FACULTEIT DIERGENEESKUNDE
accredited by EAEVE

INVITATION

PUBLIC DEFENCE

COMPARATIVE ANALYSIS OF THE IMMUNE RESPONSES ELICITED BY NATIVE VS. RECOMBINANT VACCINES AGAINST GASTROINTESTINAL NEMATODES

Ana González Hernández

5th December 2017

Promotor

Prof. Dr. Peter Geldhof
Faculty of Veterinary Medicine, UGent

Members of the Examination Committee

Prof. Dr. F. Pasmans
Faculty of Veterinary Medicine, UGent
Chairperson of the Examination Committee

Prof. Dr. F. Van Immerseel
Faculty of Veterinary Medicine, UGent

Dr. A. Nisbet
Moredun Research Institute, Midlothian, UK

Prof. Dr. G. Leclercq
Faculty of Medicine and Health Sciences, UGent

Prof. Dr. E. Claerebout
Faculty of Veterinary Medicine, UGent

Dr. B. Devriendt
Faculty of Veterinary Medicine, UGent

UNIVERSITEIT GENT

Curriculum Vitae

Ana González Hernández was born on October 29th 1989 in Salamanca, Spain.

In 2007, she completed her secondary education at the Instituto Lucía de Medrano in Salamanca. In 2012, she finished her studies of biology at the Faculty of Biology in University of Salamanca. During her studies, she carried out two research dissertations on the fields of cancer and neurobiology and was granted a one-year Erasmus scholarship to study at Ghent University.

She started her PhD track in November 2012 at the Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University. Her research was funded by the Special Research Fund of Ghent University.

Ana González Hernández is author and co-author of 4 scientific peer-reviewed publications. She has presented at several national and international conferences and symposia, particularly on the subject of vaccine-induced immunity against gastrointestinal nematodes of cattle.

Where?

The defense will take place on
Tuesday 5th December 2017 at 16:30 hours

Kliniekauditorium A
Faculteit Diergeneeskunde
Universiteit Gent
Salisburylaan 133, 9820 Merelbeke

The defense will be followed by a reception.

Attendance

Please confirm your attendance before 30th November 2017 to:
Isabelle.Despeghel@UGent.be

Summary

O. ostertagi and *C. oncophora* are the most prevalent gastrointestinal (GI) nematodes of cattle in temperate climate regions which pose a major constrain on animal welfare and production. The widespread resistance to the current anthelmintic control methods, urges the development of sustainable alternatives such as vaccines. In that context, our group has developed two protective experimental vaccines against both parasites based on activation associated secreted proteins (ASP) obtained from the excretory/secretory (ES) material of adult worms. Nevertheless, recombinant production of these protective vaccine antigens is an absolute requirement for their commercialization. To date, most of the recombinantly produced vaccine antigens against livestock GI nematodes have unfortunately failed to provide protection when compared to their native counterparts. Elucidating the vaccine-induced immune responses that correlate with protection is of great importance Therefore the overall aim of the present thesis was to unravel the immune responses associated with protection following vaccination with native and recombinant ASP-based vaccines against the gastrointestinal nematodes *O. ostertagi* and *C. oncophora.*

In first instance, the protective potential of a *O. ostertagi* recombinant ASP (pASP) produced in the *Pichia pastoris* expression system in cattle was evaluated. Moreover, the effect of antigen (native (nASP) vs. recombinant) and adjuvant (QuilA vs. $Al(OH)_3$) in the vaccine-induced immune responses was investigated in both cattle and mice. Immunization of cattle with the protective nASP+QuilA vaccine was associated with antigen-induced proliferation of natural killer (NK) cells combined with the induction of a mixed IgG1/IgG2 antibody response. This was also observed in mice following the same vaccination regime. However, replacing QuilA by $Al(OH)_3$ or nASP by pASP significantly decreased the capacity of the vaccines to trigger both NK cell activation and antibody responses and failed to induce protection against a challenge infection. In addition, antibodies raised by the native vaccine were highly specifical and preferentially bind to nASP. Finally, reducing the nASP completely abolished its ability to induce NK cell activation and antibody responses upon vaccination, suggesting an important role of protein conformation on the immunostimulatory activity of nASP.

Similarly, the cellular and humoral mechanisms underlying the vaccine-induced responses by the native (ndd-ASP) and recombinant *C. oncophora* vaccines were compared. Immunization of cattle with the native *C. oncophora* vaccine conferred significant levels of protection after an experimental challenge infection, whereas the recombinant vaccine did not. Moreover, vaccination with ndd-ASP resulted in a higher proliferation of CD4-T cells when compared with animals vaccinated with the recombinant antigen. Although both native and recombinant vaccines induced similar levels of antibodies, animals vaccinated with the native vaccine were able to raise antibodies with greater specificity towards ndd-ASP in comparison with antibodies raised by vaccination with the recombinant vaccine.

In addition, the responses elicited by *O. Ostertagi* and *C. oncophora* antigens (native vs. recombinant) and adjuvant in bovine dendritic cells (DC) were studied. Stimulation of DC with QuilA induced moderate activation reflected in the increased up-regulation of co-stimulatory molecules and the inability to take up DQ-OVA. Stimulation of DC with native but not with recombinant ASP from both parasites elicited the production of IL-12. Due to the presence of LPS in certain native-ASP batches, the question rises whether the induction of IL12 production is due to the action of the native-ASP or due to other components such as LPS.

Finally, a general discussion and future prospects are presented. While this project provided broad information on the vaccine immune responses that might correlate with protection against *O. ostertagi* and *C. oncophora*, many questions remain to be addressed. For instance, the exact mechanism by which native ASP elicit protective immune responses is yet unknown. Additional information on the interactions of native ASP with different immune cells would help not only to understand the immunological requirements for protection, but also provide essential information on crucial epitopes recognized by the immune system. Simultaneously, it will be essential to perform a detailed analysis of the structural differences between native and recombinant ASP.