academicJournals

Vol. 15(36), pp. 1986-1990, 7 September, 2016
DOI: 10.5897/AJB2016.15575
Article Number: 273864A60390
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Confirmation of antibodies against L-tryptophan-like epitope in human African trypanosomosis serological diagnostic

Silla Semballa[1,2,3], Romaric Nzoumbou-Boko[1,2,3] , Philippe Holzmuller[4] , Paul Chuchana[5] , Jean-loup Lemesre[6], Philippe Vincendeau[1,2], Pierrette Courtois[1], Sylvie Daulouède[1] and Philippe Truc[7,8]*

[1]Université de Bordeaux, IRD, UMR 177 «INTERTRYP», 33076 Bordeaux France.
[2]CHU de Bordeaux, Laboratoire de Parasitologie, 33076 Bordeaux France.
[3]Laboratoire des Sciences Biologiques et Agronomiques pour le Développement (LASBAD) Université de Bangui, Bangui, République Centrafricaine.
[4]CIRAD UMR Contrôle des maladies exotiques et émergentes (CMAEE), TA-A15/G, Campus International de Baillarguet, 34398, Montpellier Cedex, France.
[5]IRMB, Unité INSERM U1183, Hôpital Saint Eloi, 34295 Montpellier France.
[6]Institut de Recherche pour le Développement IRD, UMR 177 « INTERTRYP», Centre IRD de Montpellier, 34000 Montpellier Cedex, France.
[7]Institut de Recherche pour le Développement IRD, UMR 177 «INTERTRYP», Campus International de Baillarguet, 34398 Montpellier Cedex, France.
[8]Laboratoire de recherche sur les trypanosomoses, Unité Mixte de Recherche 177 IRD-CIRAD, Campus International de Baillarguet, 34398 Montpellier Cedex, France.

Received 20 July, 2016; Accepted 25 August, 2016

Antibodies directed against L-tryptophan epitope (WE - W for tryptophan, E for epitope), a constant epitope borne by variant surface glycoproteins (VSG), have been detected in sera of all 152 Human African Trypanosomosis (HAT) patients from Angola. The WE is present in VSG hydrophobic regions of the C terminal domains. In the assay, L-tryptophan was linked to bovine serum albumin (BSA) with glutaraldehyde to synthesize W-G-BSA conjugate which was used in an enzyme-linked immunosorbent assay (ELISA) to detect the antibodies. A significant difference was found between HAT patients and controls confirming previous results obtained with a lower number of patients in Congo. A diagnostic test based on this synthetic epitope, especially in combination with other tests, might improve the HAT diagnostic test in field conditions.

Key words: Tryptophan, enzyme-linked immunosorbent assay (ELISA), human African trypanosomosis, serological diagnostic.

INTRODUCTION

Trypanosoma brucei gambiense (*T. b. gambiense*) and *T. b. rhodesiense*, the causative agents of Human African Trypanosomosis (HAT) or sleeping sickness, are tsetse fly-transmitted protozoan parasites. Diagnosis of HAT

remains a challenge for disease control as HAT is characterized by a non-specific clinical presentation and its definitive diagnosis requires trypanosome detection by microscopy (WHO, 2013). Additionally, the detection of this parasite is difficult because of the low parasite concentration in circulating blood, despite the use of concentration methods such as the mini-anion-exchange centrifugation technique (mAECT) (Lumsden et al., 1979). Moreover, molecular diagnostics based on parasite DNA or RNA detection are more sensitive but are not adapted to field conditions.

Serological diagnostic test of HAT, caused by *T. b. gambiense*, had been performed in the field during medical surveys since 1978 using the Card Agglutination Test with stained Trypanosomes (CATT) (Magnus et al., 1978). CATT/*T. b. gambiense* is still the reference test in the field conditions although, it requires refrigeration and an electric rotator, with drawbacks of false positive and negative results (Magnus et al., 1978). Trypanosomes produce a set of variant surface glycoproteins (VSG). Thus, new diagnostic approaches are based using native surface glycoproteins although the variable nature of the VSG is not satisfactory for a serological diagnostic test (WHO, 2013).

Following an initiative of Foundation for Innovative New Diagnostics (FIND), a rapid diagnostic test is now available (Standart Diagnostic SD Bioline HAT, Sternberg et al., 2014). However, this latter is very useful for passive mode detection but not for mass screening survey (Büscher et al., 2014). The search of new biomarker candidates will permit an improvement to HAT diagnostic test based on CATT (Bonnet et al., 2015). Proteomic investigations are identifying potential immunodiagnostic parasite protein antigens and several biomarkers that are in preliminary evaluation or design for new diagnostic tests (Holzmuller et al., 2013). It has also been discussed how molecular diagnostics may contribute to the elimination of HAT (Büscher and Deborggraeve, 2015).

