PRIZE OF THE CENTRE D'ÉTUDES PRINCESSE JOSÉPHINE-CHARLOTTE 2013

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PRIZE OF THE CENTRE D'ÉTUDES PRINCESSE JOSÉPHINE-CHARLOTTE FOR SCIENTIFIC RESEARCH AGAINST VIRAL INFECTIONS 2013

This biennial Prize, which amounts 12.500 EUR, rewards a scientific research achievement in the field of viral infections, in connection with human health.

This year, the Prize is awarded to :

Laurent GILLET

Docteur en médecine vétérinaire, ULg
D.E.A. en sciences vétérinaires, ULg
Docteur en sciences vétérinaires, ULg (Aspirant du F.R.S.-FNRS, 1.10.2001 > 30.9.2005)
Chargé de recherches du F.R.S.-FNRS, ULg (1.10.2005 > 30.9.2008)
Chercheur qualifié du F.R.S.-FNRS, ULg (1.10.2008 > 30.9.2012)
Chargé de cours à l'Université de Liège

for his work :

« Study of the neutralisation evasion by gammaherpesviruses. »

Gammaherpesviruses are important animal and human pathogens which are associated with the development of cancers. In this work, we tried to understand how these viruses, that establish lifelong latency, are able to be transmitted between hosts in the presence of a specific immune response. Especially, we investigated how gammaherpesviruses evade neutralizing antibodies. As neutralization mainly blocks virus entry into the host cell, we firstly studied the mechanisms of gammaherpesvirus entry. Secondly, we showed that the glycans that are present at the viral surface contribute to the protection of some vulnerable entry epitopes from antibodies. Finally, in order to study the importance of these mechanisms in natural conditions, we developed the first experimental transmission model of a gammaherpesvirus between mice. In the future, this work could help us to develop new strategies in order to block the epidemiological cycle of gammaherpesviruses but also of other persistent viruses for which conventional vaccines have proved unsuccessful.

MEMBERS OF THE JURY 2013

Mr	CONTENT Jean *President of the Jury*	Directeur honoraire de l'Institut Pasteur de Bruxelles, Professeur honoraire à l'ULB
Mr	MUYLDERMANS Serge	Hoofddocent - VUB
Mr	PIETTE Jacques	Directeur de recherches du F.R.S. - FNRS à l'ULg
Mrs	VANDAMME Anne-Mieke	Gewoon hoogleraar - KU Leuven

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Mr	MICHIELS Thomas *Observer*	Président du Centre d'Études Princesse Joséphine-Charlotte, Professeur à l'UCL

Laurent GILLET

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"Study of the neutralisation evasion by gammaherpesviruses"

Summary

Introduction

Herpesviruses are important pathogens which are ubiquitous in both human and animal populations. We are mainly interested in gammaherpesviruses. These viruses drive lymphocyte proliferation during host colonization and consequently, they can induce some lymphoproliferative disorders. In humans, the Epstein-Barr virus (EBV) and the Kaposi's Sarcoma-associated Herpesvirus (KSHV) infect respectively up to 95% and 50% of human populations. In developed countries, EBV primary infection is associated with the development of infectious mononucleosis in adolescent and young adults. Moreover, human gammaherpesviruses are also linked with the development of several malignancies such as Burkitt's and Hodgkin's lymphomas, nasopharyngeal carcinoma, Kaposi's sarcoma and post-transplant lymphoproliferative disease [1,2]. Human cancers associated with these two viruses are particularly prevalent in Africa where they are associated to malaria [3] and human immunodeficiency virus-1 infection [4]. More generally, individuals with inherited or acquired immunodeficiency have an increased risk of developing a malignancy caused by one of these two viruses [5]. Similarly, some animal gammaherpesviruses are also associated with lymphoproliferative disorders. Especially, *Alcelaphine herpesvirus-1*, which is carried asymptomatically by wildebeest, causes malignant catarrhal fever when cross-species transmitted to a variety of susceptible species of the *Artiodactyla* order [6]. Gammaherpesviruses are therefore important human and animal pathogens and the establishment of affordable, safe and effective strategies to fight these infections is therefore an important goal.

