0.05)

55% reduction in host blood loss (P<0.05)


*Parasitological Evaluation of ASP-2 Vaccinated Hamsters (Necator americanus)*

30-40% worm burden reduction (P<0.05)
INHIBITION of L3 through TISSUE

% reduction in L3 penetration

<table>
<thead>
<tr>
<th>GROUP</th>
<th>a-irL3</th>
<th>a-ASP-2</th>
<th>Control</th>
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<tr>
<td>N</td>
<td>5</td>
<td>9</td>
<td>12</td>
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</tbody>
</table>
ANTI-ASP-2 ANTIBODIES

INHIBITION OF L3 TISSUE PENETRATION (Somatic Migration)

REDUCED NUMBER OF ADULT HOOKWORMS

REDUCTION IN HOST INTESTINAL BLOOD LOSS

REDUCTION IN FECAL EGG COUNTS

REDUCTION IN MALNUTRITION AND ANEMIA
The Neglected Tropical Diseases: Humanity’s Ancient Diseases of Stigma

- 13 Parasitic and Bacterial Infections
- Rural Areas of Low-Income Countries
- Poverty-Promoting Conditions
- Burdened humanity for centuries
- Documented in Ancient Texts
  “The Biblical Diseases”
- Notoriety as deforming and disabling diseases
- Associated with intense stigma
- Do not receive the attention of “The big 3”

- River Blindness
- Guinea Worm
- Lymphatic Filariasis
- Leprosy
# A Century of Drug Discovery for Neglected Diseases

<table>
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<tr>
<th>Sleeping Sickness</th>
<th>River Blindness Elephantiasis</th>
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<tr>
<td>Melarsoprol</td>
<td>Ivermectin</td>
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<tr>
<td>Suramin</td>
<td>Diethylcarbamazine</td>
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<tr>
<td>Pentamidine</td>
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<table>
<thead>
<tr>
<th>Leishmaniasis</th>
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</thead>
<tbody>
<tr>
<td>Stilbamidine</td>
<td>Nifurtomox</td>
</tr>
<tr>
<td></td>
<td>Benznidazole</td>
</tr>
</tbody>
</table>

**Drugs in Pipeline for 2000:**
1 for Neglected Tropical Disease
8 for ED; 7 for Obesity; 4 for Sleep Disorders
A vaccine with no commercial market

My experience has taught me that no movement ever stops or languishes for want of funds. This does not mean that any movement can go on without money, but it does mean that wherever it has good men and true at its helm, it is bound to attract to itself the requisite funds.

The Human Hookworm Vaccine Initiative
PPP Developing & Manufacturing an Orphan Product in the Non-Profit Sector

Sponsor and Program Management:
Sabin Vaccine Institute

Institutions:
The George Washington University
Oswaldo Cruz Foundation (FIOCRUZ)
Instituto Butantan
London School of Hygiene & Trop Med
Queensland Inst. Medical Research

“Our Guaranteed Money-Losing Company”
**Na-ASP-2 Hookworm Vaccine**

- **cDNA Cloned from human hookworm *Necator americanus***
  - *N. americanus* isolated from a patient in China
  - Passaged in Golden Hamsters
  - PR-1 Family of PRP superfamily, which includes Hc24 a protective antigen from *Haemonchus contortus* (function unknown)

- **Expressed in *Pichia pastoris***
  - Purified by IEC
  - 21.3 kDa (197 amino acids)
  - EAEAEF N-linked tag

- **Adsorbed to Alhydrogel®**

Goud et al. *Vaccine* 2005; 23: 4754-64

α-helix-β-sheet-α-helix
HHVI Milestones
The Na-ASP-2 Hookworm Vaccine

- 2000 (Q2) GF Funding Awarded
- 2000 (Q4) Launch of the HHVI
- 2001-2003 Selection of Na-ASP-2 based on five major preclinical criteria and small-scale expression (GWU)
- 2003-2004 Process development of Na-ASP-2 at GWU
- 2004 X-ray Crystal Structure of Na-ASP-2 (Eppley Cancer Institute)
- 2004 (Q1) cGMP Manufacture
- 2004 (Q4) IND Submission
- 2005 (Q1) FDA Approval
- 2005 Phase 1 trials (healthy adult volunteers) at GWUMC
Clinical Development Strategy

