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VSG 117 gene is conservatively present and early expressed in *Trypanosma evansi* YNB stock

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ABSTRACT

African trypanosomes, including *Trypanosoma brucei* and the closely related species *Trypanosoma evansi*, are flagellated unicellular parasites that proliferate extracellularly in the mammalian bloodstream and tissue spaces. They evade host immune system by periodically switching their variant surface glycoprotein (VSG) coat. Each trypanosome possesses a vast archive of VSGs with distinct sequence identity and different strains contain different archive of VSGs. VSG 117 was reported as a widespread VSG detected in the genomes of all the *T. brucei* strains. In this study, the presence and expression of VSG 117 gene was observed in *T. evansi* YNB stock by RT-PCR with VSG-specific primers. We further confirmed that this VSG tends to be expressed in the early stage of *T. evansi* infections (on day 12–15) by immuno-screening the previously isolated infected blood samples. It is possible that the VSG 117 gene evolved and spread through the African trypanosome population via genetic exchange, before *T. evansi* lost its ability to infect tsetse fly. Our finding provided an evidence of the close evolutionary relationship between *T. evansi* and *T. brucei*, in the terms of VSG genes.

1. Introduction

African trypanosomes, including *Trypanosoma brucei* and the closely related species *Trypanosoma evansi*, are extracellular flagellated parasites. *T. brucei*, which undergoes a complex life cycle that alternates between tsetse flies and mammalian hosts, is responsible for human sleeping sickness and the ruminant nagana in Africa. *T. evansi*, the causative agent of surra, infects most livestock and many wild animals. Although morphologically indistinguishable from *T. brucei*, *T. evansi* is mechanically transmitted by bloodsucking flies or vampire bats and spread beyond the tsetse fly belt of Africa to Asia and South America.

Within the Bloodstream and tissue spaces of a mammalian host, African trypanosomes are fully exposed to the host's immune system. They avoid immune elimination by periodically changing their variant surface glycoprotein (VSG) coat. Each trypanosome possesses a large archive of VSG genes which are highly divergent in sequence (Berriman et al., 2005) and different trypanosome strains contain different archive of VSGs. The host's immune selection and strain competition play an important role in shaping VSG divergence. The effect of host immune selection is well-illuminated by the polarity in the VSG sequence homology: the exposed N-terminal domain is hypervariable; while the hidden C-terminal domain relative conserved (Marcello et al., 2007).

Strain-specific VSGs have a potential to allow a strain to infect a host previously exposed to another different strain. In fact, the early expressed VSGs of different strains are so distinct that some of them have been used for identification and diagnostic of trypanosome species (subspecies) infection. Comparative analysis of the homologous VSGs from different strain also demonstrated that the location of divergence was more prevalent in those exposed N-terminal regions (Hutchinson et al., 2007), consistent with the strain competition selection for strain-specific VSGs.

Some VSGs are unique to an individual trypanosome strain, while others conserved among different trypanosome species and strains (Pays et al., 1981; Frasch et al., 1982). One most intensively studied example is the VSG 117 gene. This VSG gene was originally identified from a variant antigen type (MITat 1.4) of *T. brucei* strain 427, then was found widely present in many strains of *T. brucei*, including *T. b. rhodesiense*, *T. b. gambiense* and *T. evansi* (Cross, 1977; Frasch et al., 1982).

In this study, VSG 117 was found conservatively present in *T. evansi* YNB stock. This VSG was further detected expressed in the early stage of three individual rabbit infections. It is possible that the VSG 117 gene evolved and spread through the African trypanosome population via genetic exchange, before *T. evansi* lost the ability to infect tsetse fly. Our finding provided an evidence

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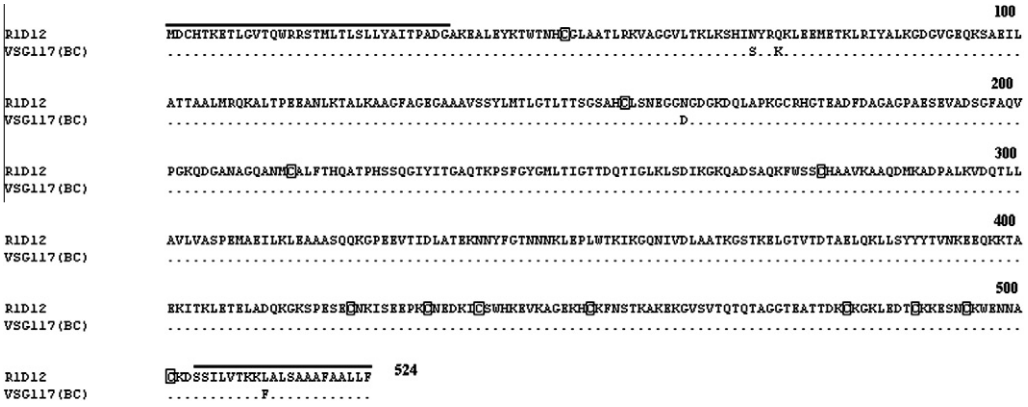

Fig. 1. An alignment of the deduced amino acids of VSG R1D12 with the VSG 117 basic copy. Identity is shown as dots in the alignment. The N-terminal signal sequences and C-terminal GPI-anchor-adding sequences are indicated by a line above the alignment and conserved cysteines by the open boxes.

of the close evolutionary relationship between *T. evansi* and *T. brucei*, in the terms of VSG.