The development of vaccines against gammaherpesviruses is particularly challenging. Indeed, during the co-evolution with their host, these viruses have developed numerous strategies to evade the immune response. Especially, gammaherpesviruses coexist with antibody and resist neutralization. Understanding how they do this and what counter-measures might be taken is likely to be important in achieving infection control [7].

Results

During the last years, our research group pursued studies on Murid herpesvirus 4 (MuHV-4) and Bovine herpesvirus 4 (BoHV-4) that had been started during my PhD (under the

supervision of Prof. A. Vanderplasschen, University of Liège) and during a postdoc (under the supervision of Dr P.G. Stevenson, University of Cambridge). Indeed, as experimental studies are difficult to perform directly with EBV or KSHV, we used related animal gammaherpesviruses such as MuHV-4 or BoHV-4 that allowed us to tackle the same fundamental questions in a more accessible form. Here are summarized three of the research areas that have been investigated during the last six years.

1. Investigation of the mechanisms involved in gammaherpesvirus entry into the host cell.

The commonest mechanism of *antibody*-mediated *neutralization* of *viral* infection is to prevent attachment and *entry* into target cells. Therefore, in order to understand how gammaherpesviruses resist neutralization we firstly analyzed their entry mechanisms.

The first step of infection is binding of the viral particle. Many herpesviruses bind to cell-surface heparan sulfate (HS) [8]. We had showed that MuHV-4 has at least two HS-binding glycoproteins, gp70 and gH/gL [9,10]. Moreover, it had been shown by de Lima *et al.* that another MuHV-4 glycoprotein, gp150 interacts with HS [11] and could participate to regulation of cell binding [9]. In order to determine if this observation could be generalized to other gammaherpesviruses, we characterized the BoHV-4 Bo10 gene which encodes the BoHV-4 positional homolog of MuHV-4 gp150. Our results showed that the Bo10 gene encodes a protein, hereafter called gp180, which is incorporated in viral particles. A Bo10 deleted strain was viable but showed a growth deficit that was associated with a reduction of binding to cell expressing HS [12]. Interestingly, gp180-deficient virions were paradoxically more infectious for cells that were not expressing HS such as circulating leukocytes that are infected *in vivo*. By analogy to MuHV-4 gp150, we proposed that BoHV-4 gp180 could regulate the attachment of virions to the cell surface by covering some high affinity receptor-binding protein until it has been displaced by HS [12]. BoHV-4 has even developed this mechanism in a more sophisticated way as we showed that this virus is able to orientate tropism of progeny virions by using alternative splicing of its Bo10 gene [13]. As a similar gene organization is observed in KSHV, we hypothesized that this mechanism could be conserved in other viruses [13].

The second step of infection by herpesviruses is fusion of their envelope with cellular membranes. Herpesviruses use a core fusion machinery composed by glycoproteins gB, gH and gL. Interestingly, we showed that BoHV-4 gL is not essential for infectivity [14], similarly to what we had observed in MuHV-4 [15]. At least in these two viruses, gL is therefore not involved in fusion, although gL-deficient virions showed poor growth

associated with an entry deficit. We showed that a major part of their entry defect appeared to reflect impaired endocytosis, which occurs upstream of membrane fusion itself. One of the major functions of gL in these viruses could be to trigger virion endocytosis [14]. Interestingly, we showed that gL-dependent epitopes are the main targets for neutralization of BoHV-4 virions suggesting that this function could be very important.

2. Identification of mechanisms used by some of these viruses to evade neutralizing antibodies.

Most of the herpesvirus envelope proteins are glycosylated. In this part of the work, we studied how the glycans that are located at the surface of the viral particle participate to the evasion of neutralizing antibodies. In the case of BoHV-4, we showed that glycans protect virions from neutralization [16]. Then, we identified by proteomic characterization of BoHV-4 extracellular virions, that gB, gL, gH and gp 180 are heavily glycosylated. Among these proteins, gp180 is potentially the most glycosylated [17] and its absence increased the sensitivity of virions to neutralization by immune sera. Antibody had greater access to gB, gH and gL on gp180-deficient virions, including neutralizing epitopes on gL. It appeared therefore that gp180 provides a glycan shield for otherwise vulnerable viral epitopes [16]. Interestingly, we had showed that the N-terminal part of MuHV-4 gB is also highly glycosylated and provides a protective and largely protected cover for vulnerable epitopes located on other proteins [18]. As these proteins and their glycans are conserved among gammaherpesviruses, we therefore proposed that these viruses could use these proteins and their glycans to form a shield against neutralizing antibodies at the surface of the viral particle. This observation is particularly interesting especially because BoHV-4 Bo17 gene encodes a glycosyltransferase [19]. In the future, we want to study the consequences of Bo17 expression on BoHV-4 neutralization.

