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Prediction of MHC-I binding epitopes in a gene encoding paraflagellar rod protein 1 (PFR1) of *Trypanosoma evansi* Izatnagar isolate

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Summary: In this study, *in silico* prediction of nanomeric epitopes of paraflagellar rod protein 1 (PFR1) of *Trypanosoma evansi* Izatnagar isolate was explored, and analyzed for CD8+ T cell binding ability based on their predicted peptide scores for their respective MHC-I specific alleles

Key words: *Trypanosoma evansi*, paraflagellar rod protein 1, MHC-I.

Introduction

Trypanosoma evansi is a hemoprotozoa that occurs in vertebrates, principally in their blood and tissue fluids. In the Indian sub –continent, trypanosomosis is more popularly known as surra, which is a typical hemolytic disease of a wide range of warm-blooded animals. The disease is a serious constraint for both livestock agriculture and human health. The zoonotic potential of *T. evansi* has been largely presumptuous and overlooked. The report of two clinical cases of *T. evansi* infection in human beings in Maharashtra and West Bengal added a new dimension to the epidemiology of *T. evansi* infection (OIE, 2006). This parasitic disease causes huge economic losses to the livestock industry (Reid, 2002). The disease has been contained mainly through chemotherapy but increasing drug resistance challenges the application of drug based control strategy. The development of vaccines against trypanosomosis based on variant surface glycoproteins was abandoned (Donelson *et al*., 1998) due to variation. Vaccine development against animal trypanosomosis based on variant surface glycoprotein no longer holds any promise (Donelson et al., 1998). This has prompted researchers to investigate the alternative novel invariant proteins like paraflagellar rod proteins present in the kinetoplastid flagellum. This is a unique structure of trypanosoma flagellum due to presence of this paracrystaline structure (PFR). One of the most unique structural features of the trypanosome flagellum is the presence of a large para-crystalline filament, the paraflagellar rod (PFR), which extends alongside the axoneme from the flagellar pocket to the flagellum tip. Unlike the axoneme, which is broadly conserved among the eukaryotes, the PFR is restricted to kinetoplastids, euglenoids

and dinoflagellates. PFR is vital for trypanosome motility (Bastin *et al*., 1998) and is unique among the kinetoplastids as their heteropolymers provide the building block of flagellum (Abdille *et al*., 2008). This PFR is an elegant and stable lattice-like arrangement of protein filaments which is composed of two major and related proteins PFR1 and PFR2. Taking into consideration of the high sequence homology between the PFR proteins of trypanosomatids (Maga *et al.*, 1999), we hypothesize that PFR1 gene could be highly conserved in *Trypanosoma* species and could be used as cross reactive candidate. Targeting the *Trypanosoma evansi* using subunit vaccine with PFR1 by assembling their minimal CD8+ and CD4+ T cell epitopes might be effective in inducing immune responses. Recently the complete sequence of *Trypanosoma evansi* PFR1 gene has been submitted to the gene bank (accession number: FJ 968743). The present study aimed towards the prediction of CD8+ antigenic peptides (9- mers) from the PFR1 protein of Izatnagar isolate of *Trypanosoma evansi* (horse strain) that binds to class I MHC CD8+ molecules. This prediction will be useful in designing new peptide molecules for antigen-based vaccine design.

Materials and Methods

The hypothetical protein of *Trypanosoma evansi* PFR1, GenBank accession FJ 968743 (589 amino acid) was used in this study. The bioinformatics tool, ProPred1 (Singh and Raghava, 2003) was used to predict MHC class I binding peptides (Cytotoxic T-Lymphocytes CTL epitopes) for 47 alleles by adjusting the threshold score of 4% in presence of Proteosome and Immuno Proteosome Filter.

Results and Discussion

Based on the translated protein information, a three dimensional computer simulated model of PFR1 protein was synthesized with the help of online available LOOPP software (with the aid of Signal P software, available online). The computer simulated recombinant PFR1 three dimensional model has a single domain. The protein conformation consisted of beta sheets with intermittent alpha chains (Fig 1&2). The template is monomer in nature. Predicted PFR1 protein of *T.evansi* possesses molecular weight of 68682.4Da. In this study, *in silico* prediction of nanomeric epitopes of *Trypanosoma evansi* PFR1 was explored, and analyzed for CD8+ T cell binding ability based on their predicted peptide scores for their respective MHC-I specific alleles. All possible overlapping 9-mer peptides were generated for a given antigen sequence. The scores of this 9-mer peptide were calculated using quantitative matrix of selected MHC alleles. All peptides having score greater than selected threshold score (4%) were assigned as predicted binders for selected MHC-I alleles as indicated by others (Bhasin, 2003; Somvanshi P *et al.*, 2008). However among 47 alleles screened, top 26 antigenic CTL epitopes (25 for Human and 1 for Cattle MHC-I) has been selected, which were showing score > 50 (table .1). Out of 26 CTL epitopes were predicted, the peptides, HLA-B*2705 (6000), HLA-A20 (cattle) (4000), HLA-A2 (1242.014), HLA-B*2702 (600), HLA-B*8102 (660) are the top five scorers, with maximum MHC-I binding ability. From the data obtained, it is clear that only HLA-B*2705 allele in human beings has maximum MHC-I binding ability for *Trypanosoma evansi* PFR1 rest alleles have weak MHC-I binding ability. In bovines, HLA-A20 has highest MHC-I binding

