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A LiTat 1.5 variant surface glycoprotein-derived peptide with diagnostic potential for *Trypanosoma brucei gambiense*

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Abstract

OBJECTIVE To evaluate the accuracy of a peptide, corresponding to the variant surface glycoprotein (VSG) LiTat 1.5 amino acid (AA) sequence 268–281 and identified through alignment of monoclonal antibody selected mimotopes, for diagnosis of *Trypanosoma brucei gambiense* sleeping sickness.
METHODS A synthetic biotinylated peptide (peptide 1.5/268–281), native VSG LiTat 1.3 and VSG LiTat 1.5 were tested in an indirect ELISA with 102 sera from patients with HAT and 102 endemic HAT-negative controls.
RESULTS The area under the curve (AUC) of peptide 1.5/268–281 was 0.954 (95% confidence interval 0.918–0.980), indicating diagnostic potential. The areas under the curve of VSG LiTat 1.3 and LiTat 1.5 were 1.000 (0.982–1.000) and 0.997 (0.973–1.000), respectively, and significantly higher than the AUC of peptide 1.5/268–281. On a model of VSG LiTat 1.5, peptide 1.5/268–281 was mapped near the top of the VSG.
CONCLUSIONS A biotinylated peptide corresponding to AA 268–281 of VSG LiTat 1.5 may replace the native VSG in serodiagnostic tests, but the diagnostic accuracy is lower than for the full-length native VSG LiTat 1.3 and VSG LiTat 1.5.

keywords diagnosis, sensitivity, specificity, sleeping sickness, *Trypanosoma brucei gambiense*, variant surface glycoprotein

Introduction

The protozoan parasite *Trypanosoma brucei (T.b.) gambiense* is the causative agent of the chronic form of sleeping sickness or human African trypanosomiasis (HAT), endemic in West- and Central-Africa. If undiagnosed, this devastating disease may persist for years until the patient dies (Brun *et al.* 2009).

At present, diagnosis of *T.b. gambiense* HAT is based on serological screening to reveal HAT suspects on whom microscopic parasite detection is performed (Chappuis *et al.* 2005). The commonly used antibody detection test, the card agglutination test for trypanosomiasis (CATT) (Magnus *et al.* 1978), detects antibodies against the variant surface glycoprotein (VSG) LiTat 1.3, a predominant variable antigen type (VAT) recognised by most *gambiense* HAT patients (Van Meirvenne *et al.* 1995). In some foci, for example in Nigeria and Cameroon, a considerable fraction of HAT patients do not react with VSG LiTat 1.3, possibly due to the absence of the corresponding gene in the repertoire of local *T.b. gambiense* strains (Dukes *et al.* 1992; Büscher *et al.* 1999). To compensate for this, newer antibody detection tests include VSG LiTat 1.5 as an additional VAT (Büscher *et al.* 1999; Lejon *et al.* 2006).

However, native VSGs may expose non-HAT-specific epitopes resulting in non-specific reactions (Jamonneau *et al.* 2010; Schwede *et al.* 2011). Moreover, production of these antigens requires culture of infective *T.b. gambiense* in laboratory rodents, posing an important health risk to the staff (Herwaldt 2001).

Peptides may replace VAT-specific epitopes. In previous manuscripts, we described how peptide mimotopes, mimicking VSG LiTat 1.3 and VSG LiTat 1.5 epitopes, were selected by phage display (Van Nieuwenhove *et al.* 2011, 2012a). Mapping of the sequence of the mimotopes against the complete primary amino acid (AA) sequence allowed us to identify an AA stretch of the native LiTat 1.3 VSG sequence with diagnostic potential (Van Nieuwenhove *et al.* 2012a). In analogy, we aligned

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mimotopes, selected with monoclonal antibodies, to the primary LiTat 1.5 VSG sequence and thus identified an AA sequence with diagnostic potential. The corresponding peptide was synthesised, and we evaluated its accuracy for *gambiense* sleeping sickness diagnosis.

Materials and methods

Identification of peptide 1.5/268–281

The panning of the anti-LiTat 1.5 monoclonal antibodies and the alignment of the selected phage displayed peptide sequences to the VSG LiTat 1.5 protein sequence [GenBank HQ662603] was described previously (Van Nieuwenhove *et al.* 2011, 2012b). Based on homology analysis of the selected mimotopes, the synthetic peptide TAQAVYKDHDPDQG-K-biotin (1.5/268–281), corresponding to AA stretch 268–281 of the VSG LiTat 1.5 protein sequence, was synthesised at >85% purity (Peptide 2.0, Chantilly, VA, US).

