Non-human infective trypanosomes expressing LiTat 1.3 or 1.5 VSGs
Towards a new diagnostic test for *gambiense* sleeping sickness

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Introduction

Immune Trypanolysis (TL)

- Reference test for detection of *T.b. gambiense* specific antibodies in blood
- Most sensitive and specific serodiagnostic test for *gambiense* HAT
- Ideal for large scale surveillance and monitoring of sleeping sickness elimination efforts

<table>
<thead>
<tr>
<th></th>
<th>CATT <em>T. b. gambiense</em></th>
<th>Immune trypanolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>69-100 %</td>
<td>97-100 %</td>
</tr>
<tr>
<td>Specificity</td>
<td>83-99 %</td>
<td>100 %</td>
</tr>
</tbody>
</table>
Introduction

Immune trypanolysis (TL)

- **Test principle**
  - Live, cloned *T. b. gambiense* populations expressing LiTat 1.3 or LiTat 1.5 Variant Surface Glycoproteins
  - Incubation with test serum/plasma
  - Addition of guinea pig complement
  - If specific anti-VSG antibodies present
    => Ab mediated complement lysis of trypanosomes

- **Disadvantages**
  - Highly virulent human infective *T. b. gambiense* clones
  - Trypanosome propagation in laboratory animals
Objective

To make non-human infective *T. b. brucei*

- that express *T. b. gambiense* VSGs LiTat 1.3 & LiTat 1.5
- that can be maintained *in vitro*
- that can be used in immune trypanolysis
Obstacle

ApoL1 (human trypanolytic factor)

Pays et al. 2006 Nature Reviews Microbiology 4, 477–486
Methods

First transfection

- Electroporation of LiTat 1.3 or LiTat 1.5 in *T. b. brucei* Lister 427
- Selection with puromycin
- *T. b. brucei* double expressors (VSG221 and LiTat 1.3 or LiTat 1.5)
Methods

Second transfection

- Knock-out of the native VSG221 from the active ES
- Selection with puromycin and blasticidin
- *T. b. brucei* non-switching single LiTat 1.3 or LiTat 1.5 expressors
Results

PCR on cDNA with specific primers

Primer pairs

<table>
<thead>
<tr>
<th>Lane</th>
<th>VSG specific primers</th>
<th>Expected amplicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VSG221</td>
<td>800 bp</td>
</tr>
<tr>
<td>2</td>
<td>LiTat 1.3</td>
<td>600 bp</td>
</tr>
<tr>
<td>3</td>
<td>LiTat 1.5</td>
<td>1000 bp</td>
</tr>
</tbody>
</table>
Results

Immunofluorescence on single expressors (SE) with specific rabbit antisera, anti-rabbit-DyLight488 and DAPI

**LiTat 1.3 SE**

- Pre-immune
- Anti-VSG221
- Anti-LiTat 1.3
- Anti-LiTat 1.5

**LiTat 1.5 SE**

- Pre-immune
- Anti-VSG221
- Anti-LiTat 1.3
- Anti-LiTat 1.5
Results

Immune trypanolysis with LiTat 1.3 and LiTat 1.5 SE and human sera

- Non antibody mediated lysis of the recombinant strains with endemic non-HAT control sera caused by ApoL1
- Ways to inhibit ApoL1 activity
  - 56°C heat inactivation of human serum
  - hypertonic medium by addition of sucrose
  - absorption of lipid fraction on filter paper
  - assay temperature 4°C
  - addition of lysosomotrophic amines (chloroquine or NH₄Cl)
  => delay of lysosomal acidification and of ApoL1 release from HDL particle
Results

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Results

Immune trypanolysis with LiTat 1.3 and LiTat 1.5 SE and human sera

*Protocol:*

- *In vitro* expansion of *T. b. b.* Lister 427 LiTat 1.3 or LiTat 1.5 SE
- Incubate 30' with 50 μM chloroquine or 8 mM NH₄Cl at 4 °C
- Mix 80 μl trypanosome suspension with 10 μl guinea pig serum and 20 μl human test serum
- Incubate 60' at 37 °C (5 % CO₂)
- Incubate at 4 °C to stop reaction until microscopic examination
- If > 50% lysis => positive
Results

Immune trypanolysis with LiTat 1.3 and LiTat 1.5 SE and human sera

- 2-8 sera from HAT patients
- 2-12 sera from endemic controls

<table>
<thead>
<tr>
<th>Percentage immune trypanolysis</th>
<th>Ammonium chloride</th>
<th>Chloroquine</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HAT sera</td>
<td>Controls</td>
</tr>
<tr>
<td>LiTat 1.3 SE</td>
<td>93 %</td>
<td>9 %</td>
</tr>
<tr>
<td>LiTat 1.5 SE</td>
<td>87 %</td>
<td>28 %</td>
</tr>
</tbody>
</table>
Conclusions

• Non-human infective trypanosomes expressing gambiense specific VSGs
  – *T. b. brucei* LiTat 1.3 SE
  – *T. b. brucei* LiTat 1.5 SE

• Cultured *in vitro* under antibiotic pressure
  => no VSG switching

• Inhibition of ApoL1 activity
  – Chloroquine
  – Ammonium chloride
  – Optimisation required!