POXVIRUS Y VACUNAS

Mariano Esteban

Summary

The aims of our group are geared to understand molecular basis in the pathogenesis of infectious agents and their interaction with the host, as well as to use this knowledge in the development of vaccines effective against diseases like AIDS, malaria and leishmaniasis.

As a model system of infectious agent and as a delivery vector for the expression of genes of interest, we used vaccinia virus (VV) a member of the poxvirus family.

We focus our research in three main areas of interest:

1. Vaccinia virus assembly.
2. Virus-host cell interactions and action of interferons;
3. Developmen of vaccines against Aids, malaria and leishmaniasis.

We would like to respond to the following challenging questions:

a) what is the structure of the different forms of vaccinia virus (VV) during morphogenesis and how these forms contribute to virus infection to cells and tissue distribution.

b) how VV gets into cells and what are the viral components involved

c) what is the structure of the viral complex A27L/A17L involved in virus attachment to cells.

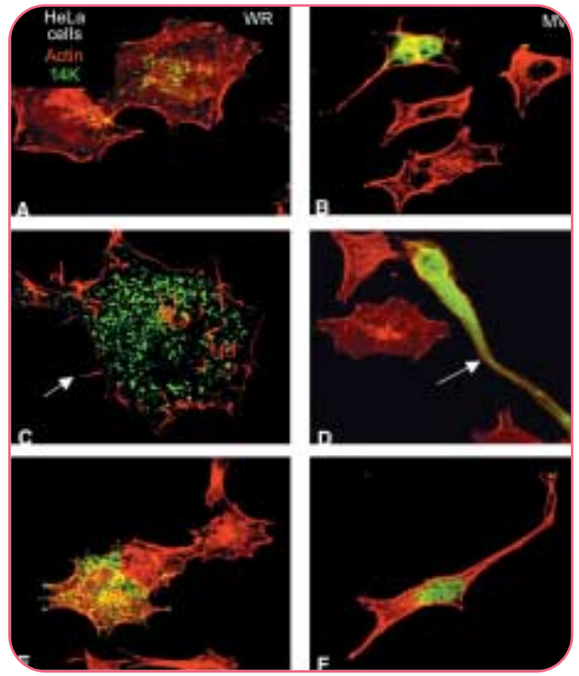

Figure 1. Gene expression of MVA in human cells.

d) how VV turns-off host cell translation

e) what is the impact of VV and its mutant viruses on host cell gene expression profiling and how some of the cellular genes facilitate or inhibit VV replication.

f) What is the role of interferon (IFN)-induced genes (i.e, PKR and the 2-5A synthetase/RNase L system) on antiviral and anticellular functions, how viruses evade the IFN system and can these viruses and/or the IFN-induced genes be used to destroy tumour cells.

g) Can we modulate the immune system (humoral and cellular) with poxvirus vectors and generate effective vaccines against relevant human diseases like AIDS, malaria, leishmaniasis, HCV and cancer.

