African trypanosomes: how do they enter the brain and cause sleeping sickness

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How can the extracellular trypanosomes affect brain functions to cause neuropsychiatric disturbances – sleeping sickness?

Do the parasites invade the brain? How and why?

Courtesy Prof Michael Duszenko, Dept Biochemistry, University of Tuebingen
Paul Ehrlich (1885) noted that systemically infused trypan dyes (to label and kill trypanosomes) did not stain brain tissue; the same dyes infused into the CSF did stain brain tissue.

Lead to the concept of a Blood-Brain Barrier (BBB)

Spinal cord and dorsal root ganglia (DRG) from a rabbit systemically infused with the blue dye.

The spinal cord and roots are not stained, while the DRG, which lack a blood-nerve tissue barrier, are stained.
Choroid plexus epithelial cells secrete the cerebrospinal fluid that circulate in ventricular and subarachnoid spaces.

Ransohoff et al Nature Rev 2003
Blood-brain and blood-cerebrospinal fluid (CSF) barriers

Heavy accumulation of *Tb brucei* (red) in choroid plexus early after infection in a mouse

Wolburg et al *Cell Tissue Res* 2008
EARLY STAGE

Parasites *(red)* inside cerebral vessels *(green)*

LATE STAGE

Parasites *(red)* outside cerebral vessels *(green)*
Sorokin et al., JCB 153:933-45, 2001

Sixt et al, JCB 153:933, 2001
Trypanosomes pass endothelial basement membranes containing laminin $\alpha$-4, but not $\alpha$-5.
Trypanosomes pass first the endothelial and then the parenchymal basement membrane (glia limitans)

In the absence of IFN-γ trypanosomes (red) are trapped between the two basement membranes (green)

Collaboration with Prof. Lydia Sorokin, Münster University
**Neuroinvasion** of *T. b. brucei* into the brain and morbidity is **determined by host genetics and not by the parasitemia** levels, e.g.: SV129 mice higher parasitemia than B6 mice, but less neuroinvasion of trypanosomes and T cells; they live longer.

**Minocycline** impedes *Tb brucei* (and T cell) neuroinvasion, and inflammatory gene expression in the brain (but not in the spleen), and morbidity, but no effect on parasitemia.

Trypanosome and T cell **neuroinvasion** may cause morbidity and be **related to expression of host genes in the brain**
Array of – 36,000 host genes comparing suramin-sensitive and suramin-resistant infection

Cluster on features with IQR > 1;
838 features

Note the marked difference in the clustering of gene expression between the 3 groups
Analyses of chemokines and their receptors

- Chemokines attract or retain inflammatory cells in a target tissue
- CXCL10 is induced by IFN-γ (IP-10)

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<thead>
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<th>Chemokine</th>
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<td>CCR5</td>
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Expression of CXCL10 and CXCL10 mRNA in astrocytes in *T. b. brucei* infected brain

Collaboration with *Christina Fenger* and *Bente Finsen*, University of Southern Denmark, Odense
Mice with deleted genes encoding CXCL10 and its receptor CXCR3 do not lose weight as WT mice do after infection, despite similar levels of parasites in the blood.

Collaboration with Prof. Allan Randrup Thomsen
The Panum Institute,
University of Copenhagen
Mice with deleted genes encoding CXCL10 and its receptor CXCR3 show less neuroinvasion of T cells and trypanosomes than wt mice.
Cerebrospinal fluid levels of CXCL10

T. b. gambiense patients
Dieudonné Mumba
INRB, Kinshasa, RD Congo

T. b. rhodesiense patients
Nkhwachi Gondwe-Mphepo
CTTBD, Lilongwe, Malawi.
The IFN-γ-inducible CXCL10 (IP-10) plays an important role for trypanosome and T cell crossing the BBB.

CXCL10 in combination with other biomarkers discovered by proteomics (J-C Sanchez et al.) may be candidates to better distinguish between Stage I and II in Human African Trypanosomiasis.

Daniel Amin & Willias Masocha
Hypothesis for brain invasion by *Tb brucei*

4. Trypanosomes (and T cells) cross the BBB as a second wave

3. IFN-γ in CSF diffuses between ependymal cells into white matter to induce IP-10 and affect astrocytic basement membrane

2. Choroid plexus epithelial barrier disrupted

1. Trypanosomes and IFN-γ-producing T cells invade choroid plexus early
Sleep disruption in African trypanosomiasis - Sleeping sickness
Sleep and sleep/wake cycle disruption in HAT

Buguet et al., 2001
African trypanosomes and white blood cells invade **Circumventricular organs**, which have fenestrated, permeable vessels.