A tryptophan-like epitope (WE) was found in VSGs from the *T. brucei* group (Semballa et al., 2007). The tryptophan residue is one of the conserved flanking residues of cysteine residues in their hydrophobic regions in the C terminal domain. Antibodies directed to WE were previously found in the sera of HAT patients detected during medical survey (active mode detection), but they were not detected in HIV infection, malaria, Chagas and Parkinson's diseases (Okomo-Assoumou et al., 1995). This latter investigation was performed in the Bouenza focus in the Republic of Congo. No other study has been done in a different HAT focus.

In the present study, anti-WE detection in sera of HAT patients from Angola detected by passive mode at the Viana hospital compared to negative controls, in order to confirm the potential value of this immune marker in another endemic country, use in setting up a new test for serological HAT mass screening.

METHODS

Patients

Informed consent was obtained from all individual participants which were included in the study. Patients were diagnosed by passive mode detection in Viana hospital in Angola (Truc et al., 2012). Only CATT positive patients with trypanosomes that were detected in one body fluid (blood, lymph juice, cerebrospinal fluid) were selected. Stage determination of the disease was done by searching trypanosomes and white blood cells count in CSF: 13 in stage 1 (early period) and 139 in stage 2 (neurological period). A clinical examination allows detection of neurological signs. Sera from CATT negative subjects living in the same endemic area were used as controls. These controls were uninfected volunteer persons. Serum aliquots were kept at -80°C until use.

ELISA based on L-tryptophan conjugates

Enzyme-linked immunosorbent assay (ELISA) was performed as previously described (Okomo-Assoumou et al., 1995). Briefly, W and BSA (bovine serum albumin) were dissolved in 1.5 M acetate buffer, pH 8 before the addition of glutaraldehyde. After dialysis, the conjugate (W-G-BSA) or control (G-BSA) was added into polystyrene well plate. The well plate was filled with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-Tween), 10% glycerol and BSA (5 g/L). The well plate was incubated for 1 hr at 37°C to saturate it. The plate was rinsed twice with PBS-Tween. The well plate was then filled with 200 µL of diluted (2,000-fold) serum plus PBS-Tween containing BSA (5 g/L) and 10% glycerol. After washings, horseradish peroxidase-conjugated goat antibodies were added to human immunoglobulins. Thereafter, substrate solution and stop solution were successively added. The absorbance in the well plate was measured at 492 nm. The specific immunologic binding of sera was obtained by subtracting blank values read on well plates coated with BSA-G from experimental absorbance values.

Statistical method

Results are expressed in OD value for each individual. Comparison between the patients and the control groups was made using the nonparametric Wilcoxon–Mann–Whitney test with median and quintiles. The difference was considered significant when $p<0.05$. This comparison was illustrated by a graph performed with Graph Pad V6.0.

RESULTS AND DISCUSSION

Anti-WE antibodies were detected in serum of 152 patients (Table 1) and in 10 controls (Table 2). The limited

*Corresponding author. E-mail: philippe.truc@ird.fr. Tel.: +33 467593919. Fax: +33 467593893.

Table 1. Values of anti-WE antibodies in optic density and clinical stage for each patient.