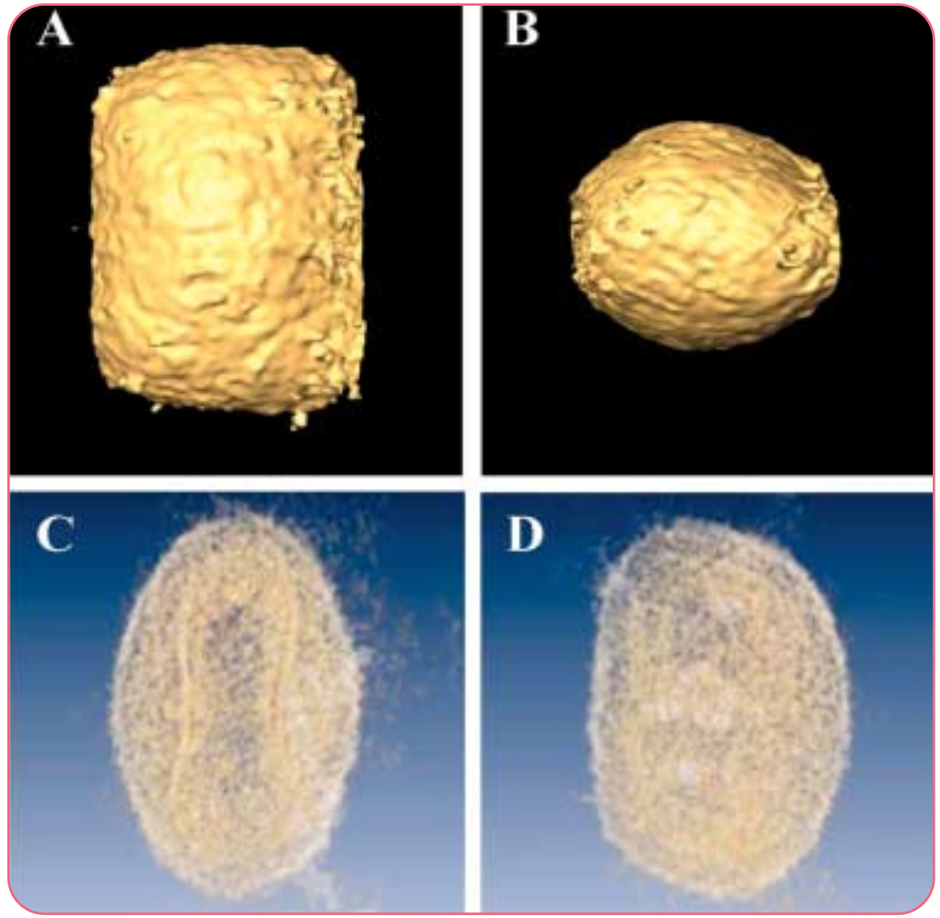

Figure 2. The structure of the infectious form (IMV) of vaccinia virus (VV) has been defined at the resolution of 4-6 nm through cryo-electron tomography.

PERSONNEL

Group Leader:
Mariano Esteban

Postdoctoral Fellows:
Alberto Fraile (Contract Ramón y Cajal)
Ivan Ventoso
Susana Guerra,
Carmen E. Gómez
Mariang García
Gracyna Kochan

Predoctoral Fellows:
Elena Domingo
José Luis Nájera
Eva Pérez
Magdalena Krupa
Andrea Vandermeeren

Technical Assistants:
Victoria Jiménez
Yolanda García

PUBLICATIONS

Gherardi, M.M., Nájera, J.L., Pérez-Jiménez, E., Guerra, S., García-Sastre, A. and Esteban, M. (2003). Prime/boost immunization schedules based on influenza and vaccinia virus (VV) vectors (MVA and WR) potentiate cellular immune responses against HIV-env protein systemically and in the genito-rectal draining lymph nodes. *J. Virol.* **77**, 7048-7057.

Guerra, S., López-Fernández, L., Pascual-Montano, A., Muñoz, M., Harsman, K. and Esteban, M. (2003). Cellular gene expression survey upon vaccinia virus infection of human HeLa cells. *J. Virol.* **77**, 6493-6506. (front cover Dec. issue).

Gallego-Gómez, J.C., Risco, C., Rodríguez, D., Cabezas, P., Guerra, S., Carrascosa, J.L. and Esteban, M. (2003). Differences in virus-induced cell morphology and in virus maturation between the MVA and other strains (WR, Ankara, NYCBH) of vaccinia virus in infected human cells. *J. Virol.* **77**, 10606-10622.

Gil, J., García, M.A., Gómez-Puertas, P., Guerra, S., Rullás, J., Alcamí, J. and Esteban, M. (2004). TRAF family proteins link PKR with NF-kB activation. *Mol. Cell. Biol.* **24**, 4502-4512.

