Humans have natural immunity against *T. brucei*


*T. brucei brucei* cells are lysed by exposure to human serum.  
*T. brucei rhodesiense* and *T. brucei gambiense* are resistant to human serum.

The Trypanosome Lytic Factor (TLF1) is a subclass of high density lipoprotein (HDL3). TLF1 are 18 nm high density particles that contain phospholipids, cholesterol, cholesterol esters, and apolipoproteins. TLF1 toxin candidates were: ApoE-I, haptoglobin-related protein (Hpr) and ApoL-I.
1. Baboon serum contains HPR and not APOL1 but lyses both serum-resistance and serum-sensitive trypanosomes.

2. HPR-specific antibodies inhibit the activity of lytic HDL particles.

3. Recent suggestion is that both HPR and APOL1 are necessary. Particles fractionated into particles containing both APOL1 and HPR could lyse trypanosomes but not particles with just one component.

4. Mechanism of lysis suggested to be damage to lysosomal membrane allowing release of proteolytic enzymes and auto-digestion.
Baboons and some other primates are immune to all African trypanosomes.

1. Jayne Raper searched for TLF1 components in Baboon blood. She only found Hpr and did not find ApoL1, and antibodies against Hpr seemed to neutralize the lytic activity of Baboon blood in vitro.

2. But when she inserted the Baboon version of Hpr into mice, they remained susceptible to the parasite. So she gave up on Hpr and began working on ApoL1.

3. She then showed that Baboon blood actually did contain ApoL1, but it was only 60% similar to the human ApoL1 and therefore was not detected by antibody. When she inserted this gene into mice, they became resistant to *T. rhodesiense* infection!
Two *T. brucei* subspecies resist TLF and cause sleeping sickness: *T. b. gambiense* and *T. b. brucei*.

In *T. b. gambiense* resistance is constitutive but in *T. b. brucei* resistance is **inducible** under selective conditions.

Resistance to human serum in *T. rhodesiense* was studied by analyzing gene expression in naturally-resistant cells and serum-sensitive derivatives. The *SRA* gene was identified by differential hybridization of cDNA libraries. (Hamers, 1989)
The SRA gene turned out to be an “expression gene associated gene” or ESAG.

SRA is found at only one ES: Etat 1.10
Gain and loss of resistance to human serum

Due to selection for SRA expression, antigenic variation only occurs by homologous recombination. SRA is not expressed in non-human serum.
The SRA protein is similar in structure to the N-terminal fold of VSG.
SRA binds to ApoL-I. The N-terminal region of SRA is responsible for resistance to NHS and interacts with a C-terminal helix of ApoL-I. This suggested that ApoL1 is the lytic factor in TLF1!
ApoL1 has N-terminal ColicinA-like pore-forming domain

ApoL1 resembles pro-apoptotic members of the Bcl2 family since they share the same ionic pore-forming domain with a Bcl2 homology signature.

It appears that in humans, proteins originally designed to organize cell death under inflammatory conditions were also used to kill extracellular pathogens.
ApoL1 is predicted to have a pH-dependent structure.

At neutral pH, such as in the blood, the two α-helices of the membrane addressing domain interact through two salt bridges (indicated by pink squares), which are formed by the side chains of the amino acids indicated. This hairpin structure shows a segregation between hydrophobic (orange) and hydrophilic (green) surfaces, as indicated by space-filling models. At acidic pH, such as in the lysosome (pH 5.3), the salt bridges are predicted to dissociate as a result of neutralization of the negatively charged residues, and this would lead to loss of the large hydrophobic surface.
Transfecting the SRA gene into sensitive *T. brucei* conferred resistance to lysis

*T.b. brucei* AnTat 1 parasites transfected with either a control plasmid (dots) or the SRA expression plasmid (squares) were incubated at 37°C, in the presence (black symbols) or absence (open symbols) of 5% normal human serum.
Mutant human sera:
1. Lacks Hpr (and Hp): Hp(r)/- HS
2. Lacks ApoL-I : apoL-I/- HS

Trypanosomes incubated for 24 hr with 10% serum ± recombinant apoL-I or recombinant Hpr

Presence of ApoL1 Correlated with Resistance to NHS
Expansion of the Lysosome in Presence of NHS or Recombinant ApoL1
Evidence for APOL1 being the only trypanolytic factor in human serum

1. Addition of recombinant APOL1 to fetal calf serum which lacks HPR rendered the serum trypanolytic with same phenotype of lysis.

2. Presence of HPR in APOL1-free serum did not trigger trypanosolysis.

3. Depletion of APOL1 from human serum without removal of HPR led to complete loss of trypanolytic activity.

4. Trypanolysis did not occur when recombinant APOL1 which was mutated in the pore-forming domain or in the membrane-addressing domain was added to APOL1-depleted serum.

