In search of a chemotherapy for sleeping sickness

Finding the Achilles heal of Trypanosomids: secretory trafficking and a lytic factor in human serum
Goals for discussion

- Identify the life stages of Trypanosoma alternating between host and the insect vector.
- Describe cell biological structures of these parasites.
- Role of public health surveillance in limiting epidemic spread of this disease.
- Identify current chemotherapeutic targets.
- Why chemotherapy and not a vaccine?
QUIZ

• Who was Paul Ehrlich?
• What are the three life stages of the African Trypanosome? Name one feature that delineates the different life stages.
• Do all sub-species of T. brucei cause human disease?
• Why might understanding the cell biology of these organisms facilitate finding a therapy for this disease?
Paul Ehrlich

- Nobel Prize in Medicine 1908: treatment for Syphilis and concept of chemotherapy for microbial pathogens.
- “When once we are acquainted with the majority of the chemioreceptors of a particular kind of parasite ... we shall have far-reaching possibilities of simultaneous attack by various agencies.” Ehrlich, 1914
The Scope of Human African Trypanosomiasis (African Sleeping Sickness)

- 60 million humans at risk
- ~30,000 cases annually
- many unreported & untreated
- fatal without treatment...always!
- kills 3-7 million cattle yearly
- $4.5 billion in annual economic losses
- treatment is very nasty!
The Problem: Chemotherapy
The Opportunity: Novel Biology
Life Stages of Trypanosomatid parasites
Surface Coats in Trypanosomatids

![Diagram of Surface Coats in Trypanosomatids]

- **T. brucei BF**
  - VSG
- **T. cruzi epimastigotes**
  - trans-sialidases
  - mucins
  - gp72
- **Leishmania promastigotes**
  - LPG
  - PPG
  - gp63

Secretory and endocytic organelles in Trypanosomatids

*T. brucei*
BF

*T. cruzi*
epimastigote

*Leishmania*
promastigote

Trypanosoma brucei Life Cycle

- Bloodstream vs procyclic stages
- No intracellular stages
- Robust culture & genetic systems
Entry of *T. cruzi* into the cytosol of the host cell

Receptor-mediated attachment

Lysosome recruitment/fusion

Trypomastigote in lysosome-derived vacuole

Replication of amastigotes

Escape from vacuole

Tc-TOX
HISTORY:
Male Caucasian, 26
Infected, Rhodesia, Sept. 1909
Admitted, Liverpool, Dec. 1909
Died, Liverpool, June, 1910

TREATMENT:
Atoxyl
Succinamide of Mercury
Trypan Red
Methylene Blue
Trypsin
Quinine
So-called Vaccine
Leukocyte Extract

“We beg to apologize for complicating the case with so many treatments...”
Waves of Parasitemia: VSG switching in response to immune pressure
VSG: A Critical Bloodstream Stage Virulence Factor

- $10^7$ molecules/cell; 10% total protein
- Forms macromolecular barrier
- >1000 VSG genes; antigenic variation
- GPI-anchored homodimer
- GPI anchor is critical for trafficking

Chattopadhyay et al.  
*JBC* 2005 280:7228
## Current Chemotherapeutic Options

### Summary of Drugs Available for Treatment of Human African Trypanosomiasis

(adapted from Bouteille et al., 2003)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Marketed</th>
<th>Spectrum of Activity</th>
<th>Stage of Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suramin (Germanin)</td>
<td>1922</td>
<td><em>T. b. rhodensiense</em></td>
<td>Stage 1</td>
</tr>
<tr>
<td>Pentamidine (Pentacarinat)</td>
<td>1937</td>
<td><em>T. b. gambiense</em></td>
<td>Stage 1</td>
</tr>
<tr>
<td>Melarsoprol (Arsobal)</td>
<td>1949</td>
<td><em>T. b. gambiense</em></td>
<td>Stage 1 &amp; 2</td>
</tr>
<tr>
<td>Eflornithine (Orindyl)</td>
<td>1981</td>
<td><em>T. b. gambiense</em></td>
<td>Stage 1 &amp; 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. b. rhodensiense</em></td>
<td></td>
</tr>
</tbody>
</table>

2010 NECT trial: nifurtimox and eflornithine combination therapy

[https://www.youtube.com/watch?v=nVmF3NKtbqs](https://www.youtube.com/watch?v=nVmF3NKtbqs): In the sleeping sickness ward
Treatment: lives vs facial hair

VANIQA® (eflornithine HCl) Cream, 13.9%

for women with unwanted facial hair
with VANIQA®, your unwanted facial hair may be easier to manage

VANIQA® is an FDA-approved prescription cream clinically proven to reduce the growth of unwanted facial hair in women. VANIQA® has only been studied on the face and adjacent involved areas under the chin of affected individuals. Usage should be limited to these areas of involvement.