Gherardi, M.M., Pérez-Jimenez, E., Nájera, J.L. and Esteban, M. (2004). Induction of HIV immunity in the genital tract after intranasal delivery of a MVA vector: enhanced immunogenicity after DNA prime-modified vaccinia virus Ankara boost immunization schedule. *J Immunol.* **172(10)**, 6209-20.

Tapia, E., Pérez-Jimenez, E., Lopez-Fuertes, L., Gonzalo, R. and Esteban, M (2003). The combination of vectors expressing IL-12+IL-18 elicits high protective immune response against cutaneous leishmaniasis after priming with DNA-p36/LACK and the cytokines, followed by a booster with a vaccinia virus recombinant expressing p36/LACK. *Microbes and Infection* **5**, 73-84.

Ramirez, J.C., Finke, D., Esteban, M., Kraehenbuhl, J.P. and Acha-Orbea, H (2003). Tissue distribution of Modified Vaccinia Virus Ankara (MVA) after mucosal or systemic administration. *Arch. Virol.* **148**, 827-839.

Gherardi, M.M., Nájera, J.L., Pérez-Jiménez, E., Guerra, S., García-Sastre, A. and Esteban, M. (2003). Prime/boost immunization schedules based on influenza and vaccinia virus (VV) vectors (MVA and WR) potentiate cellular immune responses against HIV-env protein systemically and in the genito-rectal draining lymph nodes. *J. Virol* **77**, 7048-7057.

Ramiro, M.J., Zárate, J.J., Hanke, T., Rodríguez, D., Rodríguez, J.R., Esteban, M., Lucientes, J., Castillo, J.A. and Larraga, V (2003). Protection against Leishmania infantum visceral leihsmaniasis in dogs is achieved by immunization with heterologous prime-booster regime using LACK-expressing DNA and recombinant vaccinia virus. *Vaccine* **21**, 2471-2484.

Guerra, S., López-Fernandez, L., Pascual-Montano, A., Muñoz, M., Harsman, K. and Esteban, M (2003). Cellular gene expression survey upon vaccinia virus infection of human HeLa cells. *J. Virol* **77**, 6493-6506. (front cover Dec issue).

Esteban, M., García, M.A., Domingo-Gil, E., Arroyo, J., Nombela, C. and Rivas, C. (2003). The latency protein LANA 2 from Kaposi´s sarcoma associated herpesvirus (KSHV) inhibits apoptosis induced by PKR but not RNase L activation. *J. Gen.Virol* **84**, 1463-1470.

Gherardi, M.M., Ramirez, J.C. and Esteban, M (2003). Interleukin-12 (IL-12) and IL-18 synergize to clear vaccinia virus infection: involvement of innate and adaptive components of the immune system. *J. Gen. Virol.* **84**, 1961-1972.

González-Lopez, C., Martinez-Costas, J., Esteban, M. and Benavente, J (2003). Avian reovirus sA protein is an inhibitor of the double-stranded RNA-dependent protein kinase PKR. *J. Gen. Virol.* **84**,1629-1639.

Gallego-Gómez, J.C., Risco, C., Rodríguez, D., Cabezas, P., Guerra, S., Carrascosa, J.L. and Esteban, M (2003). Differences in virus-induced cell morphology and in virus maturation between the MVA and other strains (WR, Ankara, NYCBH) of vaccinia virus in infected human cells. *J. Virol* **77**, 10606-10622.

González-Aseguinolaza, G., Nakaya, Y., Molano, A., Dy, E., Esteban, M., Rodríguez, D., Rodríguez, J.R., Palese, P., García-Sastre, A. and Nussenzweig, R.S (2003). Induction of protective immunity against malaria by prime/boost immunization with recombinant cold- adapted influenza and modified vaccinia virus Ankara viruses expressing a CD8+ T cell epitope derived from the cincumsporozoite protein of *Plasmodium yoelii. J. Virol* **77** , 11859-11866.

