A gammaherpesvirus affects type 2 innate lymphoid cells in the context of HDM-induced asthma

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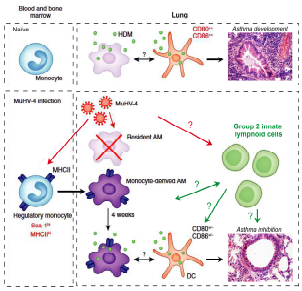
The "Hygiene hypothesis" postulates that allergic diseases could be prevented by some infections in early childhood. Gammaherpesviruses (γ-HVs) are among the most prevalent human viruses and profoundly imprint on the immune system of their hosts. Using Murid gammaherpesvirus 4 (MuHV-4), a mouse model of human γ-HV infections, we recently showed that γ-HV infection inhibits the development of House Dust Mites (HDM)-induced airway allergy (Machiels et al., *Nature Immunology*, 18(12):1310-1320 (2017)). Group 2 innate lymphoid cells (ILC2s) play a major role in the initiation, maintenance and memory of type 2 immune responses. As activation of these cells can be modulated by viruses associated with asthma exacerbation, we investigated whether MuHV-4 infection affects the lung ILC2s compartment.

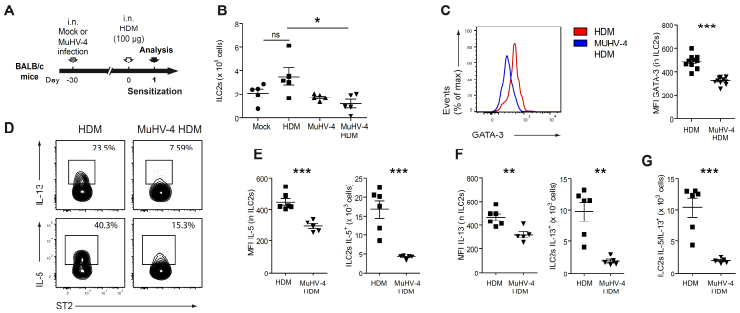
1. Gammaherpesvirus infection induces persisting changes in the alveolar niche that protect against airway allergy



MuHV-4 infection induces persisting changes in alveolar macrophages (AMs) that protect against asthma. In the absence of a previous MuHV-4 infection, resident AMs cannot prevent dendritic cells (DCs) from inducing a strong TH2 response in HDM-induced asthma. MuHV-4 virus infection induces death of AMs and a regulatory phenotype in the infiltrating monocytes that are differentiating into AMs. Long-term-persisting monocyte-derived AMs inhibit HDM-induced asthma by selectively interfering with the ability of DCs to trigger a TH2 response, without affecting TH1 response. The aim of this work is to understand whether MuHV-4 infection and/or recruited immune cells affect lung ILC2s in the context of HDM-induced asthma.

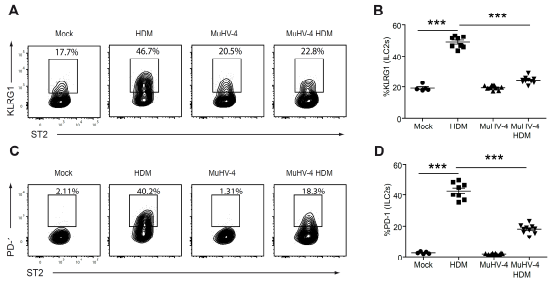
Adapted from Bérengère de Laval & Michael H Sieweke, *Nature Immunology*, News and views, 18(12):1279-1280 (2017).

