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IL-10 is up regulated in early and transitional stages in vervet monkeys experimentally infected with *Trypanosoma brucei rhodesiense*

Maina Ngotho [a,c,d], Naomi Maina [a], John Kagira [a], Felix Royo [c], Idle O. Farah [b], Jann Hau [c,d,*]

[a] *KARI-Trypanosomiasis Research Centre, P.O. Box 362, Kikuyu, Kenya*
[b] *Institute of Primate Research, P.O. Box 24481 Nairobi, Kenya*
[c] *Division of Comparative Medicine, University of Uppsala, Sweden*
[d] *Department of Experimental Medicine, Panum Instituttet, University of Copenhagen, Blegdamsvej 3B, 2200 Copenhagen N, Denmark*

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Abstract

IL-10 has been suggested as a possible parameter for human African trypanosomiasis stage determination. However, conclusive experimental studies have not been carried out to evaluate this, which is a prerequisite before a potential test can be validated in humans for diagnostic purposes. We used the vervet monkey model of trypanosomiasis to scrutinize IL-10 in blood and cerebrospinal fluid (CSF). Five adult males were experimentally infected with *T. b. rhodesiense*. The infected animals became anemic and exhibited weight loss. Parasitemia was patent after 3 days and fluctuated around 3.7×10^7 trypanosomes/ml throughout the experimental period. The total CSF white cell counts increased from pre-infection means around 3 cells/μl to a peak of 30 cells/μl, 42 days post-infection (DPI). IL-10 was not detectable (<2 pg/ml) in serum prior to infection. IL-10 serum concentrations increased to 273 pg/ml 10 DPI coinciding with the first peak of parasitemia. Thereafter the levels declined to a mean value of 77 pg/ml 34 DPI followed by a significant rise to a second peak of 304 pg/ml ($p < 0.008$) 42 DPI. There was no detectable IL-10 in CSF. IL-10 synthesis is thus stimulated both in the early and transitional stages of experimental trypanosomiasis. That IL-10 is produced in early stage disease is an interesting finding unlikely to be detected in humans where it is difficult to determine the exact time of infection. The IL-10 peak observed on day 42 of infection might indicate onset of parasite neuroinvasion coinciding with a peak in white blood cell counts in the blood and CSF.

Keywords: *Trypanosoma brucei rhodesiense*; Chlorocebus aethiops; Cercopithecus aethiops; Vervet monkey, African green monkey; Human African trypanosomiasis; Sleeping sickness; IL-10; Diagnosis

1. Introduction

Human African trypanosomiasis (HAT) or sleeping sickness occurs in two clinically distinguishable forms. One of these is caused by *Trypanosoma brucei gambiense*, and is commonly described as the Gambian or West African form characterized by slow development and progression, which may span several years. The other form is the Rhodesian or East African form of sleeping sickness, caused by *T. b. rhodesiense*, and characterized by more severe and acute symptoms that progress within weeks. The Rhodesian disease is viewed as a compressed Gambian form because most of the symptoms as well as the neuropathological changes are similar in both forms [1]. The involvement of the central nervous system (CNS) during trypanosomiasis, characterized by invasion of the brain and spinal cord by trypanosomes and abnormal cerebrospinal fluid (CSF) is called late or meningoencephalitic stage. The precise timing of the earliest penetration of the blood–brain barrier is still unknown. The discovery of clinical and biological changes, which herald the invasion of the CNS, assumes major importance in determining the appropriate choice of therapy. Recent studies in humans have shown that an increase of IL-10 in CSF is a strong predictor of late stage infection and may be used for stage determination as well as indicator of success of chemotherapy [2–5]. However, the changes in IL-10 concentrations during infection in man are not well understood since frequent sampling especially of CSF is unethical. The vervet

* Corresponding author. Department of Experimental Medicine, Panum Instituttet, University of Copenhagen, Blegdamsvej 3B, 2200 Copenhagen N, Denmark. Tel.: +45 35 32 63 73; fax: +45 35 73 99.
E-mail address: jhau@emed.ku.dk (J. Hau).

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monkey (*Chlorocebus aethiops*, synonym *Cercopithecus aethiops*) model for *T. b. rhodesiense* sleeping sickness mimics the disease in man. Three model disease stages have been described; early, terminal/transitional, and advanced late stage [6,7]. In this model, the animals are experimentally infected and treated around day 28 with diminazene aceturate, a drug that does not cross the blood–brain barrier, to provoke the meningoencephalitis or second stage. Contrary to humans infected with *T. b. rhodesiense*, vervet monkeys die spontaneously around day 50, most probably from severe pancarditis, if no treatment is given [8]. Thus vervets are more susceptible to trypanosomiasis than are humans. In the present study, the objective was to investigate progression of trypanosome infection in vervet monkeys, specifically the IL-10 profile in serum and CSF, without treatment with diminazene aceturate. This will assist in characterization of the model and may reveal early important markers of infection.