Patient Code	Stage	OD Units (anti-WE)	Patient Code	Stage	OD Units (anti-WE)
V31	1	0.546	V117	2	0.750
V34	1	0.475	V119	2	0.725
V36	1	0.536	V122	2	3.808
V38	1	0.442	V124	2	0.546
V40	1	0.291	V125	2	1.346
V42	1	0.447	V128	2	0.722
V91	1	0.345	V129	2	0.635
V198	1	0.404	V130	2	0.451
V201	1	0.225	V133	2	0.716
V203	1	0.320	V134	2	0.676
V215	1	0.259	V135	2	0.602
V217	1	0.382	V137	2	0.593
V131	1	0.267	V138	2	0.604
V32	2	0.500	V139	2	0.505
V63	2	0.514	V141	2	0.592
V120	2	0.371	V142	2	0.779
V231	2	0.540	V143	2	0.780
V02	2	0.311	V144	2	0.736
V03	2	1.317	V145	2	0.634
V05	2	1.848	V146	2	0.592
V07	2	0.345	V147	2	1.288
V10	2	1.251	V150	2	0.707
V13	2	0.628	V151	2	0.852
V15	2	0.772	V152	2	0.509
V16	2	0.914	V153	2	0.724
V17	2	0.730	V156	2	0.777
V18	2	0.911	V157	2	0.557
V19	2	1.107	V159	2	0.691
V23	2	0.792	V160	2	0.504
V24	2	0.693	V162	2	0.622
V25	2	0.426	V164	2	0.871
V26	2	0.598	V165	2	0.602
V27	2	0.733	V166	2	0.512
V35	2	0.554	V167	2	1.234
V37	2	0.683	V168	2	0.730
V43	2	0.783	V170	2	0.760
V44	2	0.784	V171	2	0.715
V46	2	1.036	V172	2	0.850
V47	2	0.775	V173	2	0.530
V48	2	0.687	V174	2	0.517
V49	2	0.588	V176	2	0.642
V52	2	0.655	V177	2	0.736
V53	2	0.603	V178	2	0.546
V54	2	0.736	V179	2	0.794
V57	2	0.856	V180	2	0.577
V61	2	0.915	V182	2	0.607
V64	2	0.728	V183	2	1.055
V65	2	0.664	V187	2	0.573
V66	2	0.582	V188	2	0.719
V67	2	0.652	V189	2	0.746
V69	2	0.659	V191	2	0.700

Table 1. Contd.

V70	2	0.846	V192	2	0.744
V75	2	0.561	V194	2	0.677
V80	2	0.684	V195	2	0.624
V82	2	1.389	V196	2	0.514
V83	2	0.685	V199	2	1.034
V85	2	0.643	V200	2	0.490
V86	2	0.594	V202	2	0.325
V87	2	1.015	V206	2	0.835
V88	2	0.558	V207	2	0.559
V89	2	0.604	V208	2	0.741
V90	2	0.898	V209	2	0.571
V92	2	0.841	V210	2	0.589
V93	2	0.843	V212	2	0.518
V94	2	0.781	V214	2	0.489
V96	2	0.364	V216	2	0.649
V98	2	1.637	V221	2	0.498
V100	2	0.996	V223	2	0.733
V104	2	0.802	V226	2	0.385
V105	2	1.099	V228	2	0.655
V106	2	0.944	V232	2	0.880
V107	2	0.700	V235	2	0.555
V109	2	0.758	V238	2	0.439
V110	2	3.090	V239	2	0.571
V113	2	0.796	V240	2	0.438
V114	2	0.719	V241	2	0.518

Table 2. Values of anti-WE antibodies in optic density afor each negative control individual.

Control Code	OD Units (anti-WE)
AG	0.109
AR	0.108
EB	0.096
MR	0.114
NF	0.089
BA	0.111
BH	0.192
MF	0.121
OE	0.117
OC	0.110

number of the control group was due to the difficulty in obtaining the people's consent at the Viana Hospital. In a survey carried out in Congo, including 22 controls and 76 patients (52 and 24 in stages 1 and 2 respectively), a very low immunological signal was detected in controls.

The comparison between patients (median= 0.6622, n=152) and controls values is highly significant P <0.0001 (Figure 1). This confirms the use of anti-WE as a potential biomarker in HAT serological diagnostic test. Further investigations are required to compare the present results with the post-cure HAT persons and other parasitic diseases. Furthermore, among 139 stage 2 patients, 86 presented neurological disorders. There is no correlation between OD values and presence of neurological disorders P=0.58. Thus, the anti-WE value is not correlated to patient clinical state.

Rapid tests are now available and are very promising but have been employed mainly for passive mode detection (Büscher et al., 2014). However, their observed specificity was relatively low. A larger study on their use in field condition in combination and in comparison with immune trypanolysis, the reference test, is under progress.

L-Tryptophan epitope conjugate production by peptide synthesis is now cheaper and easily compared to CATT antigen production, requiring *T. b. gambiense* infection in laboratory rodents, or production of recombinant proteins to develop diagnostic kits. Moreover, WE conjugate is thermo-stable and can be developed in a rapid test format. For instance, as most anti-WE antibodies are of IgM isotype, an agglutination assay is feasible. Reliably and easy to use, diagnostic tools are necessary to eliminate HAT as a public health problem in the year 2020 and zero transmission in the year 2030.

