P0875

Characterization of swine skin and lung dendritic cells and their response to influenza virus

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Purpose/Objective: Swine is a natural host of influenza virus. Swine influenza strains can contaminate humans. Once infected, swine present identical symptoms as human, such as anorexia, pyrexia, cough, fever and nasal discharge. It has been recently shown in the mouse that dendritic cells (DC), and among them inflammatory TNF/iNOS-producing DC (Tip DC), are partly responsible both for the virus clearance and for the inflammatory pathology (Aldridge, PNAS 2009). Moreover, lung DC can be infected by influenza virus, although, interestingly, only the cross-priming CD103^{pos} DC subpopulation actually releases viral particles (Moltedo, PlosPathogen 2011). To establish if these DC/influenza interactions are idiosyncratic in the mouse model or can be generalized to natural hosts of influenza virus, we wanted:

1 To characterize the swine DC subpopulations network, first in the skin and then in the lung.

2 To describe their susceptibility to influenza virus infection and their capacity to produce new virions.

Materials and methods: Primary skin and lung cells were extracted and Facs-phenotyped (CadM1, CD206, CD209, CD14). Putative DC subpopulations were cell-sorted and the expression of genes specific for DC subpopulations (Flt3, ZBTB46, MAFB, BatF3, CSF1-R, XCR1, CX3CR1, CCR2, CLEC10A) were tested by q-PCR. Lung DC were infected with swine influenza virus and effective infection and virus production were tested by NP and NS1 immunofluorescence staining and by titration of the virus release.

Results: In a previous study, we described the different DC subpopulations present in pig skin (Marquet, PlosONE 2011). The deepening of this last study allowed us to revisit the classification of human skin DC subpopulations according to phenotypic and transcriptomic similarities between swine, human and mouse subsets, by reclassifying CD14^{pos} dermal DC as monocyte-derived DC and by identifying unambiguously the CD172a^{neg}/CadM1^{pos}/XCR1^{pos} cDC as equivalent to the murine CD103^{pos} cross-priming dermal DC.

We then identified the lung counterparts of these skin DC subpopulations and described their capacities to replicate influenza virus.

Conclusions: All together these data establish the swine as a model of choice for the study of normal and pathologic immune responses against influenza infection.

P0876

Circulating dendritic cells of multiple sclerosis patients are dysregulated and their frequency is correlated with MS-associated genetic risk factors

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Purpose/Objective: Dendritic cells (DC) are widely known as professional antigen-presenting cells and provide an important link to the adaptive immune system where they regulate the balance between immunity and tolerance. Alternations in the DC compartment can ultimately lead to the induction or perpetuation of autoimmune diseases such as multiple sclerosis (MS). This study aims to identify alterations in DC phenotype and functionality in MS. Moreover, the contribution of genetic risk factors to DC alterations was determined.

Materials and methods: An *ex vivo* analysis of myeloid (mDC) and plasmacytoid DC (pDC) was carried out on peripheral blood of MS patients (n = 104) and age- and gender-matched healthy controls (HC, n = 112). Frequencies and expression of costimulatory (CD80 and CD86) and migratory molecules (CD62L, CCR5 and CCR7) were investigated. Interleukin (IL)-12p70 and interferon (IFN)-α secretion was measured following Toll-like receptor (TLR) challenge. Study subjects were genotyped for HLA-DRB1*1501 and IL-7R α.

Results: A significant decrease of circulating pDC was found in peripheral blood of patients with chronic progressive MS (CPMS) compared to relapsing-remitting (RR) MS and HC. No differences in blood frequencies of mDC were found between different study groups. Both mDC and pDC of MS patients show shifts in the expression of CD86, CCR5 and CCR7 indicating that activation and migratory patterns of DC change during MS. Moreover, RRMS patients showed a reduced upregulation of CD86 on pDC and enhanced IL-12 production by mDC after TLR ligation, indicative of altered DC responsiveness. Treatment of MS is associated with a decrease of CD62L-positive mDC and pDC. HLA-DRB1*1501 carriers have reduced frequencies of circulating mDC as compared to non-HLA-DRB1*1501 carriers. Moreover, patients not carrying the protective IL-7Rα haplotype 2 have lower frequencies of pDC in the peripheral blood, indicating that genetic risk factors may impact the DC compartment of MS patients.

Conclusions: Our data indicate that circulating DC subsets undergo changes in phenotype and functionality during MS disease. This study further provides evidence that MS-associated genetic risk factors such as HLA-DRB1-1501 and absence of IL-7Rα haplotype 2 have an impact on the DC compartment and thereby may contribute to the induction and/or maintenance of autoimmune responses.

P0878

Contrasting responses of DC and NK cells to IFN-I contribute to host resistance to viral infection

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Purpose/Objective: Type I interferons (IFN-I) play a major role in immune defense against viral infections in mammals. Depending on the target cells, IFN-I either promote or inhibit cell proliferation and survival. How IFN-I mediate such opposite effects is not well understood. On dendritic cells (DC), IFN-I promote the up-regulation of major histocompatibility class I and co-stimulatory molecules, and the trans-presentation of IL-15. On natural killer (NK) cells, IFN-I promote entry into cell cycle, survival and cytotoxicity. However, the mechanism of action of IFN-I on NK cells, either directly by IFNAR triggering or indirectly through IL-15 presentation by DC, is still controversial.

Materials and methods: We combined the use of mutant mice, functional genomics, and flow cytometry analysis of transduction molecule phosphorylation to investigate how murine cytomegalovirus (MCMV) infection modulates the transcriptome and the antiviral activity of DC subsets and NK cells, in particular the role of IFN-I.

Results: We showed that splenic DC subsets undergo similar transcriptomic changes early after infection, with the induction of many IFN-I-stimulated genes, including genes involved in the recognition of viral infection, and inhibition of genes involved in proliferation. We showed that this occurs at least in part under cell-intrinsic instruction by IFN-I. NK cells induced genes involved in proliferation, which can be accounted for by stimulation through IL-15. We demonstrated that MCMV infection *in vivo* or IL-15 stimulation *in vitro* increase E2F expression and phosphorylation in NK cells, in a phosphatidylinositol 3-kinase dependent manner. Finally, we showed that the ability of DC, but not of NK