

BRIEF REPORT

Human *Trypanosoma evansi* Infection Linked to a Lack of Apolipoprotein L-I

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SUMMARY

Humans have innate immunity against *Trypanosoma brucei brucei* that is known to involve apolipoprotein L-I (APOL1). Recently, a case of *T. evansi* infection in a human was identified in India. We investigated whether the APOL1 pathway was involved in this occurrence. The serum of the infected patient was found to have no trypanolytic activity, and the finding was linked to the lack of APOL1, which was due to frameshift mutations in both *APOL1* alleles. Trypanolytic activity was restored by the addition of recombinant APOL1. The lack of APOL1 explained the patient's infection with *T. evansi*.

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N Engl J Med 2006;355:2752-6.

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TRYPANOSOMA EVANSI IS A WIDELY DISTRIBUTED HEMOFLAGELLATE PARASITE that affects domesticated mammals (e.g., horses, cattle, camels, and water buffalo). Since its adaptation to mechanical transmission by blood-sucking insects (tabanids), the parasite has spread beyond its original distribution in sub-Saharan Africa and is now also present in South America, North Africa, and large parts of Asia, including India. Normally, humans are resistant to infection with *T. evansi*, as well as to infection with the related African trypanosomes, the prototype of which is *T. brucei brucei*.¹⁻³ Human innate immunity against *T. brucei brucei* is due to the trypanolytic activity of a human-specific apolipoprotein bound to high-density lipoproteins, termed apolipoprotein L-I (APOL1).⁴ This protein contains an ionic pore-forming domain consisting of nine alpha helices, as well as an adjacent pH-sensitive membrane-addressing domain consisting of two alpha helices.⁵ APOL1 is taken up in the parasite by endocytosis and triggers the formation of anion-selective pores in the lysosomal membrane, which induces the uncontrolled osmotic swelling of this compartment and subsequent cell death.^{5,6}

The *T. brucei* subspecies *T. brucei rhodesiense* and *T. brucei gambiense* have acquired resistance to APOL1, which enables these parasites to infect humans and cause sleeping sickness. In *T. brucei rhodesiense*, resistance to normal human serum is conferred by a single protein, termed serum resistance-associated protein (SRA), which interacts strongly and specifically with APOL1.^{4,7} SRA was found to provide specific resistance to *T. brucei rhodesiense*.^{7,8} The mechanism of resistance of *T. brucei gambiense* to APOL1 is not understood.⁶ *T. evansi* is normally sensitive to normal human serum^{2,3}; therefore, it had not been known to cause human disease until recently, when it was found to have infected a human in the Maharashtra state of India.^{9,10} The patient was an Indian cattle farmer who presented with a 5-month history of fluctuating trypanosome parasitemia associated with febrile episodes. Findings on morphologic examination of the parasites, as well as serologic and molecular tests — in particular the presence of typical kinetoplast-DNA minicircles of type A — iden-

tified the infecting species as *T. evansi*.^{9,11} The patient was treated with suramin, which led to a complete cure.¹⁰ SRA was not detected in these parasites.¹¹ We report results of the analysis of the trypanolytic activity in the serum of the infected person; our analysis allowed us to propose an explanation as to why he became infected.

METHODS

We obtained the *T. evansi* reference strains Zagora 1.17 and Vietnam-WH, taken from a camel in Morocco and a water buffalo in Vietnam, respectively, from the Institute of Tropical Medicine, in Antwerp, Belgium. The parasites isolated from the Indian patient, with his consent, could not be grown in vitro or in rodents, which prevented their physiological analysis. Genetic analyses showed that these parasites have a close relationship with the Vietnam-WH isolate.¹¹