Sera

All 102 sera from *gambiense* HAT patients originated from DR Congo (Mumba Ngoyi *et al.* 2010). Thirty-one endemic HAT-negative sera originated from the DR Congo (Lejon *et al.* 2006) and 31 from Benin (Lejon *et al.* 2006). Ethical clearance was obtained from the Ethics Committees of DR Congo and the Institute of Tropical Medicine, Antwerp (ITMA). Forty additional endemic negative control specimens from Congo were obtained from the archived specimen bank at ITMA.

Indirect ELISA

Native VSG LiTat 1.3 and LiTat 1.5 were purified from a population of *T.b. gambiense* VAT LiTat 1.3 and LiTat 1.5, respectively (Büscher *et al.* 1999). The diagnostic performance of peptide 1.5/268-281 was evaluated with human sera used in previous experiments following methods that were previously described (Van Nieuwenhove *et al.* 2012a). Briefly, ELISA plates were coated with 10 μg/ml streptavidin or with 2 μg/ml VSG, or wells were left empty (Ag0). After saturation, plates were washed and 2 μg/ml peptide was added to the wells containing streptavidin. The peptide-free wells received PBS. PBS sucrose was added to the VSG-containing and the Ag0 wells, and plates were sealed and frozen. For testing, plates were thawed and serum dilutions of 1/100 were applied. After washing, horse radish peroxidase (PO)-conjugated goat anti-human IgG (H + L), diluted 1/40 000, was added followed by washing and the chromogen/substrate solution 2.2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) for an hour. The optical density (OD) was read at 414 nm. The ODs were corrected (OD_c) by subtracting the corresponding ODs in the peptide-free or Ag0 wells.

Statistical analysis

The diagnostic accuracy was assessed by the area under the receiver operator characteristics (ROC) curve (AUC) (Bewick *et al.* 2004). The 95% confidence interval (CI) was calculated (DeLong *et al.* 1988) with STATA version 10.0 (Statacorp, USA). For the whole range of cut-offs, the Youden index (sensitivity + specificity − 1) was determined (Youden 1950), and the maximal Youden index was retained to define the cut-off with optimal sensitivity and specificity.

Modelling and molecular imaging

A protein database (pdb) model of the N-terminal domain of VSG LiTat 1.5 was created using SWISS-MODEL (Arnold *et al.* 2006; Kiefer *et al.* 2009). Modelling was based on the known structure of VSG MITat 1.2 (pdb 1vsgA), previously derived by X-ray crystallography (Freymann *et al.* 1984). Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (http://www.cgl.ucsf.edu/chimera).

Results

Peptide 1.5/268–281 had an AUC of 0.954 (0.918–0.980, Table 1). The sensitivity and specificity at OD_c 0.062, the cut-off with the highest Youden index, were 0.892 (0.815–0.945) and 0.951 (0.889–0.984), respectively. VSG LiTat 1.3 and 1.5 showed an AUC of 1.00 and 0.997, respectively, with respective CIs of 0.982–1.000 and 0.973–1.000. For VSG LiTat 1.3, sensitivity and specificity were 1.000 (0.964–1.000) at cut-off 1.318. For VSG LiTat 1.5, sensitivity and specificity were 1.000 (0.964–1.000) and 0.990 (0.947–1.000), respectively, at cut-off 1.182. The OD_c values with peptide 1.5/268–281, VSG LiTat 1.3 and VSG LiTat 1.5 are shown in Figure 1. The AUC of peptide 1.5/268–281 was significantly lower than the AUCs of VSG LiTat 1.3 and 1.5 (*P* values of, respectively, 0.003 and 0.005).

Peptide 1.5/268–281 corresponds to AA 236–249 of the mature VSG LiTat 1.5 protein sequence and was mapped on the model of VSG LiTat 1.5 near the top of the VSG (Figure 2).

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Table 1 Diagnostic performance of peptide 1.5/268–281 and native VSGs LiTat 1.3 and LiTat 1.5 with sera from 102 *Trypanosoma brucei gambiense* HAT patients and from 102 endemic HAT-negative controls

Antigen	AUC (CI)	Sensitivity (CI)	Specificity (CI)
Peptide 1.5/268-281	0.954 (0.918–0.980)	0.892 (0.815–0.945)	0.951 (0.889–0.984)
VSG LiTat 1.3	1.000 (0.982–1.000)	1.000 (0.964–1.000)	1.000 (0.964–1.000)
VSG LiTat 1.5	0.997 (0.973–1.000)	1.000 (0.964–1.000)	0.990 (0.947–1.000)

AUC, area under the curve; CI, 95% confidence interval; VSG, variant surface glycoprotein.