- IND Approved (1-05)
- Phase 1 U.S. with 36 healthy adult volunteers
  - Dose-escalating study (10, 50, 100 μg)
  - 0, 2, 4 months
  - Safety & immunogenicity
- Phase 1b/Phase 2b Brazil Minas Gerais
  - Safety & Immunogenicity in hookworm-infected populations
  - *Proof of Concept* Efficacy Study

Americaninhas
Minas Gerais
Anthelminthic Vaccinations
Na-ASP-2 Hookworm Vaccine

- Strategy that complements periodic deworming
- Combined use of chemotherapy and vaccination is the basis of a novel, more-versatile approach to control
- Prolong the intervals between treatments
- Reduce the likelihood of emerging drug resistance
Hookworm Vaccine Development: Downstream Approach

**Multi-antigen ("cocktail") vaccine**

L3 antigen (to reduce worm burden) +
Adult gut antigen (to reduce blood loss) +
Adjuvant
Dogs vaccinated with APR-1 develop antibodies which are ingested by blood feeding hookworms and reduce host blood loss & fecal egg counts.

Anti-APR-1 antibodies reduce host blood loss
Reduce fecal egg counts

Why a Global Access Plan?

• Based on experiences with Hepatitis B Vaccine
• 20-30 years before wide scale availability is achieved following proof-of-principle (Mahoney and Maynard, 1999)
• 10-15 years from the time of licensure
• Object of HHVI Global Access Roadmap is to reduce the timeframe for global deployment of HHV and to provide a mechanism for sustainable development
Major Challenges to Global Access for the HHVI

- **Magnitude/Scale of the Human Hookworm Problem**
  - 740 million people with hookworm
  - Ensuring use in all high transmission communities

- **Neglected Disease status**
  - Impoverished people are the lowest priority commercial market
  - No market for travelers or military
  - Massive funding schemes primarily for “the big three”

- **Health delivery systems**
  - School-aged children and pregnant women
  - Outside of EPI
  - School-based Vaccine
<table>
<thead>
<tr>
<th>Country</th>
<th>Country classification(*)</th>
<th>US Patents</th>
<th>GDP per capita</th>
<th>US patents per GDP per capita</th>
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<tr>
<td>1 United States</td>
<td>G8, OECD</td>
<td>50000 (est.)</td>
<td>36,006</td>
<td>1.389</td>
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<td>3 India</td>
<td>Advanced IDC</td>
<td>444</td>
<td>487</td>
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<td>15 Australia</td>
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INSTITUTO BUTANTAN

Created in 1901 to produce Antiplague and Antivenom Sera

1985: National Program for Self-Sufficiency in Immunobiologics

2005: Produces 86% of the vaccines for Brazil

483 million doses of vaccines: DPT, BCG, Hepatitis B, Rabies
Vaccine-linked Chemotherapy
An Integrated Approach

- Vaccination would follow deworming within three weeks
- Field testing of vaccine will evaluate post-deworming re-acquisition of infection
- Deworming will be factored into CEAs
- Evaluation of School-based vaccination programs
- School-based Vaccination
  - Tetanus (Td) booster
  - HPV (Cervical Cancer) Vaccines
  - Group B Streptococcal Vaccines
  - New-generation Injection disposable devices without needles
Na-ASP-2 Hookworm Vaccine
An Orphan Product for a Neglected Disease

GWU
Maria Elena Bottazzi
Jeff Bethony
Bin Zhan
Gaddam Goud
Aaron Miles
Susana Mendez
John Hawdon
Gary Simon
Ricardo Fujiwara
Vehid Deumic
Jordan Pleskiatt
Sen Liu
Yan Wang
Lilian Bueno
Rachna Patel
Suzanne Schuck
Reshad Dobardzic
Azra Dobardzic

LSHTM
Simon Brooker
Laura Rodrigues
Neal Alexander
Peter Smith

FIOCRUZ & Butantan
Paulo Buss
Isiais Raw
Rodrigo Correa-Oliveira
Iramaya Caldes
Stefan Geiger

Sabin Vaccine Institute
Philip Russell
David Diemert
Kari Stoever
Ami Shah Brown

Donors
Bill and Melinda Gates Foundation
March of Dimes
NIAID, NIH

QIMR
Alex Loukas
Angela Williamson

SAIC
Mike Roy
Maneesha Solanki