ImmunoTools *special* Award 2014

Maria Eugenia Rodriguez, PhD

CINDEFI. Applied Biotechnology Centre,
(CONICET-La Plata National University)
Calle 47 y 115, 1900 La Plata, Argentina

Bordetella pertussis **interaction with the host immune cells**

Bordetella pertussis is the etiologic agent of whooping cough or pertussis. A disease that causes more than 300,000 deaths each year. Despite high vaccination rates, whooping cough remains a serious threat to human health, and its incidence has been increasing in recent years in vaccinated populations.

Our laboratory is focused on the study of the physiopathology of *Bordetella pertussis* aiming at the better understanding of the infectious process and persistence within the population. We are investigating the regulation and interdependency of the bacterial virulence factors involved in the bacterial interaction with the respiratory tract epithelium and the immune system. We have several research projects ongoing in the Lab aiming at dissecting several issues related to this subject

One of our projects is focused on *B pertussis* interaction with host immune cells. Previous studies of our group indicated that *B. pertussis* survives the innate interaction with both human neutrophils and Macrophages. Moreover, we found that this pathogen is able to inhibit phagolysosomal fusion within human macrophages remaining in compartments with early endosomal characteristics that favor their survival and intracellular replication. Overall our findings support the hypothesis that macrophages may constitute be an effective bacterial cellular reservoir potentially contributing to its ability to persist within the populations. In order to gain a better insight into this significant finding we will next investigate whether there is the subtype of macrophages that supports intracellular growth of the bacteria.

During the host inflammatory response to infection, macrophages develop into two phenotypes depending on the cytokines they encounter. Stimulation by IFN–γ and/or LPS leads to the development of so called M1 subtype, characterized by a pro-inflammatory phenotype and enhanced bactericidal activity. These phagocytes play a role in the Th1 response of the acquired immune response. In contrast, stimulation with IL-4, IL-10 or IL-1β leads to the development of M2 subtype of macrophages characterized by a reduced bactericidal activity and secretion of anti-inflammatory

cytokines. M2 is the predominant form of macrophages inhabited by intracellular bacteria during chronic infections although there is evidence of pathogens developing persistent infections in M1 subtype. By the use of specific markers (anti-human CD80, anti-human CD14, anti-human CD16) we will investigate whether bacterial infection induces the differentiation of infected macrophages (or the surrounding cells) into M1 or M2 subtypes. Furthermore, the ability of the different subtypes of macrophages to support bacterial intracellular growth will be investigated in infection assays performed with selected macrophage phenotypes. To this end monocyte derived macrophages will be differentiated into M1 or M2 subtypes prior infection. *In vitro*, M1 originate from monocyte stimulated with GM-CSF in the presence of IFN-γ and/or bacterial products while M2 originate from monocyte stimulated with M-CSF and IL-4 or IL-13.

On the other hand, the study of the interaction of *B. pertussis* with relevant immune cells will also include Dendritic cells (DC). Dendritic cells are the most potent antigen-presenting cells (APCs). They play a key role in adaptive immunity and infection control. Interestingly, some intracellular bacteria can subvert DC function and gain the advantage of an ineffective host immune reaction. Evidence indicates that several pathogens are able not only to persist inside DCs but also to induce a not sufficiently activated state in DCs, which do not undergo maturation and do not produce the proinflammatory cytokines. In some cases, the infected DCs even display immunosuppressive behavior that may be directly linked to the induction of tolerogenicity favoring pathogen survival and persistence. The interaction of *B. pertussis* with DCs remains poorly documented. In particular, the ability of *Bordetella* to survive inside DCs, eventually interfere with the maturation process and functional activities, and influence the host immune responses remains poorly understood. We will generate DC from healthy human monocytes (cultured in the presence of IL-4 and GM-CSF) before infecting them with B. pertussis. Phagocytosis, bacterial trafficking inside the cell, and bacterial survival will be determined over the time of infection. We will also evaluate whether *B. pertussis* infected DC undergo phenotypic and functional maturation, and which type of Th cells polarization is induced by infected DC. To this end, the surface expression of the maturation markers such as CD80 and major histocompatibility complex (MHC) class II (HLA-DR) after infection with live bacteria will be evaluated. To determine the impact of *B. pertussis* infection on the host response, the induction of relevant immune regulatory cytokines produced by DC, such as IL-12, IL-4 and IL-10 will also be determined.

Our studies will help to understand the biological basis of the well-documented and poorly understood epidemiology of this persistent human pathogen. The knowledge we expect to produce with the above mentioned studies might contribute to the design of better preventive and therapeutic strategies to control pertussis spread. The ImmunoTools Award would be extremely useful for the proper development of our research

ImmunoTools *special* AWARD for **Maria Eugenia Rodriguez**

includes 25 reagents

FITC - conjugated anti-human CD1a, CD16, Control-IgG1,

PE - conjugated anti-human CD11c, CD80, Control-IgG1,

APC - conjugated anti-human CD14, Control-IgG1,

human IL-4 ELISA-set for 96 wells, IL-6 ELISA-set for 96 wells, IL12p40 total ELISA-set for 96 wells, human TNFa ELISA-set for 96 wells (each 3 reagents),

recombinant human cytokines: rh GM-CSF, rh M-CSF, rh IL-10, rh IL-4, rh IL-1beta

DETAILS more AWARDS