

Full Length Research Paper

Isolation of *Theileria parva* ring-infected erythrocyte surface antigen (RESA) homolog

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East coast fever (ECF) is a severe lymphoproliferative disease of cattle caused by the intracellular protozoan *Theileria parva* from the phylum Apicomplexa. Gene homologs encoding antigens from other apicomplexan parasites constitute a source of vaccine candidate antigens. The ring-infected erythrocyte surface antigen (RESA) protein is a vaccine candidate for malaria caused by *Plasmodium falciparum* and should be evaluated for inclusion in a subunit vaccine against ECF. Here we isolated and sequenced the *T. parva* RESA2 homolog and compared it to that of the malaria parasite, *P. falciparum*. The deduced amino acid sequence of the *T. parva* RESA2 gene is very similar to the *P. falciparum* RESA protein (38% identity).

Key words: *Theileria parva*, vaccine, ring-infected erythrocyte surface antigen 2 (RESA2), apicomplexan antigens, homolog screen.

INTRODUCTION

The causative agent of East Coast fever (ECF) in cattle is *Theileria parva*, a protozoan parasite that utilizes the brown ear tick, *Rhipicephalus appendiculatus* as its transmitting host. *T. parva* is an intracellular parasite that infects and transforms bovine lymphocytes causing ECF in eastern, central and southern Africa, causing major economic loss through lowering of cattle production and costs incurred in controlling the disease. Immune mechanisms deployed by cattle against *T. parva* have been well characterized (McKeever and Morrison, 1994; Morrison, 1986), and Katzer et al. (2007) has examined the influence of host immunity on the genotypic diversity of the parasite. Efforts are underway to develop a second generation of vaccines that would be safe, cheap and easy to deliver. Recently, progress has been made towards the development of a subunit vaccine whose efficacy lies in targeting bovine cytotoxic T lymphocyte (CTL) that lyse parasite infected lymphocytes (Graham et

al., 2006). A fundamental task required in this process has been the identification of parasite antigens that provoke T cell responses necessary for effective protection. The main approach taken in identifying these antigens has utilised the screening of parasite antigens from a *T. parva* complementary DNA (cDNA) expression library and genes selected from *T. parva* genome sequence data with *T. parva* specific CTL from integral transmembrane protein (ITM) immunised cattle.

In order to identify schizont antigens, a predictive software was applied to identify secreted (SignalP-2.0; <http://www.cbs.dtu.dk/services/SignalP-2.0/>) or membrane bound (TMHMM; <http://www.cbs.dtu.dk/services/TMHMM>) proteins of *T. parva* that would access the host cytoplasm and therefore the major histocompatibility complex (MHC) class I processing and presentation pathway. A second approach employed an arbitrary immunoscreen of approximately 50,000 clones from a cDNA library enriched for schizont sequences. These methods yielded eight CD8⁺ CTL target antigens, which were tested under laboratory conditions for immunogenicity and protective efficacy (Graham et al., 2006). Another complimentary approach to antigen identification would be to screen proteins with related amino acid sequence from related apicomplexan parasites that are known to be antigenic; this process would be referred to as a homolog screen.

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Abbreviations: ECF, East coast fever; RESA, ring-infected erythrocyte surface antigen; CTL, cytotoxic T lymphocyte; ITM, integral transmembrane protein; MHC, major histocompatibility complex; BLAST, basic local alignment search tool; PCR, polymerase chain reaction.

This study aims to determine whether *T. parva* homologs of known apicomplexan antigens are recognized by immune T cells. This will have an immediate effect in identifying genes products that are vaccine candidate antigens and may eventually contribute to the development of a subunit vaccine for ECF and other diseases. Here, we isolate and characterize the ring-infected erythrocyte surface antigen (RESA), a vaccine candidate for malaria caused by *Plasmodium falciparum* (Duarte et al., 2002).