Trypanolysis assays were conducted using 2×10^5 trypanosomes per milliliter from the *T. evansi* Zagora 1.17 and Vietnam-WH strains or the *T. brucei brucei* EATRO1125 strain, isolated from mice and incubated in HMI-9 medium¹² containing various concentrations of serum. After 24 hours, living trypanosomes were counted three times by the same person. The experiment was conducted three times. To identify serum components interacting with SRA, recombinant His₆-tagged SRA (10 μ g) was incubated with 50 μ l of serum for 4 hours at 4°C in 0.6 M sodium chloride, 0.35% CHAPS, and 0.15 M MES buffer (pH 5.8). The mixture was then incubated for 30 minutes at 4°C with 10 μ l Ni-NTA (nickel-charged agarose) beads (Qiagen). Bound material was eluted with 250 mM imidazole. Western blots were incubated overnight at 4°C with a 1:100 dilution of a goat polyclonal monospecific anti-APOL1 antibody (Santa Cruz Biotechnology) in 150 mM sodium chloride, 0.5% (weight per volume) Tween 20, and

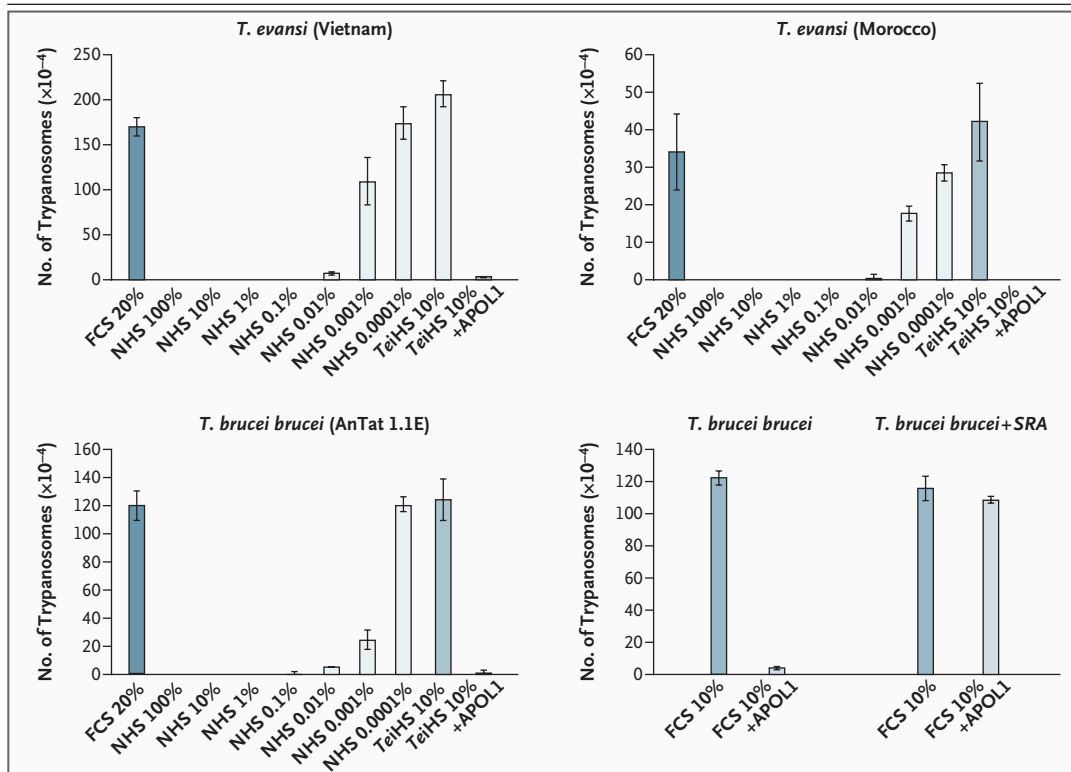


Figure 1. Lack of Trypanolytic Activity in *T. evansi*-Infected Human Serum.