3. Set up of an experimental transmission model of these viruses *in vivo*.

Although MuHV-4 had been studied for more than 30 years [20], attempts to demonstrate horizontal transmission in laboratory mice had been almost entirely unsuccessful, leaving unresolved how MuHV-4 is spread between hosts [21,22]. The objective of this part of our work was therefore to fill this gap.

We firstly showed that, in comparison with the natural host, *Mus musculus* represents a suitable host for studying gammaherpesvirus pathogenesis with MuHV-4 [23]. Then, thanks

to in vivo imaging, we have been able to observe, for the first time, transmission of MuHV-4 in mice [24]. We firstly showed that MuHV-4 excretion occurs in the genital tract of female mice at a period by which latency is considered as established. Secondly, we demonstrated the implication of sexual steroid hormones in this re-excretion process. Finally, based on these results, we tested MuHV-4 transmission in mice by creating different epidemiological conditions. Surprisingly, we were able to observe sexual transmission to naïve males. Interestingly, while horizontal transmission by saliva appears as the most common route of transmission for EBV [25,26] and KSHV [27], evidence of genital shedding has also been obtained for both viruses [27-37] suggesting that sexual transmission is possible for these viruses. Transmission of MuHV-4 between mice represents therefore a unique opportunity to study gammaherpesvirus transmission in natural settings and to define what counter-measures might be taken.

Conclusion

The gammaherpesvirus lifecycle requires that immune carriers shed sufficient infectious particles to transmit infection to naïve subjects. In this work, we tried to understand how they manage to do this. In the future, based on these results and using our recently established transmission model, we want to develop new strategies that could block the epidemiological cycle of these viruses.

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Curriculum Vitae

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University studies and diplomas obtained

- **Veterinary Medicine**

  1995- June 1998: "Candidat" in Veterinary Medicine (ULg),
  Mark: "Grande distinction".

  1998- June 2001: Doctor in Veterinary Medicine (ULg),
  Mark: "La plus grande distinction".

- **Master in Veterinary Sciences**

  2001-2002: Master in Veterinary Sciences ("Diplôme d'études approfondies") (Promotor: Prof. A. vanderplasschen, Faculty of Veterinary Medicine, ULg).

Title of the Master thesis: *In vitro* study of the interactions between Bovine Herpesvirus 4 and human cells.

Presented on the 23rd September, 2002.
Mark: "La plus grande distinction".

- **PhD thesis**

2001-2005: Doctor in Veterinary Sciences (Promotor: Prof. A. Vanderplasschen, Faculty of Veterinary Medicine, ULg).

Title of the thesis: *In vitro* study of the interactions between Bovine Herpesvirus 4 and human cells.

Presented on the 18th March, 2005.
Mark: "La plus grande distinction".

- **" Formation de Maître d'expériences catégorie C"**

2004 University of Liège
Mark: "La plus grande distinction".

Professional experience

October 2012 – present: "Chargé de cours", Vaccinology, Faculty of Veterinary Medecine, ULg.

October 2008 – October 2012: Research associate ("Chercheur qualifié", permanent position) of the F.R.S./FNRS, Immunology-Vaccinology, Faculty of Veterinary Medecine, ULg.

October 2007 - October 2008: Postdoctoral researcher ("Chargé de Recherches") of the F.R.S./FNRS, Immunology-Vaccinology, Faculty of Veterinary Medecine, ULg.

October 2005 - October 2007: Postdoctoral researcher ("Chargé de Recherches") of the F.R.S./FNRS, Division of Virology, Department of Pathology, University of Cambridge (U.K.).

October 2001 - October 2005: research fellow ("Aspirant") of the "Fond National Belge pour la Recherche Scientifique" (F.R.S./FNRS, Belgium), Immunology-Vaccinology, Faculty of Veterinary Medicine, ULg.