MATERIALS AND METHODS

Identification of antigen's homolog

The homologs of apicomplexan antigens were selected from the *T. parva* genome sequence database (<http://www.tigr.org/tdb/e2k1/tpa1/>), in the following manner:

- (1) Apicomplexan gene products that have been described as antigenic in literature were selected for analysis.
- (2) The selected gene sequences were used to perform a BLAST search (Altschul et al., 1990; Altschul et al., 1997) of the *T. parva* genome database to identify homologous genes.
- (3) Those identified in the *T. parva* database were translated to their amino acid sequences and analyzed using SignalP-2.0 and TMHMM software for the presence of signal peptides and transmembrane domains. This would identify secreted proteins or proteins located on the surface of the parasite.
- (4) The selected *T. parva* sequences were then confirmed in the GenBank database as:

- a) *T. parva* DNA sequences subjected to BLASTn to seek identical DNA sequences and BLASTx on non redundant databases that compare amino acid sequences to confirm the homologous apicomplexan antigens originally selected in 1.
- b) *T. parva* translated amino acid sequences subjected to BLASTp to also confirm the homologous apicomplexan antigens originally identified in 1. Further analysis was carried out on identified homologs e.g. predicted isoelectric point, protein motifs, etc.

Polymerase chain reaction (PCR)

For the cloning of the RESA homolog, PCR was performed in a thermocycler (MJ Research, Watertown, MA) using Taq DNA polymerase (Promega) and two primers based on the sequences identified in *T. parva* using the signal peptide analysis described above. The PCR product generated above was cloned into pGEM T-easy vector (Promega, Madison, WI). PCR was performed under the following conditions: initial denaturation at 94°C for 3 min, 35 cycles of denaturation, 94°C for 1 min; annealing at 55°C for 1 min and polymerization at 72°C for 2 min. A final round of polymerization at 72°C for 10 min was performed at the end of the 35 cycle. DNA nucleotide sequences were determined gel based sequencing at the International Livestock Research Institute sequencing unit in Nairobi.

RESULTS AND DISCUSSION

The RESA homolog along with its DnaJ domain is found on chromosome 9 in the *T. parva* genome. The full-length

cDNA of *T. parva* RESA contains an open reading frame encoding a predicted protein product of 569 amino acids and has 38% identity with the RESA2 protein from *P. falciparum* (Figure 1). The similarity between *T. parva* RESA protein and its homolog can be seen across the entire protein including both the DnaJ domain of approximately 70 amino acids and the C terminal region. East Coast fever (ECF) is a highly fatal lymphoproliferative disease of cattle caused by *T. parva*, a tick-borne intracellular apicomplexan parasite. Parasite antigens that are targets of protective cytotoxic T lymphocyte (CTL) responses are required to formulate a sub-unit vaccine against ECF. A number of CTL target antigens have recently been identified and initial evaluation has shown their vaccine potential (Akoolo et al., 2008). RESA is a vaccine candidate for malaria caused by *P. falciparum* (Duarte et al., 2002). RESA-specific antibodies efficiently inhibit *in vitro* parasite growth and immunization protects Autos monkeys against challenge (Collins et al., 1986). The RESA protein in *P. falciparum* has been classified in a group of heat shock proteins that contain a DnaJ domain. Dna J was originally identified in *Escherichia coli* as a molecule which, forming an operon with major heat shock protein Dna K (HSP 70 in eukaryotes), regulates its ATPase activity (Saito and Uchida, 1978). Since then multiple proteins have been reported to have homology to Dna J with a conserved J domain with 70 amino acids as a common denominator. Functions of these proteins include protein folding, the intracellular transport of proteins and the initiation of translation of proteins.

In *P. falciparum*, RESA and erythrocyte membrane protein 3 (PfEMP3) are associated with the red blood cell (RBC) cytoskeleton underlying the plasma membrane (Culvenors et al., 1991; Pasloske et al., 1993; Kyes et al., 1999). RESA is produced in the final stages of schizont development and is stored in apical organelles when the emerging merozoites are formed. After RBC invasion, the protein is released into the newly formed parasitophorous vacuole and is finally exported into the host cell, where it appears to associate with the red cell membrane skeleton (Foley et al., 1991; Rug et al., 2004). RESA appears to bind spectrin and stabilize the host cytoskeleton (Mills et al., 2007; Pei et al., 2007). Mills et al. (2007) also observed that RESA plays a major role in reducing deformability of host cells at the early ring stage of parasite development, but not at more advanced stage. They also showed that the effect of RESA on deformability is more pronounced at febrile temperature, which ring-stage parasite-harboring RBCs can be exposed to during a malaria attack, than at normal body temperature. Ravindran and Boothroyd (2008) have written a recent review on the process of secretion and trafficking of parasite proteins into the host cell for *Theileria*, *Toxoplasma* and *Plasmodium*.