5. Expression of SRA alone conferred complete resistance to human serum or recombinant APOL1.
Model for mechanism of lysis
Hpr strikes back!
Hp(r)^{-}/HS exhibits normal but delayed trypanolysis
ApoL-I uptake is mainly mediated by the Hpr component of the TLF HDL particles
Isolation of trypanosome glycoprotein receptor which binds Hp-Hpr with high affinity

Affinity chromatography of immobilized Hp-Hb complexes. GPI-anchored surface protein, TbHpHbR, localized to the flagellar pocket.

1. Recombinant TbHpHbR binds TLF-1, HpHb and also HprHb.

2. KO of this gene conferred resistance to human HDL-mediated lysis.

3. Receptor is normally used to acquire heme from Hp-Hb. Heme is used for p450 and b5 cytochromes that help parasite resist host oxidative defenses.

Model of trypanolysis by TLF1

1. apoL1, HDL, Hpr-Hb

2. cytoplasm (0.1 M Cl\(^{-}\))

3. (pH 5.3)

4. (DIDS) (plasma membrane)

lysosome lumen

haem

Hpr-Hb

Cl\(^{-}\) (H\(_2\)O)
Human side of science

Raper, Pays and Hajduk all agree that ApoL1 is important, but they can't seem to come together on the specifics. All three researchers permit that when a human becomes infected with T. b. brucei, Hpr (bound to hemoglobin) attaches to a receptor on the surface of the parasite, allowing TLF to gain entry into the cell. Once inside, the molecule travels to the parasite's gut-like sac—the 'lysosome'—where TLF gets broken down to its constituent parts. In this acidic environment, ApoL1 becomes activated, binds the lysosome's membrane and forms tiny pores.

That much the researchers can agree on. But exactly how TLF kills trypanosomes has been a matter of fierce debate. “I can, from personal experience, tell you that it can be very tense when people from these three labs get together,” says James Bangs, a cell biologist who studies trypanosomes at the University of Wisconsin–Madison, adding that resolving the points of disagreement will “require a little less passion and a little more objectivity.”

Raper acknowledges that the groups have their disagreements but says the rest of the field gets a kick out of it. At conferences, “it's one of the highlights,” she jokes. Hajdk points out that the groups actually agree on many important points. “It's unfortunate that this whole field comes down to what appears to be this huge controversy,” he says.
Human macrophage receptor for Hp-Hb = CD163

CD163 has similar affinity to TbHpHbR but the Tb receptor recognizes Hpr-Hb as well as Hp-Hb.

Therefore, the trypanolytic potential of TLF1 strongly depends on the relative contribution of TLF1-Hpr-Hb and competing Hp-Hb.

Hb is released in blood by intravascular hemolysis by *T. brucei* parasites. This Hb binds to both Hpr and Hp in a 100:1 ratio. But Hb-bound Hp is quickly cleared by endocytosis by macrophage CD163 receptor. This produces a ratio of Hp-Hb over TLF1-Hpr-Hb low enough to allow TLF1 uptake by the parasite. This may act as an “arming mechanism” for innate immune system of host.
1. Absence of CD163
   - *in vitro*, sample contaminated with Hb from FCS and/or during NHS sampling
   - TLF1-Hpr-Hb → TbHpHbR, Hp-Hb

2. Physiological situation
   - *in vivo* in NHS, physiological levels of Hb from intravascular haemolysis
   - TLF1-Hpr-Hb → TbHpHbR, Hp-Hb

3. Absence of Hp
   - *in vivo* in hypohaptoglobinaemic HS
   - *in vitro*, isolated TLF1 particles
   - TLF1-Hpr-Hb → TbHpHbR, Hp-Hb, CD163

Trypanolytic activity
Other pathways for uptake of TLF1:
- lipoprotein receptor
- fluid phase uptake
Correlation of ApoL1 variants with increased kidney disease in African-Americans

G1 - two single amino acid changes
G2 - 6 bp deletion

These dominant mutations are exactly at the site for binding of SRA

The variants are not protected from lysis by SRA

Positive selection for these mutations to protect against trypanosomiasis.

Correlated with a recessive mutation for increased kidney disease.
Conversion of APOLI into a drug against human-infective trypanosomes

1. Removal of C-terminal region.

2. Fusion of truncated APOLI to antibody module (nanobody) derived from single chain antibodies, targeting the toxin to invariant determinants (mannose side chains) of the trypanosome VSG.
The NbAn33 nanobody recognizes all major VSG classes and binds specifically to the trypanosomes.
Concentration-dependent lytic activity after 4 hr incubation

Trypanolytic activity of different chimeras
Injection of the modified APOLI produced decrease in acute stage parasitemia caused by either *T. brucei* or *T. rhodesiense*.

(a,c) Parasitemia and (b,d) survival of mice infected with *T. b. rhodesiense* ETat 1.2 R (a,b) or *T. b. brucei* AnTat 1.1 (c,d) and treated with 20 µg (filled squares) or 10 µg (filled circles) NbAn33–Tr-apoL-I, or 100 µg NbCEA5–Tr-apoL-I (open circles), with NHS (a,b; open squares) or PBS (c,d; open squares) at 3 d after infection (arrow).
Therapeutic effects at second wave of parasitemia