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Short communication

BoLA-6*01301 and BoLA-6*01302, two allelic variants of the A18 haplotype, present the same epitope from the Tp1 antigen of *Theileria parva*

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ABSTRACT

We have recently shown that the BoLA-A18 variant haplotype (BoLA-6*01302) is more prevalent than the BoLA-A18 haplotype (BoLA-6*01301) in a sample of Holstein/Friesian cattle in Kenya. These MHC class I allelic variants differ by a single amino acid polymorphism (Glu97 to Leu97) in the peptide-binding groove. We have previously mapped an 11-mer peptide epitope from the *Theileria parva* antigen Tp1 ($Tp1_{214–224}$) that is presented by BoLA-6*01301. Crystal structure data indicates that Glu97 in the MHC molecule plays a role in epitope binding through electro-static interaction with a lysine residue in position 5 of the epitope, which also functions as an additional anchor residue. In contrast to expectations, we demonstrate that the amino acid substitution in BoLA-6*01302 does not divert the CTL response away from $Tp1_{214–224}$. The two MHC molecules exhibit similar affinity for the Tp1 epitope and can present the epitope to parasite-specific CTLs derived from either BoLA allelic variants. These data confirm that this BoLA polymorphism does not alter Tp1 epitope specificity and that both allelic variants can be used for Tp1 vaccine studies.

1. Introduction

Theileria parva, a protozoan parasite that causes the disease East Coast fever (ECF) in cattle is responsible for major economic losses in eastern, central and southern Africa (De Leeuw et al., 1995; Mukhebi, 1992). CD8 T cells that are primed by a live *T. parva* infection and treatment method (ITM) are believed to mediate protection to disease and several *T. parva* CD8 T-cell epitopes recognized by immune CTL have been identified (McKeever et al., 1994; Morrison et al., 1995). Unlike in malaria, the specificity of the CTL response to this vaccine in individual cattle is skewed to a few epitopes and most likely contributes to the observation of strain specific immunity in ECF (MacHugh et al., 2009). Vaccination with a cocktail of *T. parva* parasite isolates provides broad-spectrum immunity to ECF (Di Giulio et al., 2009). Armed with this information, our research is focused on the identification and development of a subunit vaccine for the control of ECF.

It has been shown that ITM vaccination of Holstein/Friesian cattle homozygous for the BoLA-A18 haplotype with the Muguga isolate of *T. parva* results in approximately 70% of the CTL being directed to a single epitope on an antigen called Tp1 (MacHugh et al., 2009). The epitope maps to residues 214–224 ($Tp1_{214–224}$) and is presented by BoLA-6*01301 (Graham et al., 2006, 2008). A recombinant version of this MHC class I molecule has been crystalized together with the Tp1 peptide epitope, VGYPKVKEEML (Macdonald et al., 2010). According to this model the 11-mer peptide binds BoLA-6*01301 in a raised conformation and Glu97, an amino acid residue located in the BoLA peptide binding groove bonds with lysine at position 5 (P5) in the Tp1 peptide. An alanine substitution scan of the Tp1 epitope sequence revealed the C-terminus and the P5 position as anchor residues for binding to the MHC molecule. The BoLA-6*01301 molecule also binds nonameric peptides and an alanine scan of a high affinity self-peptide, TIMPKDIQL, revealed position P2 as an additional anchor residue to P5 and the C-terminus. This peptide binding groove appears to be quite flexible as a truncated 10-mer Tp1 peptide, GYPKVKEEML, also binds to BoLA-6*01301 but it is not recognized by Tp1-specific CTL suggesting the 10-mer peptide adopts a different conformation to the 11-mer (Macdonald et al., 2010).

In a recent survey of more than 200 Holstein/Friesian cattle in Kenya we found an A18 haplotype with a variant of the BoLA-6*01301 allele, BoLA-6*01302, to be more prevalent than BoLA-6*01301 (Svitek et al., 2015) and more recent BoLA-A18