Median eminence loaded with trypanosomes

Area postrema

Nerve cell groups in the hypothalamus regulate sleep/wakefulness. LH neurons (orexin) activate to cause wake. VPLO neurons (GABA) inhibit to cause sleep. A "flip-flip" model for rapid transitions and stability. Disturbances in this system destabilize and lead to changes in sleep pattern with uncontrolled transitions between sleep and wake.
Actigraphic recording (recording of rest-activity) in humans

Actigraphy has been already validated in humans with polysomnography as strategy to detect also sleep and wake states.

Actigraphs are wrist-worn watches relatively inexpensive (marketed e.g. for insomnia), obviously user-friendly.

Actigraphic recording can be correlated with biomarkers in biological fluids (as already demonstrated in other diseases).
24 h Actigrams – Alfred Njamnshi

healthy subject  sleeping sickness patient
Current staging relies mainly on number of white blood cells in the CSF – but most likely no linear relation between this and trypanosome brain invasion.

Are patients with no increase in CXCL10 in CSF and normal actigraphic recordings really in Stage 2?

Actigraphic recordings may in the future provide a user friendly device to obtain objective, quantitative clinical data for evaluation of course of disease.
Could less toxic and easy-to-apply drugs than Melarsoprol and Eflornithine/Nifurtimox be found???

**New targets:** Sterol biosynthesis, Thiol metabolism, Polyamine biosynthesis. **New combinations** of existing drugs or **new derivatives** of them: nitroheterocyclic derivatives-fexinidazole –oral administration, passes the BBB


Cordycepin (3´deoxyadenosine)
Possibly RNA chain terminator
Extracted from a Chinese fungi (cordyceps militaris)- cheap
Can be administered orally

Deoxycoformycin
Prevents cordycepin from being metabolically inactivated to 3´-deoxyinosine.
Used in cancer treatment, specifically for TdT+ leukemia

•96 mg/ d cordycepin and 6 mg / d deoxycoformycin are below the MTD in Man.
Treatment of *T. b. brucei* late stage infection with cordycepin and deoxycoformycin in mice

- Reinoculation into naïve immunodeficient animals
- Tbb DNA in blood and brain
- Parasites in brain sections by immunostaining

20 days

7 days

20 days

60 days

Parasitemia

Weight change
Oral and subcutaneous administration of cordycepin and deoxycoformycin

A

Cordycepin mg/kg  
- 5 mg/kg  
- 15 mg/kg  
- 5 mg/kg  
- 0 mg/kg  

Omeprazole  
- Yes  
- No  

B

- 15 mg/kg cordycepin 0.4 mg/kg deoxycoformycin  
- 5 mg/kg cordycepin 0.2 mg/kg deoxycoformycin  

C

- cordycepin/deoxycoformycin and oil  
- Oil  
- Control infection

Fraction of mice with parasitemia

Days after infection

Treatment start

Days after infection

Days after infection
• Treatment with cordycepin and deoxycoformycin cures late stage infection with rodent *T. b. brucei*

• The doublet can be administered orally or subcutaneously

• The doublet is active against human pathogenic *T. brucei* subspecies

• **Future**: Cordycepin derivatives with that are resistant to adenosine deaminase that detoxifies nucleoside analogues

*Suman Vodnala & Martin Rottenberg*
Aspirin causes severe neurodegeneration in rat brains with inflammation due to *Trypanosoma brucei* infection.
Conclusions

• Multistep entry of African trypanosomes into the brain is regulated partly by IFN-γ and IFN-γ-inducible CXCL10.

• Proteomics and gene expression studies have identified new candidate markers for better staging.

• Sleep pattern disruption in African trypanosomiasis related to parasite invasion of sleep/wake regulating hypothalmic nuclei.

• Actigraphic recordings a user friendly mean for objective measures of course of disease?

• Orally administrated drugs that can cure trypanosome infections in the rodent brain available. Will any of these be useful in Humans?????? Paul Ehrlich.
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NEUROTYP
Biology and clinical staging of trypanosome neuroinvasion in sleeping sickness