The development of a subunit vaccine requires the identification of parasite antigens that are targeted by the

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T.parva      1  MSECDTMEILASAELELEMAHSVSPPTISCFVLVSNIDSVDTPLRLSNRNVKSLTDF
P.falciparum 1  -----MDEPNVSNSSNFGEASYDNNQIVPKYNN-----Y

T.parva      61  ESISSPLISALFSTKNPKDVFSVSSGSIKSIKGVVIGAGLVLICPVVGLSNDCVKG
P.falciparum 33  TEKEEKNLISNIFSTRPKHAGACLVSGIKSVTKGIIVGTFLFLISPYLCAKAEGLNG

T.parva      121  FKGVGARMLAATNPLAGIGVSLYQVPRGVINTPHAICOKASGKVVNKRDRVWEENWYSL
P.falciparum 93  FKGFFGLSATIIFVVSIGVASYQIGRGIMNTPESITOKALCKLWDDKREWYDFYNI

T.parva      181  DEEYRQIKHYITDSSNTENFTLGNRVNNEEGEGK---KVFETELYDLEQVPTNASQEC
P.falciparum 153  DEEANEILNITDKNK--YLRKDTNDEYYNKNNGNIKVKNDLFRLLKVPDASQNE

T.parva      237  IRRSYRRLALKVHPDKNNADGSDYNEIFSRIGEAYQLGDEHRRKKYDAHGRSAIDEM
P.falciparum 211  IKROYKLAKEHPDKCS---DLKAKCFQKIGEAYQLGDEVERRRYDKEGKNAINNM

T.parva      297  PIVSLILFFSMIFGFEALPPLTGKLRMAYVLEMRDLSKTCDFYKLCQVREVQIAHY
P.falciparum 267  QFTDSTFFFTLFGSEKLDPLYTGKLRMVVYVEYEQLYKDEDVORLIIRKQNKREVQIAH

T.parva      357  LREYRSFVCEHDFRKKVIDHVKELCKNSFSVAVVEFLGWTYLYNYKEYIGKSSFLG
P.falciparum 327  LRDMMNNYIFCPDDYIIFKFSQIKELQTSFGHILLENVAWSYENCANQFTIGEVYSLFG

T.parva      417  ISGVAKTKHKTIRNFRKYFKTYVCFIKTAILESGHNETCDADLPLISDVCVN--YNEKSI
P.falciparum 387  ISGYKMQQKRFVIGTGFKFKVKTLLKSSLANQIRKEDDDDISYEKTKVNVKKEIDSL

T.parva      475  EVIDLAMLNCLIDIQNTVRAACKRLLKDMSSWBLRRAALAEIGKIFLQVAKDFRP
P.falciparum 447  ETIVETMLNCLIDIDQTIKGVCKKVFVDMGVDENMRKTRAEITLILAKIMKKIYDFRK

T.parva      535  QFSAEATNDTRQRMNMQORDNERNRNVHYSNEPFY
P.falciparum 507  NTEIMDKKLPEDACMYNIKIRNF-----

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Figure 1. An amino acid sequence alignment showing the similarities between RESA2 in *P. falciparum* and its homolog in *T. parva*.

host immune system. Recent advances in the development of a subunit vaccine for *T. parva* have focused on antigens recognized by CD8⁺ CTLs and have led to the identification of vaccine candidate antigens using an approach that incorporates using bioinformatics tools to select genes and a mass screen of a schizont cDNA library. This approach has been successful leading to the identification of several CTL target antigens. However, for some CTL reagents this approach failed to identify target antigens. Other methods of identifying these antigens are therefore important to explore. The idea of using homologs in the selection of vaccine candidates lies on the basis that two proteins similar in structure perform

comparable functions. These similarities increase the likelihood that if a parasite antigen confers protection, its homolog may have a similar role to play in closely related parasite. Though selection of homologs using comparative function may not be a very sensitive method, it is important in the identification of novel proteins whose functions and characteristics are not well understood.

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