From each parasite isolate, 2×10^5 trypanosomes per milliliter were incubated with various serum concentrations. Physiologic amounts of purified recombinant APOL1 (10 μ g per milliliter) were added in some instances. After 24 hours, living trypanosomes were counted three times. Error bars represent standard deviations from three independent experiments. *T. brucei brucei* + SRA is a transgenic *T. brucei brucei* line transfected with the SRA gene of *T. brucei rhodesiense*.⁷ FCS denotes fetal-calf serum, NHS normal human serum, and TeiHS *T. evansi*-infected human serum.

20 mM TRIS–hydrochloric acid (pH 7.5) with 1% nonfat milk. The bound antibodies were detected using peroxidase-conjugated mouse anti-goat IgG.

Genomic DNA was extracted from peripheral blood cells. The following five primer sets were used to amplify the *APOL1* coding sequences from 100 ng genomic DNA: 5'CCATCCTGGCTAACATG-GTCAAACC3' and 5'GTTAGCCTCAACTAGGATACAGCGG3'; 5'TCTGTAATGATCAGATGGCTGCCCG3' and 5'AGGTGCCACCTCCATTCTAAGTGC3'; 5'AACAAGTCCCACATCACAGCTGTCC3' and 5'AAAGTTCCCATCACCAGCAGATGGC3'; 5'AGCCACCACCGAGCCAAAAGTGC3' and 5'AGCACAAGAAAGAAGCTTACAGGGG3'; and 5'AGGGTAAATGAACCCAGCATCCTGG3' and 5'ATGGCCCCCAAGCTTGAAAGAGC3'. The polymerase chain reaction (PCR) products and seven cloned mutation-containing fragments from three independent PCRs were sequenced.

Recombinant APOL1 was purified using His₆-tagged APOL1 expressed from pET21d vector in *Escherichia coli* after a 4-hour induction at 37°C with 1 mM isopropyl β-D-thiogalactoside. After washing, inclusion bodies were dissolved in 6 M guanidium–hydrochloric acid and 50 mM phosphate buffer (pH 8.0) and incubated with Ni-NTA beads for 16 hours at 4°C.¹³ All washing steps occurred at pH 8.0. After elution and dialysis against 20 mM acetic acid, the protein was more than 96% pure, as determined by sodium dodecyl sulfate–polyacrylamide-gel electrophoresis (SDS-PAGE). The nucleotide sequence GenBank accession number for these sequences will be publicly

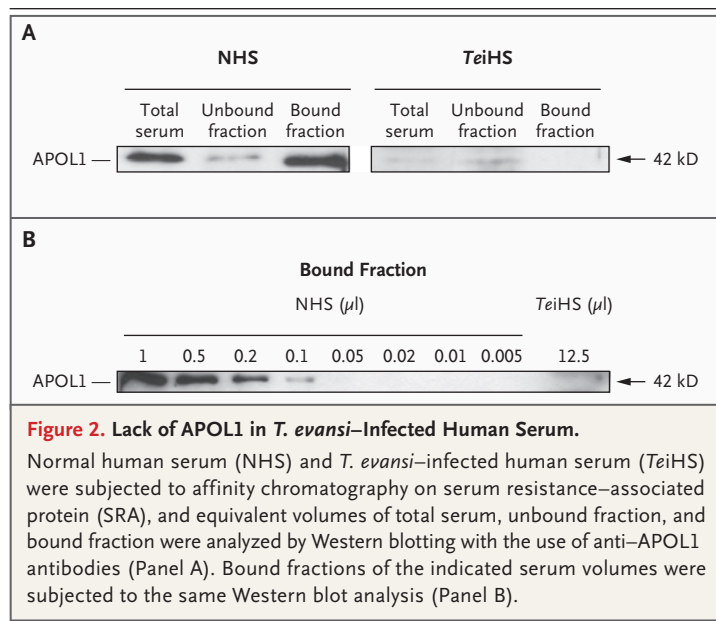
available with the release of dbSNP build 127 of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

RESULTS

The potential of the serum from the person with *T. evansi* infection to lyse *T. evansi* was evaluated in vitro. Two different parasite strains, one from Morocco and one from Vietnam, were used. No lytic activity was observed, since the parasites had normal growth in this serum. Trypanolytic activity was shown in normal human serum after dilution by a factor of 100,000 (Fig. 1). The serum from the person with *T. evansi* infection showed no lytic activity against *T. brucei brucei* (Fig. 1).