Clinically proven. FDA approved.

VANIQA® slows hair growth
With VANIQA®, you can slow the rate of facial hair growth and improve the appearance where VANIQA® is applied. Improvement in the condition occurs gradually. You may spend less time removing hair, or in the frequency of hair removal, with VANIQA®.

Approved Use
VANIQA® (eflornithine hydrochloride) Cream, 13.9% is a prescription medication applied to the skin for the reduction of unwanted facial hair in women. Usage should be limited to the face and adjacent involved areas under the chin of affected individuals.
Typanocidal drugs target protein disulfide, DNA damage and oxidative stress pathways.

- Melarsoprol
- Eflornithine
- Nifurtimox
- Benzonidazole
- Pentamidine
Transporters and targets in the endocytic pathway of Trypanosomatids

Garcia-Salcedo Frontiers in Pharmacology 2016 V7:351
Public Health Measures

• Surveillance:
  – Monitoring both cattle and human
  – Cost and limited technology: access to electricity
  – Increasing mobility of both cattle and humans

• Limiting cycling between fly and infected hosts
Why health infrastructure matters

Figure 8.2 Number of reported cases of African trypanosomiasis and population screened, 1940-1998

Looking at Uganda in particular

Conflict and Health: Civil conflict and sleeping sickness in Africa in general and Uganda in particular

Risk for cross infection by *T. b. rodesiens* and *T. b. gambiense* in conflict zones
Vector Control
CATT Test for African sleeping sickness.
New assays

**Improved Field Surveillance**
- Temperature stable
- Reliable:
- Recognize more variants
- Veterinary Tests

**Dip tests**
Simple technological solutions to diagnostics in the field

https://techcrunch.com/2017/01/10/this-20-cent-whirligig-toy-can-replace-a-1000-medical-centrifuge/
Observation

- *T.b. brucei* is the causative agent for Nagana found in cattle and Tetsi flies in close association with people, yet people are only found to be infected with the related *T.b. rhodesiense* and gambiense.
The flagellar pocket
TLF lytic assay

Hajduk and Bangs 2007
Composition of TLF

A

B

C

Purifying Antibody

Hpr | ApoL-1 | ApoA-1

Hpr tetramer

ApoL-1

ApoA-1

Hpr dimer

Tf

Hpr dimer

ApoL-1

ApoA-1
TLF lytic mechanism

J. Raper, Nature 2013
How does TLF work
A role for a cofactor in TLF lytic activity?

Assay in PBS + FBS

Assay in PBS + BSA
A role for a Hemoglobin in TLF lytic activity?

**Bos taurus** gamma Hb

- VDEVGGEALGR
- LHVDPENFR
- VVTGVANALAHé
- LLVVYPWTQR
- FGSEFSPELQASFQK

**Coomassie**

![Image of Coomassie gel with bands for Hb, BSA, and FBS]
Fenton Reaction

\[
\begin{align*}
\text{Fe}^{2+} & \quad \text{Fe}^{3+} \\
\text{H}_2\text{O}_2 & \quad \text{OH}^- + \text{OH}^-
\end{align*}
\]
TLF Resistance: Receptor mutation
TLF resistance: Neutralization

(a) DIC/DAPI T. b. brucei
T. b. brucei
TLF/lysosome

(b) Model of SRA-mediated resistance in *T. b. rhodesiense*

Key: 
- TbrHbHpR
- TLF
- SRA

Neutralization of TLF

- SRA/TLF binding
- Early endosomes
- Lysosome
- Flagellar pocket

SRA/TLF degradation
Trypanosomiasis Lytic Factor

- Protein lipid complex containing
  - ApoA-1 and are therefore high density lipoproteins (HDLs). Necessary but not sufficient
  - Haptoglobin related protein (Hpr): only found in human TLF
  - Hemoglobin (Hb): increases TLF killing >10 fold
- Found in sera of primates
- Resistance in mediated by two components
  - Serum resistance antigen (SRA): neutralization of TLF
  - Mutations in Hpr receptor blocking uptake of TLF
APOL1 evolution

J. Raper PNAS 2014

http://www.pnas.org/content/111/20/E2130.full
Broader application of TLF findings?
Take Home

• Why Chemotherapy instead of a vaccine?
• TLF provides a model and perhaps a vehicle for developing safe, effective and inexpensive anti-trypanosomoid chemotherapeutics
  – Suramin and TLF are both taken up by receptors in the flagellar pocket (Field and Horn 2013)
• TLF as a model for targeting pathogenic microbes in acidified phagosomes:
  – TB, Toxoplasma, Malaria, and the American Trypanosome T. Cruzi
The TLF investigators

Steve Hajduk

James Bangs

Jane Raper

Malcom McConville