Awards

29/6/2001: "**Prix des Annales de Médecine Vétérinaire**" award (Best scientific work of the promotion, Faculty of Veterinary Medicine, ULg) for the work headed: "Contributions à l'études du gène Bo17 de l'herpèsvirus bovin 4 : revue des rôles des β-1,6-N acétylglucosaminyltransférases et production d'une souche d'herpèsvirus bovin 4 délétée pour le gène Bo17".

26/04/2006: "**Prix Hamoir (2003-2007)**" national award for the work headed: *In vitro* study of the interactions between bovine herpesvirus 4 and human cells.

Work supervision

- **"Formations doctorales"**

  Bénédicte Machiels. Title of the manuscript "Natural antibody-complement dependent neutralization of Bovine herpesvirus 4 by human serum". 2008. Faculty of Veterinary Medicine. ULg.

  Céline Lété. Title of the manuscript "Study of the Bo17 gene of Bovine herpesvirus 4". 2008. Faculty of Veterinary Medicine. ULg.

  Sylvie François. Title of the manuscript "Comparative study of Murid Herpesvirus 4 infection in mice and in its natural host the bank voles". 2010. Faculty of Veterinary Medicine. ULg.

  Bérengère Boutard. Title of the manuscript "Study of the roles of the Myxoma virus M138L gene product in the pathogenesis of myxomatosis". 2012. Faculty of Veterinary Medicine. ULg.

  Mickael Dourcy. Title of the manuscript "Impact of latent Murid Herpesvirus 4 infection on the development of an anti-pneumovirus vaccine-induced respiratory immunopathology ". 2013. Faculty of Veterinary Medicine. ULg.

  Bilal Latif. Title of the manuscript "Study of the ORF63 gene of Murid Herpesvirus 4". 2013. Faculty of Veterinary Medicine. ULg.

- **PhD theses**

  Bénédicte Machiels. Title of the thesis "Study of the Bo10 gene of Bovine herpesvirus 4". The thesis has been presented on the 20st of May 2011. Faculty of Veterinary Medicine. ULg.

  Céline Lété. Title of the thesis "Study of the glycoprotein L of Bovine herpesvirus 4 and of its protection against neutralizing antibodies". The thesis has been presented on the 24th of May 2012. Faculty of Veterinary Medicine. ULg.

Sylvie François. Title of the thesis "Development of a transmission model of Murid Herpesvirus 4". The thesis has been presented on the 26th of October 2012. Faculty of Veterinary Medicine. ULg.

Composition of the research group

Laurent Gillet	Principal Investigator
Bénédicte Machiels	Postdoctoral Researcher
Céline Lété	Postdoctoral Researcher
Sarah Vidick	PhD Student
Bérengère Boutard	PhD Student
Bilal Latif	PhD Student
Mickael Dourcy	PhD Student
Caroline Zeippen	PhD Student

Former PhD student

Sylvie François, FRIA studentship 2008-2012 (PhD completed in 2012)

Professional societies and working groups

2004-present: Belgian Society for Microbiology (Member of the board since January 2012)

Services to the community

2009-present: Member of the Doctoral College of the Faculty of Veterinary Medicine (ULg). President since September 2011.

2011: FRIA scientific expert.

2010-present: Member of the organizing committee of the degree related to protection of experimental animals (Maître d'expérience catégorie C, FELASA). University of Liège.

List of peer reviewed publications

A complete list of publications with access to full-length texts can be found on the *ORBI* institutional website:

http://orbi.ulg.ac.be/simple-search?query=%28%28uid%3Au190544%29%29&title=Publications+et+communications+de+Laurent+Gillet+%5Bu190544%5D&sort_by0=1&order0=DESC&sort_by1=3&order1=ASC&sort_by2=2&order2=ASC

As first-, co-first or last author

1. Francois, S, Vidick, S, Sarlet, M, Desmecht, D, Drion, P, Stevenson, P. G, Vanderplasschen, A, & **Gillet, L.** (2013). Illuminationof murine gammaherpesvirus-68 cycle reveals a sexual transmission route from females to males in laboratory mice. ***PLoS Pathogens***, 9(4), 1003292. http://hdl.handle.net/2268/151328

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