SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Cellular and Molecular Immunology

has the honor to invite you to the public defense of the PhD thesis of

Francisco MORALES YANEZ

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Development of innovative nanobody-based strategies to improve the diagnosis of human toxocariasis

Promotors:

Prof. dr. Serge Muyldermans
Prof. dr. Katja Polman (ITM)

The defence will take place on

Friday July 5 2019 at 14:00h

in Auditorium D.2.01 at the Campus Humanities, Sciences and Engineering of the Vrije Universiteit Brussel, Pleinlaan 2 - 1050 Elsene, and will be followed by a reception.

Members of the jury:

Prof. dr. ir. Eveline Peeters (chairperson)
Prof. dr. ir. Wim Versées (secretary)
Prof. dr. Nick Devoogdt (VUB)
Prof. dr. Edwin Claerebout (UGent)
Prof. dr. Karolien De Wael (UA)

Curriculum vitae

11/1998-11/2006: Central University of Ecuador. Doctor in General Medicine and Surgery.

11/2011-11/2013: Vrije Universiteit Brussel. MSc. in Molecular Biology (IPMB). Contributions to unravelling the nature of the false positivity in circumsporozoite *Plasmodium falciparum* ELISA

10/2013-Present: Vrije Universiteit Brussel, PhD. in Bio-engineering Sciences

Main Publications:

An innovative approach in the detection of Toxocara canis excretory/secretory antigens using specific nanobodies. **Morales-Yanez FJ,** Sariego I, Vincke C, Hassanzadeh-Ghassabeh G, Polman K, Muyldermans S. Int J Parasitol. 49, 635-645, 2019

Identification of Useful Nanobodies by Phage Display of Immune Single Domain Libraries Derived from Camelid Heavy Chain Antibodies.Romao E, **Morales-Yanez F**, Hu Y, Crauwels M, De Pauw P, Hassanzadeh GG, Devoogdt N, Ackaert C, Vincke C, Muyldermans S. Curr Pharm Des. 22(43):6500-6518, 2016

Abstract of the PhD research

Human toxocariasis (HT) is a zoonotic disease caused by the infection of the larval stage of the dog roundworm *Toxocara canis*. Currently, laboratory diagnosis of HT relies on the ELISA-based detection of specific immunoglobulins against the *T. canis* excretory/secretory (TES) antigen (Ab TES-ELISA). TES antigen is a mix of highly glycosylated proteins released by the parasite into the tissues during the migration of larval stages in the human host. The most important drawback of the Ab TES-ELISA is its inability to distinguish between active and past infections. Moreover, cross-reactivity with other antigens from other helminths is frequent. This is particularly important in tropical regions, where polyparasitism is frequent.

In this PhD thesis, we introduce a sensitive and specific nanobody-based sandwich ELISA (Nb-ELISA) to detect TES antigen with a limit of detection of 0.650 ng/ml in serum spiked with TES antigen. This Nb-ELISA employs bivalent biotinylated nanobodies as capturing agent and nanobodies chemically coupled to horseradish peroxidase for detection. The Nb-ELISA was able to detect TES antigen in mice sera taken 3 days after they were experimentally infected with *T. canis* eggs. Even higher sensitivity was achieved by converting the ELISA into an electrochemical magnetosensor assay, whereby the reaction takes place on the surface of streptavidin-precoated paramagnetic beads. In this configuration, the assay had a limit of detection of 10 pg/ml in serum spiked with TES antigen. This assay was evaluated in serum samples from children of remote rural communities in the province of Esmeraldas (Ecuador). We found that 38% (33/84) of the sera were positive for TES antigen. Positivity was significantly correlated with eosinophilia. Additionally, the test showed no cross-reactivity with antigens from other helminths. To our knowledge, this is the most sensitive and specific immunoassay to diagnose HT currently available. As the electrochemical assay provides evidence of active Toxocara infections, it has great potential to significantly improve HT diagnosis. It also provides opportunities to develop point of care diagnostic systems for other diseases where high sensitivity and specificity are required. This research highlights the impressive versatility of nanobodies for the development of innovative immunoassays.