International Scholars Journals

International Journal of Animal Breeding and Genetics Vol. 6 (10), pp. 001-004, October, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

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

Nyerhovwo J. Tonukari[1]* and Richard T. Kangethe[2]

[1]Department of Biochemistry, Delta State University, P. M. B. 1, Abraka, Delta State, Nigeria.
[2]Department of Biochemistry, University of Nairobi, P. O. Box 30197, 00100, Nairobi, Kenya.

Accepted 02 September, 2017

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 apendiculatus* 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.

*Corresponding author. E-mail: tonukari@gmail.com.

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

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.

REFERENCES

Akoolo L, Pellé R, Saya R, Awino E, Nyanjui J, Taracha EL, Kanyari P, Mwangi DM, Graham SP (2007). Evaluation of the recognition of *Theileria parva* vaccine candidate antigens by cytotoxic T lympho-

cytes from Zebu cattle. Vet. Immunol. Immunopathol, 121(3-4): 216-221.

Collins WE, Pye D, Crewther PE, Vandenberg KL, Galland GG, Sulzer AJ, Kemp D J, Edwards SJ, Coppel RL, Sullivan JS, Morris CL, Anders RF, (1994). Protective immunity induced in squirrel monkeys with recombinant apical membrane antigen-1 of Plasmodium fragile. Am. J. Trop. Med. Hyg., 51 (6) : 711-719.

Culvenor JG, Day KP, Anders RF (1991). *Plasmodium falciparum* ring infected erythrocyte surface antigen is released from merozoite dense granules after erythrocyte invasion. Infect. Immun. 59 : 1183-1187.

Duarte EC, Gyorkos TW, Pang L, Avila S, Fontes CJ (2002). Inter-test reliability of the anti-RESA indices based on ELISA tests using eluates from whole blood spots dried on filter paper. Epidemiol. Infect. 129 (1): 139-145.

Foley M, Tilley L, Sawyer WH, Anders RF (1991). The ring-infected erythrocyte surface antigen of *Plasmodium falciparum* associates with spectrin in the erythrocyte membrane. Mol. Biochem. Parasitol., 46: 137-147.

Graham SP, Pella R, Honda Y, Mwangi DM, Tonukari NJ, Yamage M, Glew EJ, de Villiers EP, Shah T, Bishop R, Abuya E, Awino E, Gachanja J, Luyai AE, Mbwika F, Muthiani AM, Ndegwa DM, Njahira M, Nyanjui JK, Onono FO, Osaso J, Say RM, Wildmann C, Fraser CM, Maudlin I, Gardner MJ, Morzaria SP, Loosmore S, Gilbert SC, Audonnet JC, van der Bruggen P, Nene V, Taracha ELN (2006). *Theileria parva* candidate vaccine antigens recognized by immune bovine cytotoxic T lymphocytes. Proc. Natl. Acad. Sci. USA., 103 (9): 3286-3291.

Katzer F, Ngugi D, Schnier C, Walker AR, McKeever DJ (2007). Influence of host immunity on parasite diversity in *Theileria parva*. Infect. Immun., 75 (10) 4909–4916.

Kyes SA, Rowe JA, Kriek N, Newbold CI (1999). Rifins: a second family of clonally variant proteins expressed on the surface of red cells infected with *Plasmodium falciparum*. Proc. Natl. Acad. Sci. USA. 96: 9333–9338.

McKeever DJ, Morrison WI (1994). Immunity to a parasite that transforms T lymphocytes. Curr. Opin. Immunol., 6, 564–567.

Mills JP, Diez-Silva M, Quinn DJ, Dao M, Lang MJ, Tan KS, Lim CT, Milon G, David PH, Mercereau-Puijalon O, Bonnefoy S, Suresh S (2007). Effect of plasmodial RESA protein on deformability of human red blood cells harboring *Plasmodium falciparum*. Proc. Natl. Acad. Sci. USA. 104: 9213–9217.

Morrison WI, Goddeeris BM, Teale AJ, Baldwin CL, Bensaid A, Ellis J (1986). Cell-mediated immune responses of cattle to *Theileria parva*. Immunol. Today. 7: 211-216.

Pasloske BL, Baruch DI, van Schravendijk MR, Handunnetti SM, Aikawa M Fujioka H, Taraschi TF, Gormley JA, Howard RJ (1993). Cloning and characterization of a *Plasmodium falciparum* gene encoding a novel high-molecular weight host membrane-associated protein, PfEMP3. Mol. Biochem. Parasitol., 59: 59-72.

Pei X, Guo X, Coppel R, Bhattacharjee S, Haldar K, Gratzer W, Mohandas N, An X, (2007). The ring-infected erythrocyte surface antigen (RESA) of *Plasmodium falciparum* stabilizes spectrin tetramers and suppresses further invasion. Blood 110, 1036–1042.

Ravindran S, Boothroyd JC (2008). Secretion of proteins into host cells by Apicomplexan parasites. Traffic, 9 (5), 647 – 656.

Rug M, Wickham ME, Foley M, Cowman AF, Tilley L (2004). Correct promoter control is needed for trafficking of the ring-infected erythrocyte surface antigen to the host cytosol in transfected malaria parasites. Infect. Immun. 72: 6095–6105.

Saito H, Uchida H (1978). Organization and expression of the dnaJ and dnaK genes of *Escherichia coli* K12. Mol. Gen. Genet., 4, 164 (1), 1–8.