2. MuHV-4 infection affects the function of lung ILC2s after HDM sensitization



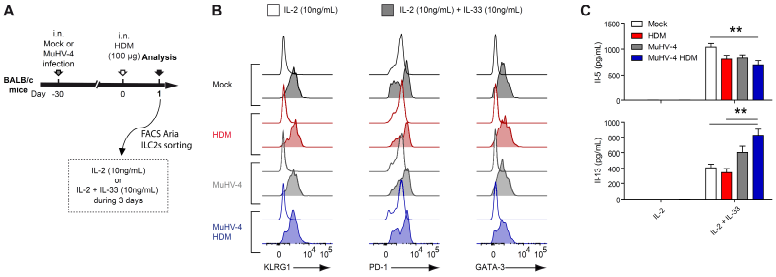
(A) Experimental model of MuHV-4 infection (1.10^4PFU/50µL) and HDM sensitization (100µg/50µL) in 8-week-old BALB/c mice (n = 6 to 10 in each group). **(B)** Quantification of lung ILC2s (live Lin⁻CD45⁺CD127⁺CD90.2⁺ST2⁺CD25⁺) cells, after HDM sensitization (lineage markers : B220, CD11c, CD3, CD4, CD49b, CD5, CD8α, F4/80, FcεR1, Gr1 and Siglec F). **(C)** Representative histograms and mean fluorescence intensity (MFI) of GATA-3 staining in ILC2s of mice infected or not with MuHV-4 and treated with HDM. **(D)** Representative contour plots of intracellular stainings of IL-5 and IL-13 on ILC2s isolated from HDM-treated mice, after 4h of *ex vivo* restimulation with PMA/ionomycin. **(E-F)** MFI of IL-5 **(E)** and IL-13 **(F)** stainings and numbers of IL-5 **(E)** and IL-13 **(F)** producing lung ILC2s after HDM sensitization. **(G)** Numbers of IL-5 and IL-13 producing ILC2s after HDM sensitization.

3. MuHV-4 infection affects activation of ILC2s as revealed by lower expression of PD-1/KLRG1 after HDM sensitization



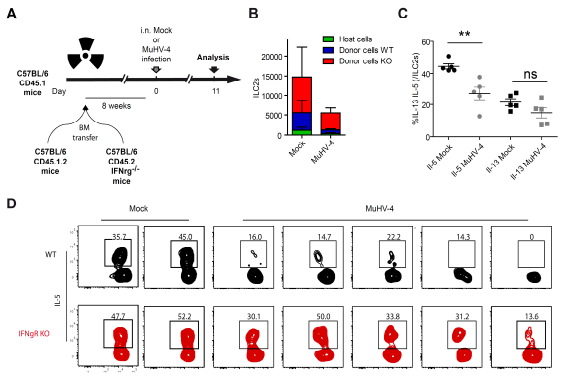
BALB/c mice (n= 5 to 10 in each group) were infected or not with MuHV-4 and submitted 30 days after infection to HDM sensitization (100µg/50µl) **(A)** Representative contour plots of KLRG1 expression on lung ILC2s from the different groups. Numbers in outlined area indicate percent KLRG1+ILC2s. **(B)** Percentages of KLRG1+ lung ILC2s among the different groups. **(C)** Representative contour plots of PD-1 expression on lung ILC2s from the different groups. Numbers in outlined area indicate percent PD-1+ILC2s. **(D)** Percentages of KLRG1+ lung ILC2s among the different groups.

4. The reduced activation of lung ILC2s following MuHV-4 infection is not due to an intrinsic defect but is dependent on the microenvironment



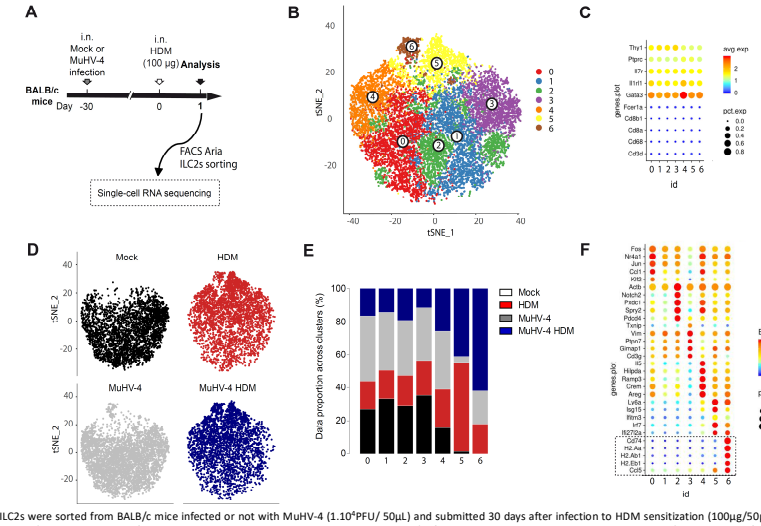
(A) Lung ILC2s were sorted from BALB/c mice infected or not with MuHV-4 and submitted 30 days after infection to HDM sensitization (100µg/50µl), cultured *ex-vivo* during 3 days with IL-2 or IL-2 + IL-33 (n=6) **(B)** Representative histograms of KLRG1, PD-1 and GATA-3 expression in sorted ILC2s cultured during 3 days with IL-2 or IL-2 + IL-33. **(C)** ELISA of cytokines in supernatant of ILC2s cultured during 3 days with IL-2 or IL-2 + IL-33

