1. **Final publishable summary report**

The *Bunyaviridae* constitute a large family of enveloped animal viruses, many members of which cause serious diseases in humans. Currently there are no available vaccines or treatments. However, relatively little is known about *Bunyaviridae*. Their strategies for transmission and infection remain largely uncharacterized. The main objective of this proposal was to investigate the transmission and infection mechanisms used by these viruses to contaminate host.

To achieve this aim, we utilized Uukuniemi virus (UUKV), a non-pathogenic bunyavirus classified in the *Phlebovirus* genus, as a model virus to study entry of bunyaviruses into mammalian cells. We developed reliable and accurate microscopy- and FACS-based assays to study UUKV infection, endocytosis, and membrane fusion in fixed and living animal host cells. We combined all these assays with a large panel of perturbants of cellular entry pathways. Together these approaches allowed us to carry out the objectives of this project.

Most bunyaviruses are transmitted to mammalian hosts by arthropods such as mosquitoes, ticks etc. Dermal dendritic cells (DCs) are probably the first cells to encounter these viruses. These cells are localized in the host dermis which is the anatomical site for the virus transmission during blood feeding of insects. We identified the C type lectin CD209, naturally expressed at the surface of DCs, as a true endocytic receptor for UUKV. We extended this finding to human pathogenic members of the *Bunyaviridae* family such as Rift valley fever virus (RVFV). Moreover, we could also demonstrate that the human primary DCs, which naturally express this receptor, are also sensitive to RVFV in a CD209 dependent manner. We pursue this investigation and characterize the mechanisms by which bunyaviruses subvert the immunological function of this cell receptor. We found that virus attachment to CD209-positive cells induces the recruitment of additional receptor molecules at the virus binding site. The particles are then endocytosed through endocytic activity of CD209 within 10-15 min. Following uptake and endocytosis, viruses dissociate from the receptor and penetrate cells in a different cellular compartment 10-20 min later.

After transmission, and then a first round of infection in DCs, these viruses target several other tissues in hosts, which do not express CD209. We found that virus attachment to the surface of CD209-negative cells was specific but inefficient, with 25% of bound viruses being endocytosed within 10 min, mainly via non-coated vesicles. The viruses entered Rab5a+ early endosomes and, subsequently, Rab7a+ and LAMP-1+ late endosomes. Acid-activated penetration, occurring 20–40 min after internalization, required maturation of early to late endosomes. The pH threshold for viral membrane fusion was 5.4, and entry was sensitive to temperatures below 25°C. Together, our results indicate that UUKV penetrates host cells by acid-activated membrane fusion from late endosomal compartments.

To complete this project, we performed an automated high-throughput siRNA screening against the whole human genome (19,062 genes, QIAGEN) to identify cellular factors involved in UUKV infection. We identified 759 potential hits involved in all steps of the virus cycle (~4% of the human genome). The most important functions required for infection were related to endocytic trafficking, transcription and translation.

This study gives new insights on the spread of these viruses in hosts during the very early stages of the transmission from insects. It is the first time that a receptor is described for most bunyaviruses. It is also the first report providing a detailed view of the different stages of bunyavirus entry, including transmission, binding, internalization and penetration. In addition, our results also contribute to a better understanding of the general cellular endocytosis machinery hijacked by other families of viruses. For instance, investigating UUKV entry, we highlighted the importance of the degradative branch of the endocytic pathway in facilitating entry of late-penetrating viruses. Another example is the characterization of the biological function of CD209 which allows a better understanding about how other pathogenic microbes could subvert the immunological functions of this receptor to infect host.

This proposal supplies a large quantity of information on the cellular factors involved in the establishment of bunyavirus infection. These factors are many potential targets and new ways to counteract these viruses which should help to develop better strategies to block bunyavirus transmission and infection. By focusing on cellular rather than on viral activities, it will be possible to avoid the rapid emergence of resistance, a problem encountered in the development of many antiviral drugs. By inhibiting commonly used cellular proteins, it will also become possible to block infection, not only by UUKV, but also by pathogenic bunyaviruses, and possibly by other family of viruses, that use the same pathways. The extension of the finding about interaction between UUKV and CD209 to RVFV and other bunyaviruses is the best example.

Numerous factors induced by worldwide human activity favor the emergence and rapid spread of virus diseases. These events usually lead to important public health problems and tragic situations in farming as recently illustrated with the epidemics of SARS, Chikungunya, foot-and-mouth disease, and influenza viruses. The economic consequences can be a real disaster, impacting touristic attractiveness, farming budget, and medical and veterinary fees. Bunyaviruses are present all around the world, even in certain rich countries such as USA or Europe. This makes them serious potential emerging agents of disease. With this study, we expect to participate in helping Europe to face up such problems. Moreover, the siRNA whole genome screen presented in this investigation will provide many projects/leads for research during the coming years and will represent attractiveness for researchers from European and other countries.