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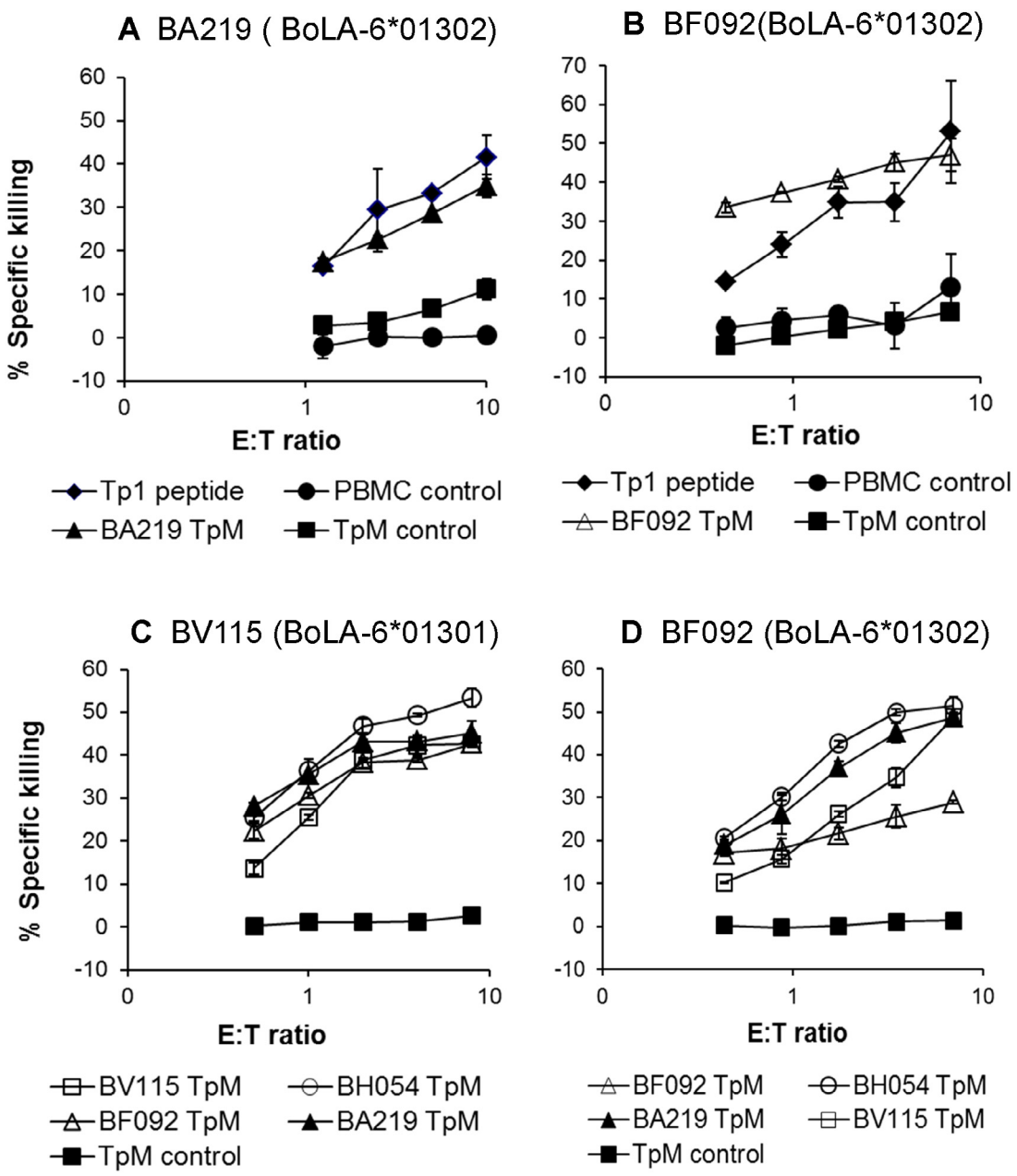

Fig. 1. Characterization of CTL recognizing $Tp1_{214–224}$ in cattle of BoLA-6*01302 and BoLA 6*01301 type. CTL from BA219 (A), BF092 (B and D), BV115 (C) were tested for lysis of $Tp1_{214–224}$ pulsed PBMC and of *T. parva* (Muguga) infected cell lines (TpM). (A and B): CTL from BA219 and BF092 (BoLA-6*01302) were tested for lysis of autologous PBMC pulsed with 1 μM Tp1 peptide, PBMC alone, autologous TpM and control TpM. (C and D): BV115 CTL (BoLA 6*01301) and BF092 CTL (BoLA 6*01302) demonstrate that the CTL can kill *T. parva* infected target cells expressing either BoLA-6*01301 (BV115 TpM and BH054 TpM) or BoLA-6*01302 allele (BF092 and BA219). The experiments were performed twice with similar result.

typing indicate that the BoLA-6*01302 is more than 90% prevalent compared to the BoLA-6*01301 allele (data not shown). There are two single nucleotide differences between these alleles but only one translates into an amino acid substitution, Glu → Leu at position 97 (Immuno Polymorphism Database (part: IPD-MHC): http://www.ebi.ac.uk/ipd/mhc/bola/align.htm). This is a change from a negatively charged amino acid to a hydrophobic one raising concerns that this polymorphism could lead to a change in the primary CTL specificity in BoLA-6*01302 cattle vaccinated by ITM.

As part of a newly established East Coast fever consortium, it is planned to use the Tp1 antigen as a model antigen for generating CTL in cattle with various delivery systems and adjuvants. Because the BoLA-6*01302 allele is much more prevalent in European cattle in Kenya and possibly also in other African countries, it is very difficult to source sufficient numbers of animals of the BoLA-6*01301 type for experiment. Hence, we decided to elucidate if BoLA-6*01302 animals also generated CTL toward the Tp1 epitope and if there were any major differences in peptide–BoLA binding assays.

2. Material and methods

2.1. Cell lines

Cell lines infected with *T. parva* were established by infection of PBMC *in vitro* with sporozoites as described previously (Goddeeris and Morrison, 1988) Cryopreserved sporozoites stabilates of the *T. parva* Muguga 3308 were used for the production of infected cell