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Curriculum Vitae

Shunchuan Zhang was born on 30th March 1986 in Sichuan, China.

In June 2009, he obtained his Bachelor Degree in Veterinary medicine from Sichuan Agricultural University in China. In the same year, he continued his master studies at the Faculty of Veterinary Medicine, Sichuan Agricultural University on the project *"The molecular characteristics, prokaryotic expression, protein expressing kinetics of duck enteritis virus (DEV) UL53 gene and the localization of DEV gK in infected cells and tissues"*. In June 2011, he obtained a Master Degree in Preventive Veterinary Medicine. During his master studies, he published three papers in 'Virology Journal' and a review in 'Reviews in Medical Microbiology'.

In November 2011, he started his PhD training under supervision of Prof. H. Nauwynck in the Laboratory of Virology, Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University on the project "*Pathogenesis of the highly passaged MCMV Smith strain and low passaged HaNa1 strain in Balb/c mice mimicking natural infection upon oronasal inoculation"*. His PhD project was sponsored by the Chinese Scholarship Council (CSC), the Concerted Research Action 01G01311 of the Research Council of Ghent University, and Belgian Science Policy (BELSPO). He is the author and co-first author of several publications in international peer-reviewed journals, and he also participated and presented his work during several national and international conferences.

UNIVERSITEIT GENT

INVITATION

Public Defence of the Doctoral Thesis of

Shunchuan Zhang

May 10th, 2016

Laboratory of Virology
Department of Virology, Parasitology and Immunology
Faculty of Veterinary Medicine, UGent

FACULTEIT DIERGENEESKUNDE
approved by EAEVE

You are kindly invited to attend the public defence of the doctoral thesis of

Shunchuan Zhang

Title of the thesis:

Pathogenesis of the highly passaged MCMV Smith strain and low passaged HaNa1 strain in Balb/c mice mimicking natural infection upon oronasal inoculation

The public defence will take place on
Tuesday, May 10th, 2016
at 17:30 hours
Auditorium Hoogbouw
Faculty of Veterinary Medicine
Salisburylaan 133, 9820 Merelbeke

After the public defence a reception will be held in the Museum of Anatomy

Please confirm your attendance before May 3rd, 2016 to
Shunchuan.Zhang@Ugent.be

Summary of the thesis

Human cytomegalovirus (HCMV) is a betaherpesvirus that causes subclinical infections in immunocompetent hosts but clinically significant diseases in immunocompromised individuals. HCMV can certainly be transmitted from infected to susceptible hosts via oronasal route. However, it remains elusive how the virus spreads throughout the body during a natural infection. Due to the strict species-specificity of HCMV, it is impossible to study the pathogenesis of HCMV infections in experimental animals. Therefore, animal CMV infection models are used. Murine cytomegalovirus (MCMV) infection in mice is a widely used animal model to study HCMV infection. However, knowledge about the invasion strategies of MCMV upon oronasal exposure was lacking.

In **Chapter 1**, the current knowledge on MCMV is reviewed. First, an introduction is given on the history, the classification, the virus characteristics, and the replication cycle of MCMV. Further, the epizootiology, the pathogenesis of MCMV infection and the immune response during an infection of MCMV are reviewed. Finally, the anatomy of oral & nasal cavities and lymph nodes associated with alimentary & respiratory tracts in mice are briefly introduced.

In **Chapter 2**, the aims of this thesis were formulated.

In **Chapter 3**, the pathogenesis of an infection with the highly passaged MCMV Smith strain was compared with that of an infection with a low passaged Belgian MCMV isolate HaNa1 in BALB/c adult mice following oronasal inoculation with either a low (10^4 $TCID_{50}$/mouse) or high (10^6 $TCID_{50}$/mouse) inoculation dose. Both strains were mainly replicating in nasal mucosa and submandibular glands for one to two months. In lungs, both strains showed a restricted replication. Only the Smith strain established a low level of productive infection in spleen, liver and kidneys. The infected cells were identified as olfactory neurons and sustentacular cells in olfactory epithelium, macrophages and dendritic cells in NALT, acinar cells in submandibular glands, and macrophages and epithelial cells in lungs for both strains. Antibody analysis demonstrated for both strains that MCMV-specific IgG_{2a} was the main antibody subclass that was raised. Overall, our results showed that significant phenotypic differences exist between the two virus strains. MCMV HaNa1 is a promising strain to use in mouse models in order to get better insights for HCMV infections in immunocompetent humans.

In **Chapter 4**, virus titration showed a productive virus replication of both HaNa1 and Smith in the nasal mucosa from 1 dpi until the end of the experiment (14 dpi), in lungs from 5 until 14 dpi, and in submandibular glands from 7 until 14 dpi. In contrast to MCMV HaNa1, MCMV Smith established a low level productive infection in abdominal organs (spleen, liver and kidneys). Co-culture showed that for both strains, cell-associated virus was detected in a non-infectious form in nasopharynx-associated lymphoid tissues (NALT) from 1 until 14 dpi, in submandibular lymph nodes from 3 until 5 dpi, in deep cervical lymph nodes from 3 until 14 dpi, in mediastinal lymph nodes from 7 until 14 dpi, in spleen from 5 until at least 10 dpi and in the peripheral blood mononuclear cells (PBMC) at 7 and 10 dpi. This study showed that upon oronasal exposure, MCMV first enters the nasal mucosa and NALT, from where the virus disseminates to the spleen possibly via the draining lymphatic system and blood; a subsequent cell-associated viremia transports MCMV to submandibular glands and for MCMV Smith also to liver and kidneys, where a second productive replication starts.

In **Chapter 5**, the role of the spleen during an MCMV infection was investigated by the comparison of intact and splenectomized Balb/c mice. Both highly passaged MCMV Smith and low passaged MCMV HaNa1 were used. Various samples were collected at 7, 14, and 21 days post inoculation (dpi) for analyses by virus isolation/titration, co-cultivation and qPCR. The results showed that for both virus strains, 1) cell-associated virus in PBMC (determined by co-cultivation) was detected in intact mice but not in splenectomized mice; 2) the mean viral DNA load in PBMC of splenectomized mice was 4.4-(HaNa1)/2.7-(Smith) fold lower at the peak viremia (7 dpi) in contrast to that of intact mice; and 3) infectious virus in the submandibular glands was detected later in splenectomized mice (14 dpi) than in intact mice (7 dpi). Moreover, the average virus titers in submandibular glands of splenectomized mice were 10-(HaNa1)/7.9-(Smith) fold lower at 14 dpi and 1.7-(HaNa1)/2.1-(Smith) fold lower at 21 dpi compared with that of intact mice. Upon inoculation with MCMV Smith, infectious virus was found in the kidneys and liver of intact mice, but not in splenectomized mice. Taken together, all these data clearly demonstrate that virus dissemination to distant organs is reduced in splenectomized mice, further confirming the importance of the spleen as a viremia booming site for a natural MCMV infection.

In **Chapter 6**, all data obtained in the present thesis were reviewed and discussed. A general hypothetical model for MCMV dissemination throughout the body was proposed: upon oronasal exposure to MCMV, the nasal mucosa and NALT serve as portal of entry for MCMV. From these primary replication sites, the virus is transported in non-productively infected leukocytes to the draining lymph nodes (submandibular LN and deep cervical LN) and finally to the spleen and lungs possibly via lymph and blood circulation. In the spleen, a subsequent cell-associated viremia is regulated, from where a second replication is initiated in various tissues such as submandibular glands (for both Smith and HaNa1), liver and kidneys (only for Smith).