Gómez, C.E., Abaitua, F., Rodríguez, D., Duarte, C. and Esteban, M (2003). Efficient CD8+ T cell response to HIV-env V3

loop epitope from multiple isolates by a DNA prime/vaccinia virus boost (rWR and rMVA strains) immunisation regime and enhancement by the cytokine IFN-γ. Virus Research 105, 11-22

Diderlaurent, A., Ramirez, J.C., Graff, M., Gherardi, M., Orbea, H-A., Wagner, H., Esteban, M., Kraehenbuhl, J-P, and Sirard, J-C (2004). Attenuated poxviruses expressing a synthetic HIV protein stimulate HLA-A2 restricted cytotoxic T cell responses. *Vaccine* **22**, 3395-3403.

Gil, J., García, M-A., Gómez-Puertas, P., Guerra, S., Rullás, J., Alcamí, J. and Esteban, M (2004). TRAF family proteins link PKR with NF-kB activation. *Mol. Cell. Biol.* **24**, 4502-4512.

Guerra, S., Lopez-Fernandez, L.A., Conde, R., Pascual-Montano, A., Harshman, K. and Esteban, M. (2004). Microarray Analysis Reveals Characteristic Changes of Host Cell Gene Expression in Response to Attenuated Modified Vaccinia Virus Ankara Infection of Human HeLa Cells. *J Virol* **78(11)**: 5820-34.

Gherardi, M. M., Pérez-Jimenez, E., Nájera, J.L and Esteban, M. (2004). Induction of HIV immunity in the genital tract after intranasal delivery of a MVA vector: enhanced immunogenicity after DNA prime-modified vaccinia virus Ankara boost immunization schedule." *J Immunol* **172(10)**: 6209-20.

Esteban, M. (2004). Conceptos y futuras aplicaciones de la genómica, proteómica y bioinformática en el campo de la salud. En Genoma España, *Salud Humana*, pp 99-104

Esteban, M. (2004). Desarrollo de nuevas vacunas basadas en poxvirus. En "Real Expedición Filantrópica de la Vacuna. Doscientos años de lucha contra viruela". Biblioteca de Historia de América, CSIC. p 333-345.

Gil, J. and Esteban, M. (2004). Vaccinia virus recombinants as a model system to analyze interferon-induced pathways. *J. Interferon and Cytokine Research* **24**, 637-646

RESEARCH PROJECTS

Mariano Esteban. Principal Spanish Investigator.
Effector and memory anti-malaria CD8+ cell responses.
National Institutes of Health (NIH), 1 RO1 AI44375-01, 1999-2003, US $165.000.

Mariano Esteban. Principal Spanish Investigator.
Visceral Leishmaniasis vaccine: murine model studies.
National Institute of Health (NIH), USA. RO1 AI45044. 2000-2003.US $ 50.000

Project Leader of the EuroVac Cluster, European Vaccine Effort Against HIV/AIDS, Fifth Framework Programme, QLRT-PL1999-01321, Euros 329.065, 1999-2004.

Concerted Action, Fifth Framework Programme, European Vaccine against Aids (EVA) CFAR, QLRT-PL1999-00609, 2000-2003.

Mariano Esteban. Principal Investigator.
Contract wih GALENICA , USA, 2003-2004.

Mariano Esteban. Principal Investigator.
Premio IBERDROLA Ciencia y Tecnología, Profesores Visitantes, 2000-2003.

Mariano Esteban. Principal Investigator.
Desarrollo de nuevas herramientas moleculares para el estudio del virus de la hepatitis C y su aplicación a morfogénesis, estructura, resistencia del virus a interferon y caracterización de la respuesta inmune al virus.
BIO2000-0340-P4, 2001-2003. 171.649 Euros.

Mariano Esteban. Principal Investigator.

Diseño y utilización del virus vaccinia como vacuna contra distintas enfermedades: análisis de la interacción virus-célula y modulación de la respuesta inmune.
BIO2001-2269, 2001-2003, 170.000 Euros.