ability. So, the highest scorers can be used to accelerate research into the design of vaccines and diagnostic tests by exploiting genome sequences. Moreover, the predicted nanomeric epitopes can be evaluated *in vivo* and confirmed further *in vitro* for their binding ability to MHC-I in inducing strong immune responses and to be used as potent vaccine candidates in future.

Table 1. The predicted MHC-I epitopes using the ProPred1 for *Trypanosoma evansi* PFR1

Alleles	Predicted MHC-I epitopes sequence	Amino acid position	Score	Predicted binder /non binder
HLA-A1	RCETDLKHI	274	225.000	Predicted binder
HLA-A2	TKVQLQERL	218	1242.014	Predicted binder
HLA-A*0201	KIQDLERQL	324	232.745	Predicted binder
HLA-A*0205	KIQDLERQL	324	75.600	Predicted binder
HLA-A24	YFRMLYLTL	427	90.000	Predicted binder
HLA-A3	HLKAEELVA	53	60.000	Predicted binder
HLA-A*3302	TSQDLAALR	409	45.000	Predicted binder
HLA-A68.1	EVASQHKKL	365	180.000	Predicted binder
HLA-A20 cattle	**TKVQLQERL**	**218**	**4000.000**	**Predicted binder**
HLA-A2.1	WNLTEVYDL	69	126.900	Predicted binder
HLA-B14	LRQGVEEEL	484	300.000	Predicted binder
HLA-B*2702	LRQGVEEEL	484	600.000	Predicted binder
HLA-B*2705	LRQGVEEEL	484	6000.000	Predicted binder
HLA-B*3701	VDDATGLEA	3	60.000	Predicted binder
HLA-B*3801	RHDKTSQDL	405	90.000	Predicted binder
HLA-B*3901	RHDKTSQDL	405	360.000	Predicted binder
HLA-B40	KESEEALDA	507	80.000	Predicted binder
HLA-B*4403	AEREEIKRA	555	240.000	Predicted binder
HLA-B*5101	AAVDDATGL	1	346.060	Predicted binder
HLA-B*5102	AAVDDATGL	1	660.000	Predicted binder
HLA-B*5103	GAHLKAEEL	51	132.000	Predicted binder
HLA-B*5301	WNLTEVYDL	69	122.630	Predicted binder
HLA-B*5401	YFRMLYLTL	427	178.300	Predicted binder
HLA-B*51	YFRMLYLTL	427	132.650	Predicted binder
HLA-B*5801	KTSQDLAAL	408	198.000	Predicted binder

HLA-B60	WNLTEVYDL	69	352.000	Predicted binder

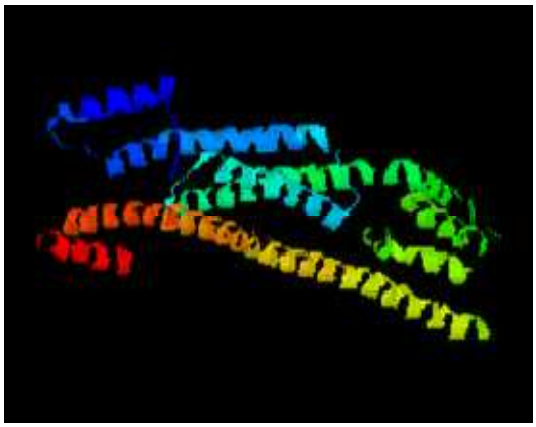

Fig1: 3D ribbon model of PFR1 protein

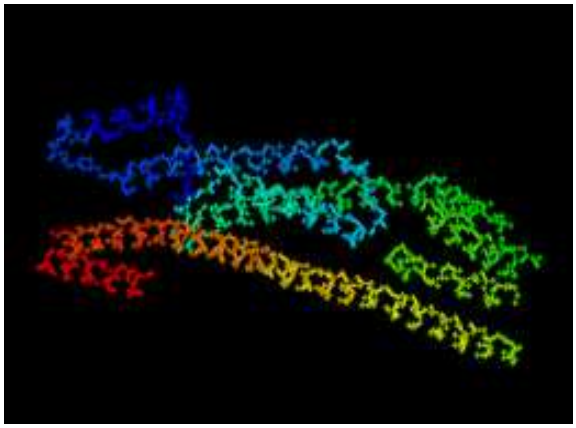

Fig **2: 3D ball stick model of PFR1 protein**

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