APOL1 is the known factor in normal human serum that kills *T. brucei brucei*.⁴⁻⁶ This protein is not present in nonhuman serum such as fetal-calf serum, but the addition of recombinant APOL1 is sufficient to render fetal-calf serum lytic for *T. brucei brucei*, except when the gene for the APOL1-neutralizing *T. brucei rhodesiense* protein SRA is transfected into this parasite⁷ (Fig. 1). The presence of APOL1 in the serum from the person with *T. evansi* infection was analyzed by incubating Western blots with anti-APOL1 antibodies, using normal human serum as a control. To assess the presence of APOL1, these serum samples were subjected to affinity chromatography on SRA, since APOL1 is known to bind strongly and specifically to this protein.⁴ As compared with normal human serum, *T. evansi*-infected human serum appeared to be largely devoid of APOL1 (by a factor of at least 125, as determined by densitometry) (Fig. 2). A band weakly detected at approximately the expected size in unfractionated *T. evansi*-infected human serum was unlikely to be APOL1, because it did not bind to SRA and was also found in the SRA-unbound fraction of normal human serum (Fig. 2A).

To determine the reason for the lack of APOL1 in serum from the person with *T. evansi* infection, DNA was extracted from his blood cells and PCR analysis was conducted with the use of five pairs of oligonucleotide primers that allowed the amplification of the complete *APOL1* coding sequence. Two different mutations were detected with equal frequency in each of three independent PCR assays and are thus likely to characterize each allele (Fig. 3A). For one allele, the absence of two bases resulted in a frameshift mutation from residue 142, leading to a premature stop at position 149,





located in helix 5 of the pore-forming domain (Fig. 3A and 3B). In the other allele, the absence of a single base resulted in a frameshift mutation from residue 266, located between the two alpha helices of the membrane-addressing domain, leading to a premature stop at position 268 (Fig. 3A and 3B). In both cases, the putative truncated proteins are predicted to be unable to trigger trypanolysis, because the simultaneous presence of intact pore-forming and membrane-addressing domains is required for this activity.⁵

The addition of normal levels of purified recombinant APOL1 to serum from the person with *T. evansi* infection was sufficient to restore its lytic potential (Fig. 1). Therefore, the lack of APOL1 is responsible for the lack of trypanolytic activity of this serum.

DISCUSSION

This case of human infection with *T. evansi* could be due either to acquired resistance to normal human serum by the parasite or to deficient trypanolytic activity of the host. *T. evansi*-infected hu-

man serum did not affect the two *T. evansi* strains (from Morocco and from Vietnam) that we tested. This serum was remarkably devoid of APOL1, a protein identified as a trypanolytic factor of normal human serum in the case of *T. brucei brucei*.⁴⁻⁶ The lack of APOL1 was due to the combination of two different frameshift mutations, one in each allele, which led to premature translational stops. The inability of *T. evansi*-infected human serum to kill *T. evansi* was probably due to this lack of APOL1, for the following reasons: this serum did not lyse *T. brucei brucei*, APOL1 is known to be the lytic factor of these parasites in normal human serum, and the addition of recombinant APOL1 restored the lytic potential of this serum.⁴⁻⁶ These data show that this case of *T. evansi* infection in a human is probably due to mutations in the APOL1 gene.

Supported by the Belgian Fonds National de la Recherche Scientifique (FNRS), the Special Program for Research and Training in Tropical Diseases (cosponsored by the United Nations Development Program, the World Bank, and the World Health Organization), and the Interuniversity Attraction Poles Program (managed by the Belgian Science Policy).

No potential conflict of interest relevant to this article was reported.

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