Imperial College London

CSIC Consejo Superior de Investigaciones Científicas

UAM Universidad Autonoma de Madrid

CENTRO DE BIOLOGÍA MOLECULAR "SEVERO OCHOA"

James S Porterfield Prize in International Virology 2016 – Report form

Name:	Alberto Domingo López-Muñoz
Address:	Molecular Biology Centre Severo Ochoa CBMSO (CSIC-UAM) C/ Nicolas Cabrera 1 Cantoblanco UAM Campus Postal Code: 28049 Madrid, Spain
Tel. and email (fax):	Telephone: +34 911964590 Email: adlopez@cbm.csic.es
Name of host / supervisor:	Host supervisor: Prof. Peter O'Hare Thesis supervisor: Prof. Antonio Alcamí
Host laboratory:	Imperial College London (Faculty of Medicine, Department of Medicine) Norfolk Place, St Mary's Campus W2 1PG London (UK)
Period in host laboratory:	from: 31-10-2016 to: 23-12-2016

TITLE OF REPORT:

Novel activities of herpes simplex virus glycoprotein G: cell migration and activation

ABSTRACT

Glycoprotein G (gG) from herpes simplex virus (HSV) types 1 and 2 (gG1 and gG2, respectively) interacts with chemokines and enhance their activity. Our laboratory demonstrated that gG also binds neurotrophic factors, where gG2 is proteolytically cleaved and secreted into the medium, promoting axonal growth.

gGs are present in the virion and at the plasma membrane of infected cells, but their ability to trigger intracellular signalling through their cytoplasmic tail is presently unknown. By yeast-two-hybrid assay, we showed that gG, through its cytoplasmic tail, interacts directly with cytoplasmic kinases, which are related to cellular motility and migration.

During this short research stay, we have observed interesting differences in the protein levels and location patterns of these kinases, between uninfected and HSV infected cells.

By characterising changes during early and late infection stages, we will be able to understand how herpesviruses modulate the cytoskeletal machinery, where gG could have a potential role contributing differently to the tropism and pathogenesis of HSV types 1 and 2.

INTRODUCTION

Large DNA viruses (herpesviruses and poxviruses) encode a unique array of proteins that mimic host immune molecules or target key components of the immune system (Alcami 2003). These viral immunomodulatory proteins contribute to viral pathogenesis in animal models. Glycoprotein G (gG) encoded by herpes simplex virus (HSV) binds neurotrophic factors (Cabrera 2014) and chemokines (Martinez-Martin 2015, Viejo-Borbolla 2012), enhancing their activities in the virion surface (Martinez-Martin 2016) and at the plasma membrane of infected cells. gG is the only viral chemokine binding protein (vCKBP) known to potentiate chemokine activity and to enhance the inflammatory response. Also, the novel function reported by our laboratory on the activation of neurotrophic factors by gG has not been described for any other virus. The ability of binding NGF (by gG1 and gG2) and enhancing axonal grown (gG2) may facilitate HSV invasion of the nervous system, where the virus establishes latency.

On the other hand, HSV induces the polarized migration of human keratinocytes into the site of infection, where uninfected surrounding cells migrate to the site of infection, spreading over infected cells in order to become infected. Moreover, by transwell migration assay, using spatially separated monolayer of cells in presence of neutralizing serum, these human keratinocytes migrated more actively (remaining uninfected) as a cytotactic response due to the presence of a paracrine stimulation from HSV infected cells (Abaitua 2013).

The main route of HSV transmission in mucocutaneous tissue is cell-to-cell spread. The majority of the viral progeny remains cells associated and transmits across cell junctions. In human skin keratinocytes, an epithelial cell type that mimics the physiological conditions for HSV infection, uninfected cells sense the spreading infection by cell-to-cell contact but also indirectly by an unknown factors which induce polarized migration, where gG and/or other viral protein could modulate directly this directional migration or through the induction of an endogenous cellular factor.

RESULTS

We compared the expression levels and localisation patter of different motility kinases in a human skin keratinocyte line (HaCaT) by immunofluorescence. Monolayers in coverslips were infected with ~0.001 PFU of different HSV-1 and HSV-2 strains and then incubated in the presence of 3% of human serum (HS) to neutralize extracellular virus and limit plaque formation. Plaques were observed after 24 hpi by fluorescent microscopy by immunostaining. We observed interesting changes of signal intensity against those motility proteins at the developing HSV plaques and in the surrounding uninfected cells, for both HSV types. That increase of signal was compared against the basal levels of these kinases in uninfected (same fields) and mock cells (data not shown).

In order to discard a potential cross-reaction between the studied cellular proteins and a viral factor during HSV replication in HaCaT, we tested different antibodies, blocking also with human or rabbit serum (5% of each for each case in combination with 5% of FBS). We made sure in this way that the observed differences in HSV developing plaques and in their surrounding cells were not due to cross-reactivity.

We studied the localisation patter of those kinases in COS1 (actively migrating cells) and VERO cells (traditional model of *in vitro* HSV infection). Cells were infected with ~5 PFU of HSV-1 and HSV-2 will type strains and then incubated in the presence of 3% of neutralizing serum until fixation and staining, 2 and 4 hpi. We found a dramatic change in the cytosolic patter of the studied cellular proteins in comparison to uninfected cells.

