Discovery of novel CTL epitopes by peptide library screening of CTL lines from *Theileria parva* immune animals

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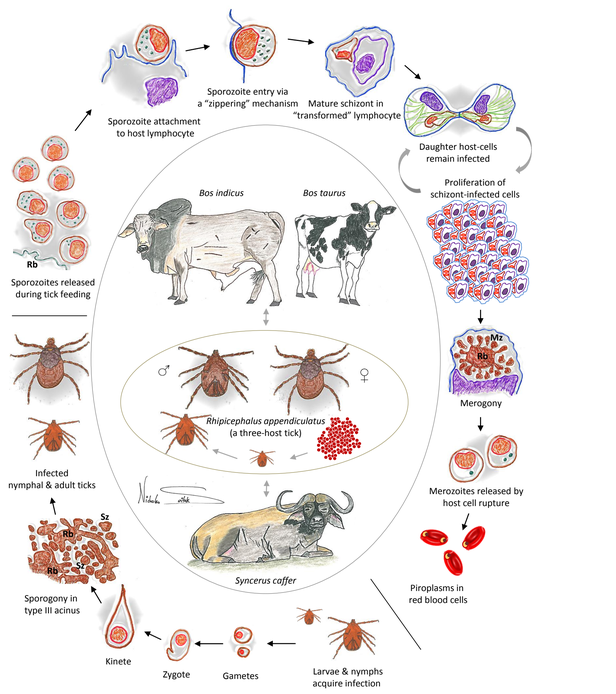
LIFE CYCLE OF *T. PARVA*

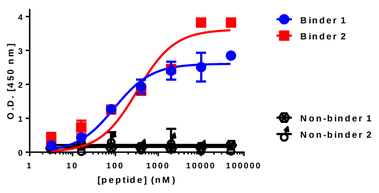


1 The figure illustrates the different life cycle stages of the parasite as it cycles through the mammalian and tick host.

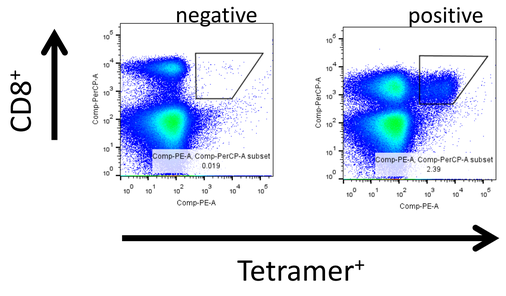
SUMMARY & PERSPECTIVE

- 4 new epitopes under identification;
- Restricting BoLA class I molecule need to be identified;
- Minimal epitope sequence will be confirmed by ELISpot, cytotoxicity assay as well as by:

A) BoLA class I monomer binding assay



B) peptide-BoLA class I tetramer staining of positive cells



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PARTNERS

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DTU Technical University of Denmark
ILRI INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

SELECTION OF PEPTIDES

Theileria parva$_{Muguga}$ → 4 chromosomes ≈ 4,000 genes →

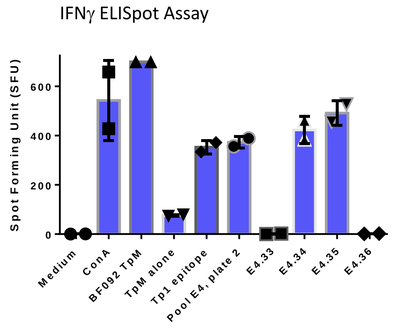
- Abundance
- Signal peptide
- Divergence to *T. annulata*
- Promiscuity to BoLA class I molecules (NetMHCpan)

2 **Selection of 500 genes ≅ 40,000 peptides**

ABSTRACT

The parasite *Theileria parva* claims the life of approximately 1 million cattle every year. Immune animals to the parasite develop a lifelong immunity based on a cytotoxic T lymphocyte (CTL) response with a strong immunodominance restricted by the bovine leukocyte antigen (BoLA) class I molecules. In our goal of developing a next-generation vaccine against *T. parva*, we have undertaken to identify new CTL inducing antigens that can be included in a recombinant vaccine. A peptide library of 18-mer peptides overlapping by 12 amino acids and covering 500 genes of the whole parasite genome was synthesized; giving approximately 40,000 peptides aliquoted in pools of 50 peptides. Genes were selected based on the presence of a signal peptide, abundance, and divergence to the related parasite *T. annulata*, as well as by a selection using immunoinformatic tools predicting, by artificial neural network, peptides binding with strong affinity to the BoLA class I molecules present in cattle of Africa. A bank of ten CTL lines from cattle immunized with the live parasite expressing different BoLA class I specificities were screened by IFN-γ ELISpot assay. Among these cells, four secreted IFN-γ in the presence of a different peptide library pool. Peptides from these pools were synthesized and positive individual 18-mer peptides were identified. Dissection of the minimal epitope is underway using IFN-γ ELISpot, cytotoxicity as well as peptide-BoLA class I flow cytometry assays. These newly identified antigens will hopefully allow us to develop a vaccine towards *T. parva* giving wide coverage in cattle population with diverse BoLA class I molecules.

