Reducing the risk of tick-borne diseases – identification of vaccine candidates against *Ixodes ricinus*

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Introduction

Ixodes ricinus is the most important vector of human and animal pathogens in Western Europe causing diseases such as tick-borne encephalitis (TBE), Lyme Borreliosis and babesiosis. In Europe, a vaccine licensed for use in humans is available only against TBE. The development of vaccines against other human tick-borne diseases is complicated by the high diversity of pathogen species. In contrast, vaccination of the primary host (e.g. deer) against *I. ricinus* could significantly reduce tick population size and thereby reduce the incidence of tick borne diseases. A cattle vaccine targeting proteins in the midgut of the tick *Rhipicephalus microplus* was shown to decrease feeding duration, egg numbers and tick population size, consequently reducing the incidence of tick-borne cattle diseases. Our study investigates the applicability of this approach for the identification of vaccine candidates against *I. ricinus*. Antibodies directed against tick midgut proteins are identified and their impact on tick feeding and survival is investigated in a mouse challenge/protection experiment. The genome and midgut transcriptome of *I. ricinus* are sequenced in the framework of a larger project and will be used here as a nucleotide database for peptide mass fingerprinting (PMF).

I. ricinus genome and midgut transcriptome sequencing

Sequences have been generated by next generation sequencing with the Illumina® Hi Seq™ 2000 (Illumina, San Diego, USA) and the IonTorrent™ PGM (life technologies, California, USA) using paired-end and transcriptome sequencing. Resulting sequences are assembled *de-novo* (Table 1). Published sequences from *I. ricinus* and related species are used as references for annotation (Blast2Go, BioBam Bioinformatics S.L., Valencia, Spain).

Table. 1: *De-novo* assembly of next-generation sequencing data from *I. ricinus*

Genome sequencing		Midgut transcriptome sequencing	
Number of sequences from Illumina® HiSeq™ 2000	998,100,906	Number of sequences from IonTorrent™ PGM	30,578,101
Number of *de-novo* assembled contigs (analysis still ongoing)	201,072	Number of *de-novo* assembled contigs	60,693
Size of *de-novo* assembled contigs	1-25 Kb	Number of annotated contigs (analysis still ongoing)	2,404

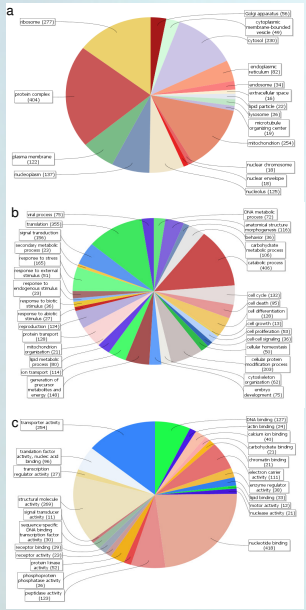

Fig. 1: *I. ricinus* midgut RNA sequence distribution for cellular components (a), biological processes (b) and molecular functions (c)

Conclusions

A first insight into the *I. ricinus* genome and midgut transcriptome has been gained and potential vaccine candidates directed against midgut proteins have been identified. In the light of climatic changes, a further spread of ticks and increase of tick density is predicted. A vaccine capable of reducing population size may have the potential to reduce these effects, thus preventing an increase in tick-borne diseases in human as well as in animals.

Immunization, challenge and proteomic analysis

An immunization study was performed on BALB/c mice (3 mice/group) using midgut extract from *I. ricinus* females (Charles River Laboratories, Ballina, Ireland). Mice were challenged with *I. ricinus* nymphs to observe the impact of immunization on tick survival and feeding behaviour. Putative antibody targets are identified by Western Blot and PMF, using the generated transcriptome information of *I. ricinus* midgut as database. Results were validated by Mascot search (Matrix Science, Boston, USA) on NCBI (National Center for Biotechnology Information, Bethesda, USA) non-redundant protein database.

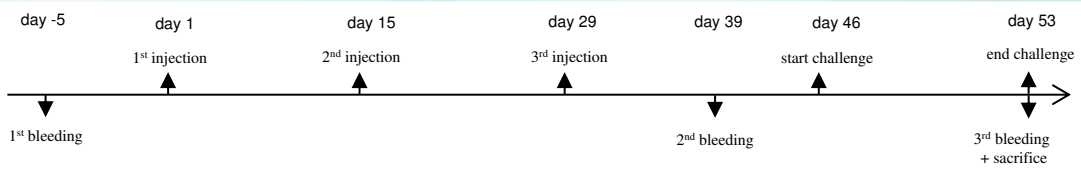

Fig. 2: Shedule of immunization study

Challenge experiment

Feeding success of *I. ricinus* nymphs on immunized and control mice was analysed by challenge. Mice immunized with *I. ricinus* midgut extract showed clearly lower feeding rates than control mice (Fig. 3).

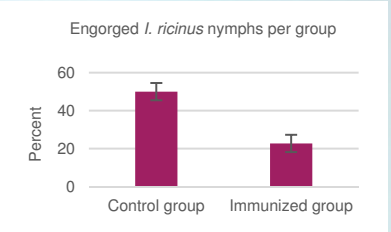

Fig. 3: Feeding success of *I. ricinus* nymphs on immunized and control mice

Identification of *I. ricinus* vaccine candidates

Vaccine candidate proteins have been detected by Western Blots with serum from mice immunized with *I. ricinus* midgut extract. Furthermore, an overlap between proteins detected by serum antibodies from immunized mice and human donors with high tick bite numbers against whole tick extract was observed (Fig. 4). Candidate proteins were identified by PMF and will be analysed further for their protective capacity.

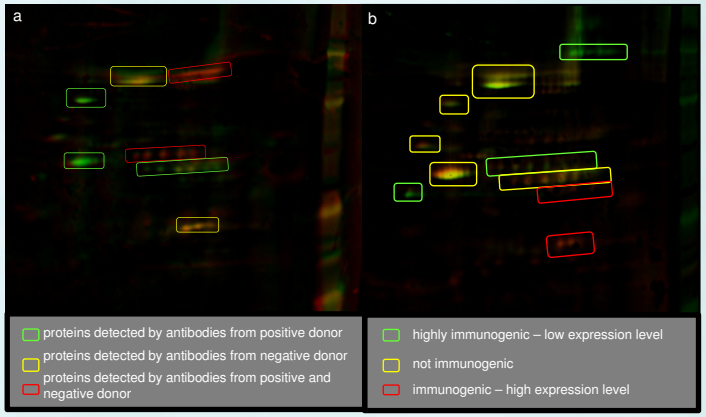

Fig. 4: Overlap between proteins detected by serum antibodies from immunized mice and high risk donors: a) *I. ricinus* whole tick total protein + serum from *Borrelia* positive (green) and negative (red) high risk group donor and b) *I. ricinus* whole tick total protein (red) + serum from immunized mouse (green)

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