DISCUSSION

Cell-to-cell transmission and cellular migration in viral infections represent a network of modulation processes, where viral proteins play a key role controlling the host immune and cytoskeletal systems (Alcami 2003, Welch 2013). The potential overexpression and/or overactivation of migration pathways mediated by HSV infection could enhance the viral spread during early infection stages, when the virus starts to replicate but infected cells are able to properly migrate. HSV could be modulating the cytoskeletal machinery immediately after infection through viral proteins present at the virion surface and by expression of immediate-early proteins, promoting a fleeting dissemination of viral particles in order to colonize quickly a nerve ending. It must be a paracrine effector induced by HSV infection that may manipulate the migratory response of those cells to attract them to be infected (Abaitua 2013). These processes can be assisted and potentiated by gG through the enhancement of cytokine activity and therefore, recruitment of immune and epithelial cell to the infectious focus by a chemotactic gradient (Viejo-Borbolla 2012). Further investigation would elucidate whether gGs could recruit a cytoskeletal complex to the leading edge of migrating cells by their cytoplasmic tail or by indirect interaction through an unknown membrane protein.

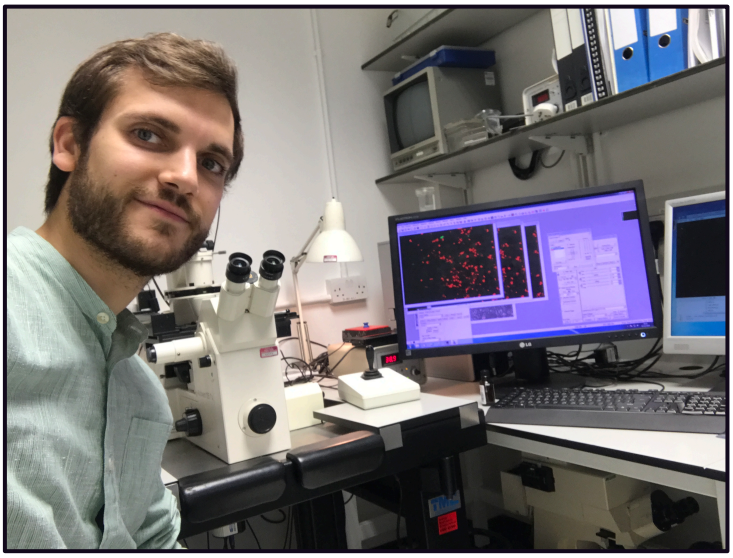
ACKNOWLEDGMENTS

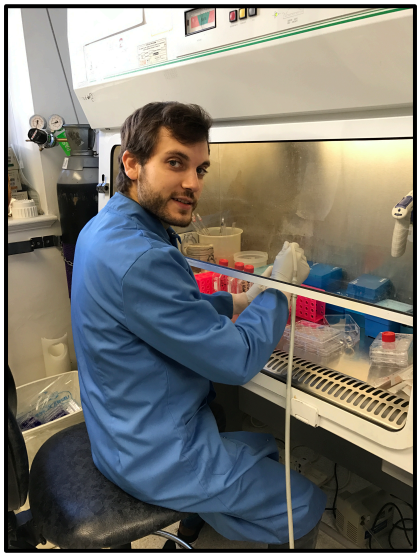
We are grateful to Professor Peter O'Hare for his helpful assistance and for giving me the opportunity to visit his laboratory at the Imperial College London as a visiting PhD student. We thank the Sir William Dunn School of Pathology (Oxford University) for the granting of one of the 2016 James S. Porterfield Prize in International Virology and the Federation of European Microbiological Societies (FEMS) for support this short stay by a Research Grant (FEMS-RG-2016-0066).
I am thankful to Professor William James for his hospitality during my short visit to his laboratory at the Sir William Dunn School of Pathology (Oxford University) by the end of my research stay in London.
Alberto Domingo López is financially supported as PhD student by a FPU studentship from Ministerio de Educación, Cultura y Deporte of Spain.

REFERENCES

- Abaitua, F., Zia, F. R., Hollinshead, M. and O'Hare, P. 2013. Polarized Cell Migration during Cell-to-Cell Transmission of Herpes Simplex Virus in Human Skin Keratinocytes. **J Virol.** 87:7921-7932
- Alcami, A. 2003. Viral mimicry of cytokines, chemokines and their receptors. **Nat Rev Immunol.** 3, 36-50
- Cabrera, J. R., Viejo-Borbolla, A., MartinezMartín, N., Blanco, S., Wandosell, F., Alcamí, A. 2015. Secreted Herpes Simplex Virus-2 Glycoprotein G Modifies NGF-TrkA Signaling to Attract Free Nerve Endings to the Site of Infection. **PLoS Pathog.** 11 (1): e1004571
- Martinez-Martín, N., Viejo-Borbolla, A. and Alcami, A. 2016. Herpes simplex virus particles interact with chemokines and enhance cell migration. **J. Gen. Virol.** 97: 3007-3016, doi: 10.1099/jgv.0.000616
- Martinez-Martin, N., Viejo-Borbolla, A., Martín, R., Blanco, S., Benovic, J. L., Thelen, M., and Alcamí, A. 2014. Herpes simplex virus enhances chemokine function through modulation of receptor trafficking and oligomerization. **Nat Commun.** 6:6163
- Viejo-Borbolla, A., Martinez-Martín, N., Nel, H. J., Rueda, P., Martín, R., Blanco, S., Arenzana-Seisdedos, F., Thelen, M., Fallon, P. G. and Alcami, A. 2012. Enhancement of chemokine function as an immunomodulatory strategy employed by human herpesviruses. **PLoS Pathog.** 8(2): e1002497
- Welch, M. D. and Way, M. 2013. Arp2/3-mediated actin-based motility: a tail of pathogen abuse. **Cell Host Microbe.** 14(3): 242–255

The St Mary’s Hospital and Imperial College London Campus





Working at the Cat. II microscope room and biosafety cabinet