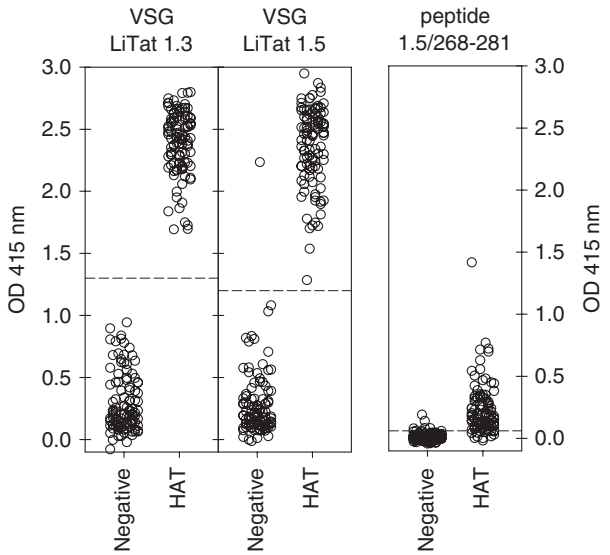

Figure 1 Performance of native variant surface glycoprotein (VSG)s and peptide 1.5/268–281 for diagnosis of HAT. The corrected optical densities of VSG LiTat 1.3, VSG LiTat 1.5 and peptide 1.5/268–281 in indirect ELISA with sera from 102 endemic negative controls and 102 *gambiense* HAT patients are shown. The cut-offs are indicated by the dashed lines.

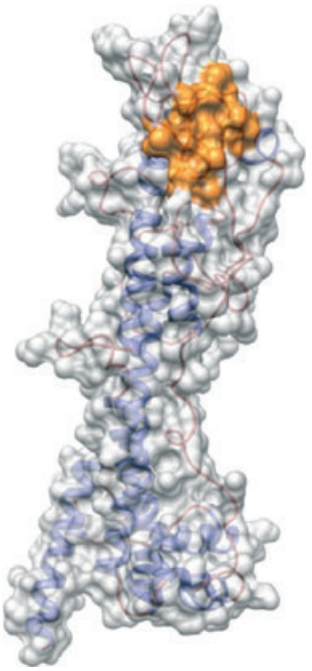

Figure 2 Localisation of peptide 1.5/268–281 (orange) on the model of a variant surface glycoprotein (VSG) LiTat 1.5 monomer (prepared with Chimera).

Discussion

We showed that a peptide stretch based on AA 268–281 of the VSG LiTat 1.5, located near the surface of the VSG, may replace the native protein in serodiagnostic tests, although it performed less well than the full-length native VSG LiTat 1.3 and VSG LiTat 1.5.

However, our study has some limitations. Indeed, previous screening of the serum for reactivity with VSG LiTat 1.3 and VSG LiTat 1.5 may have caused a bias towards higher AUC for the native proteins. In addition, the ELISA used for peptide 1.5/268–281 was not extensively optimised, while the ELISAs for VSG LiTat 1.3 and VSG LiTat 1.5 were. This might have resulted in suboptimal ODs and separation of positive and negative samples. The choice for antibody detection for screening of HAT implies a functional antibody response against the LiTat 1.5 VSG. Furthermore, we limited our tests to a single peptide and did not include peptide combinations.

Nonetheless, the present approach has an important advantage compared with the previously identified mimotopes for VSG LiTat 1.5. It enables the use of an indirect ELISA, in contrast to the complicated inhibition ELISA needed to detect antibodies against mimotopes identified using monoclonal antibodies (Van Nieuwenhove *et al.* 2011). This simplified detection system facilitates translation into ASSURED compliant rapid diagnostic tests (Mabey *et al.* 2004). Moreover, the AUC of peptide 1.5/268–281 is similar to the highest AUC obtained with mimotopes identified using human sera (Van Nieuwenhove

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et al. 2012a). Peptide 1.5/268–281 may therefore complement or replace mimotopes identified earlier as antigen in serodiagnostic tests.

Peptide 1.5/268–281 is located in the N-terminal domain of VSG LiTat 1.5, at the border of the surface-exposed loops with high sequence divergence (Hutchinson *et al.* 2007). This region, at the top of the VSG, is believed to comprise the primary targets for the antibody-mediated immune response directed against the intact living trypanosome (Hsia *et al.* 1996), which is in line with its diagnostic potential.

Although so far performance of identified mimotopes and peptides remains inferior to the native VSGs from which they were derived, the use of peptides as a serodiagnostic test to replace the native proteins has three advantages: (i) peptides can be mass produced in a standardised way; (ii) time-consuming *in vivo* culture of infective *T.b. gambiense* is no longer required; and (iii) the risk of exposing laboratory personnel is eliminated. Even though further optimisation of the assay is needed, it is possible to narrow down the critical residues of the peptide sequence by alanine scanning mutagenesis. An optimised peptide target sequence would increase binding affinity of serum antibodies and thus enhance serodiagnostic performance (Yang *et al.* 2005).

In conclusion, peptide 1.5/268–281 demonstrated diagnostic potential almost identical to the currently used native protein and may be incorporated in diagnostic tests for *T.b. gambiense* HAT.

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