In vitro polarization of bovine macrophages, an optimized protocol

Carinne Puech, Isabelle Chantal, Valérie Rodriguez, David Berthier

POSTER: Towards a better definition of the immuno-proteome in the frame of contagious or vector-borne animal diseases

Bernard Fernandez, Armelle Peyraud, Edith Demttre, Martial Seveno, Alexandre Adersen, Nathalie Vachiery, Philippe Holzmuller, Francois Thiaucourt.

Defining the repertoire of antigenic targets is central to better understanding the immune responses against wether contagious pathogens and those transmitted by arthropod vectors. Traditional molecular approaches of antigen discovery have identified many immunodominant antigens, but they afford limited proteome coverage. Advances in proteomic technologies that are based on peptide library and the increase in genome sequencing that enriched molecular databases, allowed the definition of new analytical strategies with interrogation of the entire proteome for antigens. At the same time, improved technologies for antibodies purification for serum as well as antigens immunocapture lead scientists to revisiting the characterization of immuno-proteomes, particularly in the frame of contagious or vector-borne animal diseases. Here, we propose an analytical workflow to illustrate how to deepen the definition of the immuno-proteomes, and illustrate the proof of concept targeting *Mycoplasma mycoides*, the causative agent of contagious bovine pleuropneumonia (CBPP).

PRÉSENTATION ORALE, SESSION "IMMUNOMODULATION" - 17 MARS À 19H30
Immunomodulating properties of Amblyomma variegatum saliva and role on tick-borne disease transmission

Léo Chamayou, Valér ie Rodrigues, Bernard Fernandez, Alexandre Andersen, Edith Demettre, Martial Seveno, Rosalie Aprelon, Ken Giraud-Girard, Frederic Starchurski, Nathalie Vachiery, **Philippe Holzmuller.**

The tropical bont tick, *Amblyomma variegatum*, is a major pest of ruminants, causing direct skin lesions, vectoring the obligate intracellular ricketsiale *Ehrlichia ruminantium*, the causative agent of heartwater, and being able to reactivate dermatophilosis. In general, tick salivary proteins are the result of host blood feeding adaptation and are known to contain inhibitors of blood clotting, platelet aggregation and angiogenesis, as well as vasodilators and immunomodulators. The general objective of this study was to better understand the role of the saliva in the tick interaction with the host but also in pathogen transmission or diseases activation. We therefore first analysed the immunomodulating properties of semi-fed *A. variegatum* female saliva on bovine peripheral blood mononuclear cells (PBMCs) *in vitro*. Flow cytometry and cytokine ELISAs have been used to evaluate the saliva impact on PBMCs. We focused on immunosuppressive properties by analysing both

lymphocytes proliferation and monocyte-derived macrophages phenotype characterised by inhibition or induction of stimulatory and co-stimulatory molecules such as MHC-II, CD40, CD80 or CD86, and production of pro- or anti-inflammatory cytokines such as interleukin (IL)-2, IL-4, IL-6, IL-10, IL-12B, and tumor necrosis factor (TNF)-α. Moreover, a proteomics exhaustive molecular characterisation of *A. variegatum* saliva was performed to cluster data set according to the evidenced immunomodulatory properties, and allowed refined characterisation of *Amblyomma sialome*. Bioinformatics functional analysis of tick saliva highlighted molecular determinants that could, at least in part, explain the biological effects observed on bovine PBMCs. Our results bring new insights for a better understanding of tick-ruminant interactions, and open new perspectives to develop integrative strategies to interfere with the infectious pathoimmunological process of such tick-borne infections.

POSTER: *In vitro* polarization of bovine macrophages, an optimized protocol

Carinne Puech & Isabelle Chantal, Valérie Rodrigues, David Berthier.

Macrophages are major cells of the innate immunity. Macrophages derived from monocyte precursors undergo specific differentiation depending on the local tissue environment. Similar to the T helper type 1 and T helper type 2 polarization, two distinct states of polarized activation for macrophages have been defined in mouse and humans: the classically activated (M1) macrophage and the alternatively activated (M2) macrophage phenotypes. On the other hand, these different patterns of macrophage differentiation drive adaptive responses during the stages of infection, hence restraining inflammation and favoring tissue repair. In vitro generation and characterization of these subpopulations are essential to perform relevant studies understanding the host-pathogen interactions. Currently, several in vitro differentiation and polarization protocols are used to induce M1 or M2 mouse and human macrophages but none have been developed for the bovine species. We developed a method for in vitro differentiation and polarization of bovine macrophages using GM-CSF and IFNγ to induce M1, and IL-4 to induce M2 phenotype. We characterized M1/M2 macrophages by specific morphology, production of cytokines (IL-10, IL-12 and TNF-α), NO production, and phenotypic marker such as CD206.

PRÉSENTATION ORALE, SESSION "IMMUNOMONITORING" - 18 MARS À 14H30

Whole blood transcriptome analysis *of Mycoplasma mycoides* subsp. *mycoides*-infected cattle confirms immunosuppression but does not reflect local inflammation.

Valérie Rodrigues, Philippe Holzmuller, Carinne Puech, Hezron Wesonga, Francois Thiaucourt, Lucia Manso-Silvan

Contagious bovine pleuropneumonia (CBPP), caused by *Mycoplasma mycoides* subsp. *mycoides* (Mmm), is a severe respiratory disease of cattle responsible for major economic losses in sub-Saharan Africa. Disease control relies mainly on the use of empirically attenuated vaccines that provide limited protection. Thus, understanding the virulence mechanisms used by Mmm as well as the role of the host immune system in disease development, persistence, and control is a prerequisite for the development of new, rationally designed control strategies. The aim of this study

was to assess the use of whole blood transcriptome analysis to study cattle-Mmm interactions, starting by the characterization of the bovine response to Mmm infection during the acute form of the disease. For that purpose, we compared the transcriptome profile of whole blood from six cattle, before challenge by contact with Mmm-infected animals and at the appearance of first clinical signs, using a bovine microarray. Functional analysis revealed that 680 annotated genes were differentially expressed, with an overwhelming majority of down-regulated genes characterizing an immunosuppression. The main bio-functions affected were "organismal survival", "cellular development, morphology and functions" and "cell-to cell signaling and interactions". These affected functions were consistent with the results of previous in vitro immunological studies. However, microarray and qPCR validation results did not highlight pro-inflammatory molecules (such as TNFα, TLR2, IL-12B and IL-6), whereas inflammation is one of the most characteristic traits of acute CBPP. This global gene expression pattern may be considered as the result, in blood, of the local pulmonary response and the systemic events occurring during acute CBPP. Nevertheless, to understand the immune events occurring during disease, detailed analyses on the different immune cell subpopulations, either *in vivo*, at the local site, or *in vitro*, will be required. Whole blood transcriptome analysis remains an interesting approach for the identification of bio-signatures correlating to recovery and protection, which should facilitate the evaluation and validation of novel vaccine formulations.