Mariano Esteban. Principal Investigator.
Desarrollo de nuevas herramientas moleculares para el estudio del virus de la hepatitis C y su aplicación a morfogénesis, estructura, resistencia del virus a interferon y caracterización de la respuesta inmune al virus.
BIO2000-0340-P4. 2000-2003, 171.649 Euros.

Mariano Esteban. Principal Investigator.
Mecanismo de acción de los interferones: análisis structural y funcional de la proteína quinasa PKR, un activador de apoptosis e inhibidor viral.
BMC2002-03246, 2002-2005, 196.650 Euros.

Mariano Esteban. Principal Investigator.
Analysis of the molecular mechanism of hepatitis C virus (HCV) resistance to antiviral therapy.
EU QLK2-CT-2002-00954. 2002-2005, 124.313 Euros.

Mariano Esteban. Coordinator.
Increasing the potency of vaccinia MVA vaccines.
EU QLK2-CT-2002-01867. 2002-2005. 220.000 Euros.

Mariano Esteban. Principal Investigator.
Potenciación de la respuesta inmune (sistémica y de mucosas frente al virus de la inmunodeficiencia humana (VIH-1).
FIPSE, 2002-2005, 209.365 Euros.

Mariano Esteban. Principal Investigator.
Vaccine strategies for combined targeting of innate and adaptive immune pathways (VaccTIP).
EU-2004-012161. 177.000 euros. 2004-2006.

Mariano Esteban. Principal Investigator.
Diseño de nuevas vacunas tanto preventivas como terapéuticas para las enfermedades de mayor prevalencia: sida, hepatitis C y cáncer de próstata.
BIO2004-03954,180.000 euros. 2004-2007.

Mariano Esteban. Principal Investigator.
Desarrollo de vacunas contra enfermedades prevalentes.
Fundación Botín, 200.000 euros/year. 2005-2010.

Mariano Esteban. Principal Investigator.
Desarrollo de una vacuna contra Leishmaniasis.
Comunidad de Madrid. 41000 euros. 2005.

Mariano Esteban. Principal Investigator.
Contract with GRIFOLS. 2005-2006.

DOCTORAL THESES

Juan Carlos Gallego Gómez (2003).
Biología celular de la infección y morfogénesis de mutantes atenuados del virus vaccinia.
Universidad Autónoma de Madrid. Sobresaliente *cum laude*.

Carmen E. Gómez (2003).
Respuesta inmune generada por sistemas combinados de vacunación frente a péptidos de la envuelta del VIH-1 incluidos en la proteína multiepitópica TAB-13.
Universidad Autónoma de Madrid. Sobresaliente *cum laude*.

María Angel García Chaves (2004).
Mecanismo de acción y regulación de la proteína quinasa inducida por interferon, PKR.
Universidad Autónoma de Madrid. 30 Abril de 2004. Sobresaliente *cum laude*. Premio Extraordinario UAM.

CONTRACTS

Empresas:

Analisis de anticuerpos contra el virus vaccinia en preparados de inmunoglobulinas humanas (IGIVs).
GRIFOLS, S.A , 2004-2006.

Fundaciones:

Principal investigator.
Potenciación de la respuesta inmune (sistémica y de mucosas) frente al virus de la inmunodeficiencia humana (VIH-1).
FIPSE, 2002-2006.

Principal Investigator.
Desarrollo de vacunas contra enfermedades prevalentes.
Fundación Botín, 2005-2010.

PATENTS

Pérez-Jiménez, E. y Mariano Esteban, M.
VECTORES RECOMBINANTES BASADOS EN EL VIRUS MODIFICADO DE ANKARA (MVA) COMO VACUNAS CONTRA LEISHMANIASIS.
Solicitud de invención Nº 200501886.

Gómez, C.E., Nájera, J.L., Jiménez, V. y Esteban, M.
VECTORES RECOMBINANTES BASADOS EN EL VIRUS MODIFICADO DE ANKARA (MVA) COMO VACUNAS PREVENTIVAS Y TERAPEUTICAS CONTRA EL SIDA.
Solicitud de invención Nº 200501841.