Identification of crucial host-Mycobacterium molecular interactions in myeloid core of IL-17A and TNF-alpha-dependent granuloma

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Background: *Mycobacterium tuberculosis* (Mtb) is responsible for 1.7 millions death annually, targets the lung and remains latent for years. Dormant bacteria currently represent the problem in the TB pandemics: adapted immune strategies to eliminate these bacilli are urgently needed. Granulomas are the hallmark of mycobacterial infection and are crucial to restrict mycobacterial growth. However, they are unable to destroy Mtb bacilli and upon anti-TNF-α therapy for example, granuloma dizorganize and allow TB reactivation and dissemination. During mycobacterial infection IL-17A-secreting T cells are primed. Their role in protection and pathology is still debated, but IL-17A and TNFα synergize to induce a local inflammation. Mouse models demonstrated that IL-17A positively impacts granuloma formation through unknown mechanisms. We hypothesize that giant cells of Mycobacterium granuloma may result from IL-17A-dependent DC fusion.

Our objective is to investigate host-pathogen interactions between IL-17A-dependent giant cells and different strains of Mycobacteria with or without TNF-alpha to characterize molecular mechanisms up and downstream cell fusion which could be manipulated to affect granuloma formation and/or Mycobacterium survival.

Research program:

We will study the effect of TNF-alpha and CD40-activation on IL-17A-induced genetic program in monocyte-derived DC: we have performed genechip (Affymetrix, whole human genome "U133 plus2.0") large scale studies to understand the genetic program engaged by IL-17A-treatment of monocyte-derived DC which have been either infected by Mycobacteria or not. We will perform genechip studies of two new conditions, closer to the Mycobacterium granuloma: IL-17A+ IFNγ + TNFa +/- CD40L.

- ✓ Our second aim is to identify the molecular mechanisms of the regulation of IL-17A-induced DC fusion by TNF-alpha and CD40: DC are cultured in the presence of IL-17A, IFNγ with or without TNF-alpha and CD40L with different neutralizing antibodies directed against molecular targets previously identified as regulators of DC clustering, survival and fusion. Number of nuclei, number of giant cells, mean size of giant cells and fusion efficiency will be quantified after tartrate-resistant acidic phosphatase and Hoechst staining.

- ✓ Third, we will investigate the molecular mechanisms supporting microbicidal and invasive functions of IL-17A-dependent giant cell facing Mycobacteria in the presence of TNF-alpha and CD40-activation. We have set up *in vitro* cell culture conditions of IL-17A-dependent human DC granuloma on a layer of fibroblasts and our preliminary experiments showed that these granuloma are able to digest fibroblastic layer. According to previous chip results, we will use specific inhibitors to understand the contribution of MMP family or new GC. This study could explain

molecular mechanisms used by granuloma with MGC to kill Mycobacteria, but also to destroy surrounding tissue.

- ✓ Finally, we will look for key Mycobacterium molecules regulating microbicidal and invasive functions of IL-17A-dependent granuloma including MGC. From previous studies, we will set up two functional bioassays to screen different strains of Mycobacteria from the complete collection (150 strains) of Centre Hospitalier Lyon Sud (analysis laboratory, Drs JP Flandrois & M. Chomarat). After selection of a limited number of strains which are good or bad inducers of NADPH oxidase and/or invasive functions, transcriptome studies will be realised both on human and Mycobacterium genechips to discover molecules regulating NADPH oxidase activity and invasive functions of IL-17A-dependent granuloma. Expression of identified Mycobacterial genes will be screened in other strains and these results will be compared to the first functional screening to focus on a reasonable number of genes, important in the genius Mycobacterium for host-pathogen interaction in the myeloid core of the granuloma. Then bioinformatics studies on these genes will be performed to identify human-Mycobacterium interactors for further blocking studies.

Perspectives: Understanding the mechanisms of granuloma formation and myeloid cell fusion in Mycobacterium infection is of great importance since this structure controls bacilli during latent infection. From this project, we expect to learn how to regulate myeloid cell fusion. We could also understand what advantages the fusion provides. Knowing giant cell functions, we expect to monitor and limit tissue destruction. We could have access to new way to protect against Mycobacterium infections by manipulating the microbicidal function of the myeloid core inside Mycobacterium granuloma.