2. Materials and methods

2.1. Trypanosomes

Trypanosoma b. rhodesiense KETRI 2537 (derivative of EATRO 1989) was used. The parasite was isolated from a patient in Uganda and propagated in monkeys by direct inoculation of blood and lymph node aspirate [9]. In this study, the trypanosomes were multiplied in gamma irradiated Swiss White mice. At the rising wave of parasitemia, the mice were sacrificed during anesthesia in CO_2 and trypanosomes harvested through cardiac puncture.

2.2. Animals

Seven adult male vervet monkeys weighing 3.7–4.5 kg were acquired from the Institute of Primate Research (IPR, Karen) in Kenya. They were initially housed in quarantine for a minimum of 90 days while being screened for evidence of disease, including zoonotics. During this quarantine period the monkeys became accustomed to handling and housing in individual squeeze-back stainless steel cages. They were fed twice daily with commercial monkey pellets (Monkey pellets®, Unga Feeds Ltd, Kenya), fresh fruits and vegetables. Drinking water was provided *ad libitum*. Before experimental infection, the animals were transferred to experimental wards and allowed to settle for another 2 weeks before the commencement of experiments.

2.3. Experimental design

Five male vervets were infected with 10^4 *T .b. rhodesiense* KETRI 2537 by intravenous injection. Two animals were not infected and were used as controls. The monkeys were anaesthetized with diazepam (May and Baker, UK) at 1 mg/kg body weight and ketamine hydrochloride (Rotexmedica, Trittau, Germany) at 15 mg/kg bwt for clinical examination and sampling. A clinical examination was carried out every 6 days during the experimental period. Such procedures were also carried out prior to infection and served as pre-infection self-controls. The presence of trypanosomes in ear-prick blood was determined daily and parasitemia estimated using the method of Herbert and Lumsden [10]. The animals were sampled for blood as follows: from 6 to 36 days after infection (DPI), every 48 h; and finally on day 42 post infection. The total peripheral white blood cell (WBC) counts were determined using a hematology analyzer (Coulter A^c.T diff, Beckman coulter, Miami, USA). Simultaneously, approximately 1.2 ml CSF sample was obtained by lumbar puncture every 6 days throughout the experimental period. A drop of fresh CSF was collected into a capillary tube, immediately transferred into a hemocytometer chamber and the number of white cells counted. If trypanosomes were not seen in the counting chamber, approximately 1.2 ml of CSF was collected in a Pasteur pipette whose tip had been heat-sealed. This was centrifuged and the sealed end examined using a microscope as described by Gould and Sayer [11]. The experiment was terminated after 42 DPI by euthanasia of the animals using 20% pentobarbitone sodium (Euthatal®, Rhone Merieux). A post mortem examination was undertaken and major organs preserved in 10% formalin. The tissues were trimmed, processed and stained with Hematoxylin and Eosin before being examined under microscope for histological changes.

All experimental protocols involving animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of TRC.

2.4. IL-10 ELISA

An indirect sandwich enzyme linked immunosorbent assay (ELISA) was used to determine the levels of IL-10 in the serum and CSF samples according to manufacturer's instructions (Human IL-10 Cytoset kit, Biosource International, Sweden) with sensitivity of 2.0 pg/ml. All the serum and CSF samples were aliquoted before storage at −20 °C. The samples were transported in dry ice to Uppsala where the assays were carried out. The aliquots were thawed overnight at +4 °C before laying out the plates. All assays were done in duplicate. The plates were read with an ELISA-reader (Multiscan RC, Labsystems, Sweden) at 450 nm.

2.5. Statistical analysis

Data are presented as line graphs depicting means ± SEM. Significance of differences was determined by ANOVA using Statview for Windows Version 5.0.1 (SAS Institute Inc, 1995–1998, Cary, NC). p values < 0.05 were considered significant.

3. Results

3.1. Parasitemia and Packed Cell Volume (PCV) changes

The pre-patent period of the isolate was 3 days in all the five monkeys. The parasites multiplied rapidly, giving a first parasitemia peak (10^8 trypanosomes/ml) 6 DPI (Fig. 1a). The parasitemia dropped slightly (4.2×10^7) but peaked (1.2×10^8 trypanosomes/ml) again on day 10. Thereafter the parasitemia remained high with slight fluctuations for the rest of the study period. Trypanosomes were first detected in the CSF 12 DPI and thereafter throughout the infection.