POSITIVE PEPTIDES IDENTIFIED WITH BF092 CTL



Positive peptides (TpMuguga_01g01091 [Tp12]):

DTLDSN**REQLLNQFYQLF**G (E4.34)
REQLLNQFYQLFNTPVDQG (E4.35)

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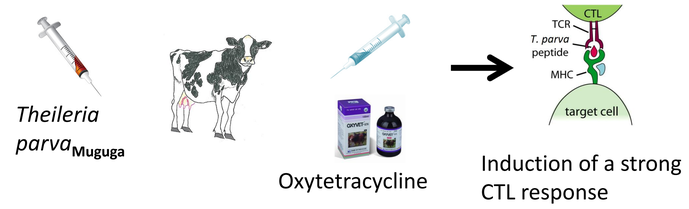
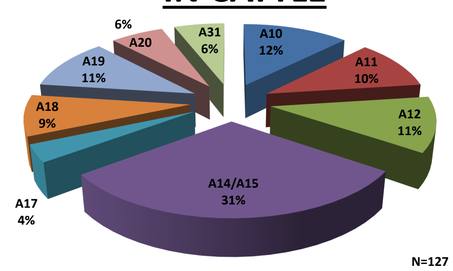
INFECTION & TREATMENT METHOD (ITM) OF VACCINATION



3 Induction of a strong CTL response

MHC CLASS I HAPLOTYPE FREQUENCIES IN CATTLE



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CTL LINES FROM *T. PARVA*$_{Muguga}$ IMMUNIZED ANIMALS

Cell line	MHC Haplotype	Epitope	Screening
F100 (B)	A10/KN104	N.A.	**Positive**
BX64 (B)	A10/KN104	N.A.	**Positive**
BF091	A18/A11/A19	$Tp1_{214-224}$	Negative
BB007	A18/?	$Tp1_{214-224}$	Negative
BF092	A18/A12	$Tp1_{214-224}$	**Positive**
BK173	A14	Not reacting to Tp9	Negative
BA219	A18	$Tp1_{214-224}$	Negative
BH055	A11/A15v	$Tp5_{214-224}$	Negative
BG042	A10/A12	Not reacting to Tp2	Negative
BW012(B)	T7/?	$Tp7_{206-214}$	**Positive**

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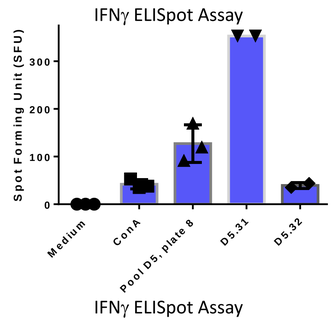
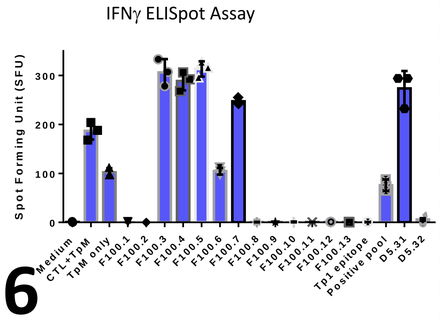
NEW ANTIGEN IDENTIFIED WITH F100 CTL



Positive peptides (TpMuguga_04g00772):

TLTLQG**HRMKVTNLKWNP**G (D5.31)
HRMKVTNLKWNPTVDYALG (lD5.32)



Looking for the minimal epitope:

F100.1	TLTLQGHRMKVT
F100.2	LTLQGHRMKVTN
F100.3	TL**QGHRMKVTNL**
F100.4	L**QGHRMKVTNL**K
F100.5	**QGHRMKVTN**LKW
F100.6	G**HRMKVTNLKWN**
F100.7	**HRMKVTNLKWN**P
F100.8	RMKVTNLKWNPT
F100.9	MKVTNLKWNPTV
F100.10	KVTNLKWNPTVD
F100.11	VTNLKWNPTVDY
F100.12	TNLKWNPTVDYA
F100.13	NLKWNPTVDYAL

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