5. IFN-γ production following MuHV-4 infection rapidly modifies ILC2s cytokines production



(A) Experimental model of bone marrow chimeras : CD45.1⁺ BALB/c mice were exposed to lethal irradiation protocol (6Gy). These recipient mice were then given 5 x 10^6 BM cells isolated from femur and tibia at a ratio of 50% for CD45.1.2⁺ WT and 50% of CD45.2⁺ IFN-γR⁻/⁻ congenic donors. 8 weeks after BM transfer, those mice were submitted to MuHV-4 infection and analyzed 11 days later (n=5). **(B)** Representative contour plots for the evaluation of chimerism in lung ILC2s. **(C)** Percentage production of IL-5 and IL-13 in lung ILC2s and **(D)** representative contour plots of intracellular stainings of IL-5 on ILC2s, after 4h of *ex vivo* restimulation with PMA/ionomycin.

6. MuHV-4 infection affects the transcriptional profiles of lung ILC2s clusters



(A) ILC2s were sorted from BALB/c mice infected or not with MuHV-4 (1.10^4PFU/ 50µL) and submitted 30 days after infection to HDM sensitization (100µg/50µl) (n= 6 in each group) and they were profiled by droplet-based single cell RNA-sequencing. ILC2s (defined as Lin⁻CD45⁺CD127⁺CD90.2⁺ST2⁺CD25⁺ cells) were sorted after negative enrichment against lineage markers (B220, CD11c, CD3, CD4, CD49b, CD5, CD8α, F4/80, FcεR1, Gr1 and Siglec F) using MojoSort™ Mouse anti-APC Nanobeads (Biolegend) and magnetic separation using LD columns (Miltenyi) according to the manufacturer's protocol. Gene counts were obtained by aligning reads to the mm10 genome using CellRanger software (10× Genomics). **(B)** *t*-Distributed stochastic neighbor embedding (*t*-SNE) plots show 15,846 ILC2s and clustering using top 20 canonical vectors after canonical correlation (CCA) of the 4 conditions. Clusters were named based on the genes that were differentially expressed. **(C)** Genes expression for markers genes of ILC2s and other immune cell types. **(D)** *t*-SNE of ILC2s using top 20 canonical vectors after canonical correlation (CCA) for the 4 conditions. **(E)** Proportion of treatment condition in each clusters **(F)** Representative differentially expressed genes (*y* axis) by clusters and conditions (*x* axis). Dot size represents the fraction of cells in the cluster that express the gene; color indicates the mean expression (logTPX) in expressing cells, relative to other clusters. Analysis were performed using R package Seurat.

Our results showed that MuHV-4 respiratory infection imprints the function of lung ILC2s following HDM treatment and reduces their capacity to produce type 2 cytokines IL-13 and IL-5. Micro-environment is needed to maintain this phenotype and IFN-γ plays a role in the decrease production of IL-5 cytokine. Single-cell RNA sequencing revealed shared general pattern upon HDM sensitization between mock and MuHV-4 infected groups and highlighted the presence of a sub-population of ILC2s expressing higher MHC-II in MuHV-4 HDM mice compared to their mock HDM counterparts. These differences may have a determining role in the subsequent development of immune responses against respiratory allergens.

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