2. Materials and methods

2.1. Animals and trypanosomes

Female ICR mice and lop-eared rabbit were obtained from the Center of Experimental Animals, Peking University Health Science Centre. The animals were fed and watered *ad libitum*. Mice were immunosuppressed with cyclophosphamide treated (0.3 g/kg body weight; Sigma) 24 h before trypanosome infection. Experiments were performed in compliance with relevant laws of china and institutional guidelines.

A total number of 30 cryopreserved infected blood samples, derived from three individual rabbit infection with *T. evansi* stock YNB, were used in this study. The procedure of variants isolation has been described in detail previously (Yang et al., 2007; Jia et al., 2010). Each infected blood sample was given a two-part name (e.g., R1D6), indicating the rabbit of infection (R1 to R3) and the day of rabbit infected blood sample collection (D6 to D33).

2.2. Parasite propagation and VSG amplification

Cryopreserved infected blood sample R1D12 was thawed and injected intraperitoneally (i.p.) into a cyclophosphamide-treated mouse. When parasitemia reached peak, mouse was sacrificed and trypanosomes were purified from infected blood by DEAE column chromatography (Lanham and Godfrey, 1970). Total RNA was prepared from the purified trypanosomes with Trizol reagent (Invitrogen), according to the manufacturer's instructions. VSG cDNA was produced from total RNA by RT-PCR as previously described (Jia et al., 2010). The expected PCR product (~1.5 kb) was cloned into pEASY-Blunt cloning vector (TransGen Biotech, Beijing, China) prior to being sequenced.

To obtain 5′ untranslated region (5′UTR) of VSG R1D12, primers (117F 5′-CACTAAACAGCGGAGCAG-3′ and 117R 5′-TATCAGAGTGG CAAAGT-3′) were designed according to the sequence of the 117 basic copy (L34415). Trypanosome genomic DNAs were prepared from purified trypanosomes using a TIANamp Genomic DNA Kit (Tiangen Biotech Co. Ltd., Beijing, China), according to the manufacturer's protocol. PCR reaction was performed using Phusion High-Fidelity DNA polymerase kit (Finnzymes Oy.) as follows: an initial denaturation step of 30 s at 98 °C, followed by 25 cycles of 7 s of denaturation at 98 °C, 30 s of annealing at 53 °C, 40 s of extension at 72 °C, and a final 7 min final extension at 72 °C. The PCR product was cloned into pEASY-Blunt cloning vector (TransGen Biotech, Beijing, China) prior to being sequenced.

2.3. Cloning and expression of a recombinant rR1D12 in E. coli and antibody generation

The N-terminal domain of VSG R1D12 (amino acids 23–313) was amplified using primers 117FB*am*HI 5′-CGCGGATCCT-CACTGCTTTACGCCATCA-3′ and 117RX*ho*I 5′-CCGCTCGAGCAG-TATTTCCGCCATCT-3′ (introduced restriction sites B*am*HI and X*ho*I were underlined) and cloned in fusion with the GST protein into the pGEX 4T-1 (Pharmacia). The derived fusion protein was expressed in BL21 (DE3) expression cells, purified from bacterial inclusion bodies and inoculated a male lop-eared rabbit for the generation of antibodies. Anti-VSG R1D12 antibodies were collected 10 days post final boost and the increase of antibody titer was controlled by ELISA test.

2.4. Immuno-screening and VSG amplification

Thirty cryopreserved infected blood samples (10 μl each, containing $\sim 1 \times 10^5$ parasites) were thawed, boiled in loading buffer for 5 min, run on 12% SDS–PAGE gels and transferred onto Polyvinylidene fluoride (PVDF) membranes (immobilon –P, Millipore).

Western blots were performed as described previously (Jia et al., 2010) with a 1/2000 dilution of rabbit anti-VSG R1D12 antisera followed by a 1/2000 dilution of the goat anti-rabbit IgG HRP-labeled secondary antibody (Signalway Antibody, Pearland, TX, USA). The ECL chemiluminescence detection system (Applygen Technologies Inc., Beijing, China) were used to visualized antigens.

The immuno-screening positive blood samples (R2D12 and R3D15) were thawed and parasites were propagated in cyclophosphamide-treated mice for RNA extraction and VSG amplification by RT-PCR.

3. Results

3.1. Conservative presence of VSG 117 gene in T. evansi

The VSG R1D12 cDNA coded a polypeptide with all the recognized VSG features (N-terminal signal peptide, GPI-anchor-adding sequence and conserved cysteine pattern). When searched the available sequences in the GenBank database, the deduced amino acid sequence was almost identical to *T. brucei* strain VSG 117 (Fig. 1).

The nucleotide sequence of VSG R1D12 cDNA, removing the splice leader sequence, is identical to the basic copy of VSG 117 (L34415), with eight random point mutations. To examine the