**PhD PROPOSAL FOR THE**

**PASTEUR - PARIS UNIVERSITY INTERNATIONAL DOCTORAL PROGRAM**

Time for applicants to contact host laboratories: September 13 – November 2, 2017

Deadline for full application: November 13, 2017

Interviews: January 30, February 2, 2018

Start of the Ph.D.: October 1, 2018

**Title of the PhD project:** Functional study of the TIFA-dependent innate immune response during *Chlamydia trachomatis* infection

**Keywords:** innate defense, signaling cascade, female genital tract, *Chlamydia trachomatis*, bacterial infection

**Department:** Cell Biology & Infection

**Name of the lab:** Cellular Biology of Microbial Infection

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***Doctoral school affiliation and University*:** Complexité du Vivant (ED515 Université Pierre et Marie Curie)

Presentation of the laboratory and its research topics:

Our laboratory studies the interactions between bacteria and their host cells, with the long-term goal of finding novel targets to fight infection, as well as of gaining knowledge on basic cell biology processes. We focus on an intracellular bacterium called *Chlamydia*. *Chlamydiae* species pathogenic to humans, mainly *Chlamydia trachomatis* and *Chlamydia pneumoniae*, cause a number of diseases, including trachoma, pelvic inflammatory disease and pneumonia. Throughout their cycle in the host cell, chlamydiae remain in a membrane-bound compartment referred to inclusion. The work of the laboratory focuses mainly on the functional study of proteins secreted by the bacteria into the host cytoplasm, and on the innate response to infection. In particular, we aim at better understanding how epithelial cells, the main target of *Chlamydia*, respond to *C. trachomatis* infection and the mechanisms by which the bacteria manipulate host defense to evade immune clearance.

Description of the project:

*Chlamydia trachomatis* is a particularly prevalent human pathogen responsible for loss of eyesight through trachoma and the most common sexually transmitted disease of bacterial origin. Most of the patients infected by *C. trachomatis* are asymptomatic and remain untreated, leading to chronic or repeated infection in the female genital tract with severe outcomes such as infertility and pelvic inflammatory disease. *C. trachomatis* develops exclusively within host cells, mainly epithelial cells, inside a membrane-bound compartment called the inclusion. Epithelial cells sense and respond to the infection, for instance with the secretion of cytokines and chemokines aiming at eradicating the bacteria. However, the signaling pathways involved, and to which extent the bacteria manipulate this host response, remain poorly understood. TNF-α receptor-associated factor (TRAF)-interacting protein with a forkhead-associated (FHA)domain(**TIFA**) is a molecule interacting with TRAF2 and TRAF6(1,2). In human embryonic kidney (HEK) 293 cells, TIFA has been shown to promote oligomerization and ubiquitination of TRAF6, thereby activating IκB kinase (IKK) and inducing NF-κB activation(3). TIFA oligomerization mediated by intermolecular binding between TIFA-FHA domain and TIFA-phosphorylated threonine 9(4), has been shown to initiate the TRAF6 oligomerization upon TNF-α stimulation and upon infection with gram-negative bacteria in HEK293 and vascular endothelial cells(4-6). TIFA was also recently reported to be required for the detection of heptose-1,7-bisphosphate (HBP), a newly discovered pathogen associated molecular pattern derived from gram-negative bacteria during lipopolysaccharide synthesis(5). The HBP/TIFA axis contributes to IL8 production in response to the infection by enteroinvasive bacteria *S. flexneri* and *S. typhimurium* in intestinal epithelial cells(7). While TIFA implication in the detection of *C. trachomatis* infection has not yet been characterized, our preliminary observations show that TIFA localizes at the periphery of the C*hlamydia* inclusion.

The present studies will focus on the innate immune response of the host upon *C. trachomatis* infection in the female genital tract. In particular, we will investigate the role of TIFA during *C. trachomatis* infection. Given that the stimulations/infections induce TIFA oligomerization and subsequent activation of NF-κB cascades(1-3), we will examine the expression and oligomerization of TIFA in human cervical epithelial Hela cells infected by *C. trachomatis*. Different TIFA constructs(3,4,7) including WT, mutation of threonine 9, FHA mutant KRN and TRAF6-binding-defective mutant E178A, will be used and the localization and oligomerization of TIFA will be determined by immunofluorescence and immunoblot using native gel electrophoresis, respectively. TRAF2 and TRAF6 localization and NF-κB activation will also be examined. The level of *C. trachomatis*-elicited inflammation in Hela cells expressing these constructs, or when TIFA expression is silenced by siRNA, will be measured. We will also examine the signaling pathways involved in TIFA activation during *C. trachomatis* infection. The results will be confirmed in primary epithelial cells isolated from the genital tract of female patients, following a protocol already established by the host laboratory. The potential importance of TIFA in *C. trachomatis* infection of the female genital tract will be examined in a mouse model of infection, using TIFA knock-out mice. The inflammation and bacterial burden in the local genital tract will be monitored. Finally, using pull-down and co-immunoprecipitation approaches, we will study the mechanism by which TIFA is recruited to the *C. trachomatis* vacuole, possibly by binding one of the numerous bacterial proteins inserted in this compartment.

Collectively, the project will permit to better understand the contribution of TIFA to the innate immune response upon *C. trachomatis* infection in epithelial cells, and to identify the mechanism of TIFA activation during this infectious process. This may help to develop the potential new strategies by interfering with TIFA signaling cascades to fight infection by *C. trachomatis* in the female genital tract. If we discover that *C. trachomatis* manipulates the HBP/TIFA signaling axis, and the underlying molecular mechanisms, this work could suggest novel strategies to modulate TIFA-induced inflammation in infectious contexts.

References:

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4. *5 Gaudet, R. G. et al. INNATE IMMUNITY. Cytosolic detection of the bacterial metabolite HBP activates TIFA-dependent innate immunity. Science* ***348****, 1251-1255, doi:10.1126/science.aaa4921 (2015).*
5. *Huang, C. C. et al. Intermolecular binding between TIFA-FHA and TIFA-pT mediates tumor necrosis factor alpha stimulation and NF-kappaB activation. Mol Cell Biol* ***32****, 2664-2673, doi:10.1128/MCB.00438-12 (2012).*
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7. *Milivojevic, M. et al. ALPK1 controls TIFA/TRAF6-dependent innate immunity against heptose-1,7-bisphosphate of gram-negative bacteria. PLoS Pathog* ***13****, e1006224 (2017).*

Expected profile of the candidate (optional):

The student will be highly motivated, hard working, and with a good background in cell biology.

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