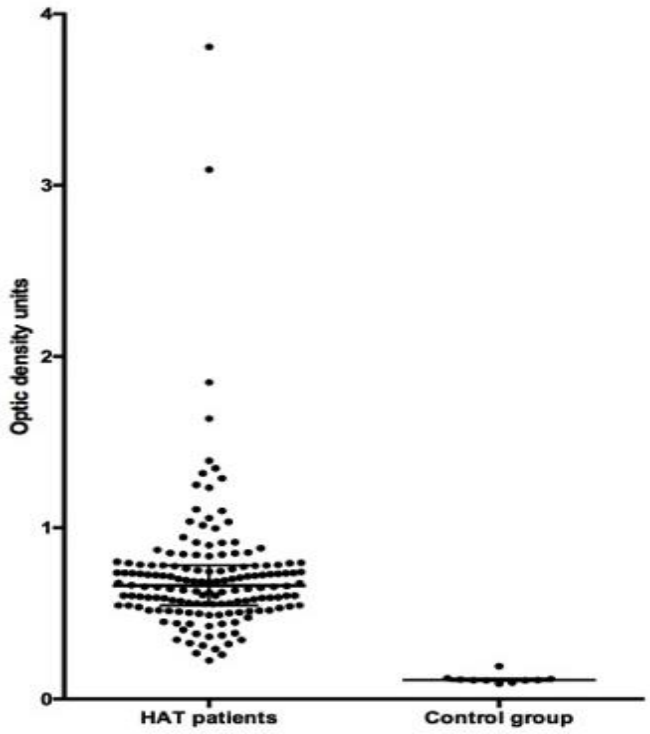

Figure 1. Level of anti-WE antibodies, as assayed by ELISA, in the HAT patients and control groups. Black lines indicates median and interquartiles (Q25 and Q75).

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors are grateful to the staff of Instituto de Combate e Controlo das Tripanossimiases, Ministry of Health, Angola.

REFERENCES

Bonnet J, Boudot C, Courtioux B (2015). Overview of the Diagnostic Methods Used in the Field for Human African Trypanosomiasis: What Could Change in the Next Years? Biomed. Res. Int. Volume 2015, Article ID 583262, 10 pages.

Büscher P, Deborggraeve S (2015). How can molecular diagnostics contribute to the elimination of human African trypanosomiasis? Expert Rev. Mol. Diagn. 15(5):607-615.

Büscher P, Mertens P, Leclipteux T, Gilleman Q, Jacquet D, Mumba-Ngoyi D, Pyana PP, Boelaert M, Lejon V (2014). Sensitivity and specificity of HAT Sero-K-SeT, a rapid diagnostic test for serodiagnosis of sleeping sickness caused by *Trypanosoma brucei gambiense*: a case-control study. Lancet Glob. Health 2(6):e359-363.

Holzmuller P, Grébaut P, Semballa S, Gonzatti MI, Geiger A (2013). Proteomics: a new way to improve human African trypanosomiasis diagnosis? Expert Rev. Proteomics 10(3):289-301.

Lumsden WH, Kimber CD, Evans DA, Doig SJ (1979). *Trypanosoma brucei*: Miniature anion-exchange/centrifugation technique for detection of low parasitaemias: Adaptation for field use. Trans. R. Soc. Trop. Med. Hyg. 73(3):312-317.

Magnus E, Vervoort T, Van Meirvenne N (1978). A card-agglutination test with stained trypanosomes (CATT) for the serological diagnosis of *T. b. gambiense* trypanosomiasis. Ann. Soc. Belge Méd. Trop. 58:169-176.

Okomo-Assoumou MC, Geffard M, Daulouede S, Chaugier C, Lemesre JL, Vincendeau P (1995). Circulating antibodies directed against tryptophan-like epitopes in sera of patients with human African trypanosomiasis. Am. J. Trop. Med. Hyg. 52(5):461-467.

Semballa S, Okomo-Assoumou MC, Holzmuller P, Büscher P, Magez S, Lemesre JL, Daulouede S, Courtois P, Geffard M, Vincendeau P (2007). Identification of a tryptophan-like epitope borne by the variable surface glycoprotein (VSG) of African trypanosomes. Exp. Parasitol. 115:173-180.

Sternberg JM, Gierlinski M, Biéler S, Ferguson MAJ, Ndung'u JM (2014). Evaluation of the Diagnostic accuracy of prototype Rapid Tests for Human African Trypanosomiasis. PLoS Negl. Trop. Dis. 8(12):e3373.

Truc P, Lando A, Penchenier L, Vatunga G, Josenando T (2012). Human African trypanosomiasis in Angola: clinical observations, treatment, and use of PCR for stage determination of early stage of the disease. Trans. R. Soc. Trop. Med. Hyg. 106(1):10-14.

WHO Control and surveillance of human African trypanosomiasis (2013). WHO Technical Report Series 984 p.