

# Cloning, production and characterization of specific allergens causing insect bite hypersensitivity in the horse

Contribution to YSC Davos 2008

Thematic topic: natural science

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*Key words:* IgE, insect bite hypersensitivity, phage surface display

## INTRODUCTION / BACKGROUND

Insect bite hypersensitivity (IBH) defines recurrent, seasonal pruritic dermatitis of the horse mainly caused by bites of midges of the genus *Culicoides* species. IBH occurs with a prevalence of 3-5% in all parts of the world where horses and these insects coexist. However, for so far unknown reasons, the incidence of IBH can be much higher in some horse families and in Icelandic horses born in Iceland and imported into continental Europe as adults. IBH does not occur in Iceland due to the absence of *Culicoides*. Circumstantial evidences indicate that salivary gland proteins of *Culicoides nubeculosus* could be crucial in eliciting IgE responses responsible for the development of IBH.

The aim of our research project is the identification, production and characterization of specific allergens from the salivary glands of *Culicoides nubeculosus* potentially involved in the pathogenesis of IBH.

## MATERIAL & METHODS

### *Identification of Culicoides allergens in salivary gland extracts*

For the identification of *C. nubeculosus* allergens present in salivary glands, salivary glands were dissected from laboratory bred female *C. nubeculosus* midges as described (1). Proteins were separated by SDS-PAGE, blotted onto nitrocellulose membranes and probed with serum of IBH-affected horses to identify IgE-binding proteins.

### *Construction of a C. nubeculosus cDNA library displayed on phage surface*

Total mRNA was extracted from 1400 salivary glands using a commercial kit (Stratagene, La Jolla, USA). cDNA was synthesised according to standard protocols starting from poly(A)<sup>+</sup> mRNA selected from total mRNA (2). In a first step the cDNA was cloned into the Eco R I / Xho I restriction sites of the  $\lambda$ -ZAP II vector (Stratagene), packaged *in vitro*, and titered. The library had a complexity of  $3 \times 10^6$  primary clones and was used to generate a cDNA library displayed on the M13 phage surface as described (3, 4).

### *Library screening and isolation of IgE-binding phage*

The phage surface displayed cDNA library was screened for phage expressing IgE-binding proteins by affinity selection using serum IgE from IBH affected horses immobilized to solid phase. Successive rounds of phage selection and amplification allow selective enrichment of phage displaying gene products with affinity to the ligand and, as a consequence of the physical linkage between genotype and phenotype, also of the genetic information encoding the product integrated into the phage genome (5).

#### *Characterization of inserts and sequencing*

Single *E. coli* colonies obtained by infection with selected phage were grown on liquid culture and used to prepare phagemid DNA. After restriction with Eco R I / Xho I, inserts were analyzed by agarose gel electrophoresis and inserts differing in size directly sequenced. All sequences were submitted to multiple BLAST analyses to identify the gene products.

#### *Expression of recombinant proteins*

cDNAs containing IgE-selected *Culicoides* salivary gland proteins were subcloned into suitable expression vectors, transformed into competent *E. coli* cells and, after sequence verification, used to produce the corresponding recombinant proteins.

#### *Testing of recombinant Culicoides proteins*

The pure recombinant proteins were tested with sera of 30 IBH-affected and 20 healthy control horses to confirm their ability to specifically bind serum IgE.

## RESULTS

SDS-PAGE and Western blot analyses of *C. nubeculosus* salivary gland protein extracts showed a complex pattern of IgE-binding proteins potentially corresponding to allergens involved in the pathogenesis of the disease.

Affinity enrichment of a phage surface displayed cDNA library generated from mRNA of *C. nubeculosus* salivary glands with serum IgE from IBH-affected horses yielded 16 discrete sequences. BLAST analyses showed that most of the cDNAs coded for proteins homologous to salivary gland proteins of *Culicoides sonorensis*. However, most of the sequences are derived from truncated cDNAs. We have expressed and purified five of these truncated proteins and demonstrated that all are able to bind serum IgE from IBH-affected horses.

## CONCLUSION & OUTLOOK

A comparison of the IgE-binding bands detected in salivary gland extract with the number of sequences derived by affinity enrichment of phage isolated for affinity to IgE of serum from IBH affected horses indicates that we were able to clone most of the allergens contained in salivary glands. Furthermore we have demonstrated that seven truncated proteins derived from affinity-selected cDNAs are able to bind serum IgE of affected horses. We will now complete all 16 sequences detected, produce the corresponding recombinant proteins, and test their ability to bind serum IgE. The involvement of the allergens in the pathogenesis of IBH will be investigated *in vitro* assessing their ability to induce mediator release from horse basophils and *in vivo* by their ability to induce type I skin reactions as well as by their ability to induce eczematous reactions. The identification, production, and characterization of specific allergens from salivary glands will allow clarifying the role of insect bites in the induction of IBH and to develop pure reagents suitable to improve diagnosis and treatment of the disease.

## REFERENCES

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