FACULTY OF VETERINARY MEDICINE
accredited by EAEVE

INVITATION

PUBLIC PHD DEFENCE

IT'S ALL PART OF THE MASTERPLAN – Pseudorabies Virus Passage across the Basement Membrane and Modulation of Plasmacytoid Dendritic Cell Activation

Jochen Lamote

Promotor

Prof. Dr. ir. Herman Favoreel
Faculteit Diergeneeskunde, UGent

Members of the examination committee

Prof. Dr. E. Cox, DVM
Faculteit Diergeneeskunde
UGent
Voorzitter van de examencommissie

Prof. Dr. T. Michiels
de Duve Institute
Université Catholique de Louvain

Dr. ir. N. De Regge
Enzootic and (re)emerging diseases
CODA-CERVA

Dr. ir. S. Glorieux
Weefselbank
UZ Gent

Prof. Dr. H. Nauwynck, DVM
Faculteit Diergeneeskunde
UGent

UNIVERSITEIT GENT

Curriculum Vitae

Jochen Lamote was born on April 15, 1988 in Bruges.

In 2006, Jochen started studying at the Faculty of Bioscience Engineering, Ghent University, where he pursued a career in science. It was there where he encountered the classes "General Virology" of professor Favoreel, and the idea for a Master thesis at the Faculty of Veterinary Medicine started growing. In 2011, Jochen obtained the diploma of "Master of Science in Bioscience Engineering: Cell & Gene Biotechnology"

Being intrigued by the lack of an effective vaccine against the herpes simplex virus (despite decades of research), he started his PhD at the Faculty of Veterinary Medicine, where he focused on the interactions of alphaherpesviruses with the innate antiviral defense.

Jochen is author and co-author of three papers in the peer-reviewed international Journal of Virology and has presented his work at several (inter)national congresses.

Where and When

Friday, September 8, 2017 at 17h

Auditorium Hoogbouw
Faculty of Veterinary Sciences
Ghent University
Salisburylaan 133, Merelbeke

After the defence you're kindly invited to the reception. Please confirm your attendance to the reception (Jochen.Lamote@UGent.be).

Summary of the PhD Thesis

Alphaherpesviruses are a group of closely related double-stranded DNA-viruses, which cause several disease symptoms in both humans and animals. In humans, symptoms are generally mild (e.g. cold sores), but infections may occasionally lead to more severe disease symptoms (e.g. encephalitis). The goal of the current thesis was to elucidate interactions of alphaherpesviruses – using the porcine pseudorabies virus (PRV) as a model pathogen - with two important elements of the innate antiviral defense, which may contribute to the design of novel vaccination strategies and treatment against these viruses. On the one hand, we investigated how alphaherpesviruses cross the basement membrane (BM), a crucial structural barrier to overcome, to invade the host, particularly focusing on the role of the viral US3 protein kinase during this process. On the other hand, we investigated the poorly understood interactions between alphaherpesviruses and plasmacytoid dendritic cells (pDC), which are important sentinels of the immune system and are able to produce massive amounts of type I interferon (TI-IFN) upon viral encounter.

Chapter 1 gives a general introduction on the three main subjects of this thesis: alphaherpesviruses, the BM and pDC.

Chapter 2 describes the aims of this PhD thesis.

In chapter 3, we show that the US3 protein kinase of PRV is crucial for the virus to transmigrate the BM. For this purpose, ex vivo porcine nasal mucosa explants were infected with wild-type (WT) PRV, ΔUS3 PRV (a mutated isogenic strain, lacking expression of US3) or US3R PRV (an isogenic strain in which the previous mutation has been restored). Analysis of PRV plaques led to two intriguing observations: (1) in line with earlier findings in vitro, US3 plays a role in efficient cell-to-cell spread ex vivo, as ΔUS3 PRV showed significantly smaller plaques compared to WT PRV at 48h; and (2) US3 is of paramount importance for PRV to transmigrate the BM as only very few ΔUS3 PRV viruses were capable of breaching this barrier (compared to 100% invasion by WT PRV). Furthermore, using specific inhibitors to counteract and simulate the earlier described US3-mediated effects on cellular Rho GTPase signaling, we showed that the role of US3 in viral BM passage is (at least in part) mediated by the ability of this protein to modulate Rho GTPase signaling.

In chapter 4, several PRV strains were assessed for their capacity to induce TI-IFN production in pDC. To this end, pDC were successfully enriched/isolated from porcine blood and cultivated with PRV-infected epithelial cells. Strikingly, we observed a hyperactivation of pDC when they were cultured with epithelial cells infected with the attenuated PRV vaccine strain Bartha. The PRV Bartha strain contains multiple well-characterized genomic differences with PRV WT strains. Using several isogenic mutants, which lack expression of one or several of the genes deleted in the Bartha genome, we found that the absence of the gE/gI complex accounts at least in part for the hyperactivating effect of Bartha on TI-IFN production by pDC. Furthermore, we found that the ability of gE (in conjunction with gI) to suppress TI-IFN production by pDC correlates with its earlier described ability to modulate Erk1/2 phosphorylation, a critical cell signaling molecule.

In Chapter 5, the potential impact of different PRV (glyco)proteins on TI-IFN production by pDC was assessed. A screening of several PRV mutants was performed, which led to the identification of US3 and gB as modulators of IFN-α production by pDC: we found that US3 stimulates TI-IFN production by pDC, whereas gB suppresses this TI-IFN production. Regarding the ability of the viral gB protein to suppress TI-IFN production by pDC, using epithelial cells which constitutively express PRV gB on their cell surface, we found that expression of PRV gB, without expression of other viral proteins, was sufficient to downregulate TI-IFN production by pDC that were stimulated with a TLR9 agonist (CpG-rich oligodeoxynucleotides). Furthermore, we showed a differential expression, modification and localization of the viral gB protein in cells infected with the Bartha vaccine strain compared to cells infected with wild type PRV, suggesting that alterations in Bartha gB might also contribute to the hyperactivating effect of this vaccine strain on TI-IFN production by pDC.

In chapter 6 the interactions between PRV and the innate antiviral defense established in the current thesis are thoroughly discussed, based on existing literature and/or preliminary results. A hypothetical model of US3-mediated BM breakdown is given, the newly gathered information regarding interactions of alphaherpesviruses with pDC are discussed, and we reflect on possible implications of this research towards the development of new antiviral treatments and/or vaccines against alphaherpesviruses.

Conclusion

In the current thesis, we unraveled some novel aspects of the PRV Masterplan to overcome the innate antiviral defense. First, we showed that the conserved alphaherpesvirus US3 protein kinase is of paramount importance to allow PRV to efficiently breach the BM. This depends at least in part on the ability of US3 to interfere with the cellular Rho GTPase signaling pathway, which possibly may lead to breakdown of the BM. During alphaherpesvirus infection of the epithelium, pDC migrate towards the site of infection and produce TI-IFN to limit viral spread and activate other components of the immune system. We found that the gE/gI glycoprotein complex and the gB glycoprotein inhibit, while the US3 protein kinase stimulate IFN-α production by pDC. Furthermore, we found that the vaccine strain PRV Bartha hyper-activates pDC, which may help to explain the immunogenicity and success of this vaccine. The hyperactivating effect of Bartha on TI-IFN production by pDC was in part attributed to its lack of gE/gI expression and may perhaps also be related to its differential expression of gB compared to wild type PRV. Altogether, these results might – further down the road - contribute to the rational design of new treatments and vaccines against alphaherpesvirus infections.