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A Gammaherpesvirus Affects Lung Resident Group 2 Innate Lymphoid Cells in the Context of HDM-Induced Asthma

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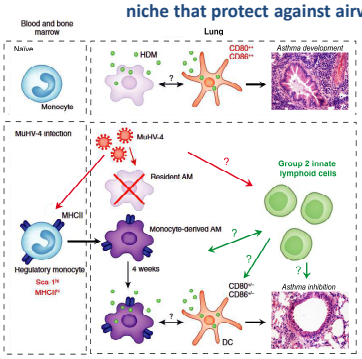
The "Hygiene hypothesis" postulates that allergic diseases could be prevented by some infections in early childhood. Gammaherpesviruses (γ-HVs) are among the most prevalent human viruses and profoundly imprint on the immune system of their hosts. While infection by these viruses generally occurs during childhood, an increased age of seroconversion to these viruses has been observed in westernized countries suggesting that delayed γ-HV infection could contribute to the increased burden of allergic diseases. 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.

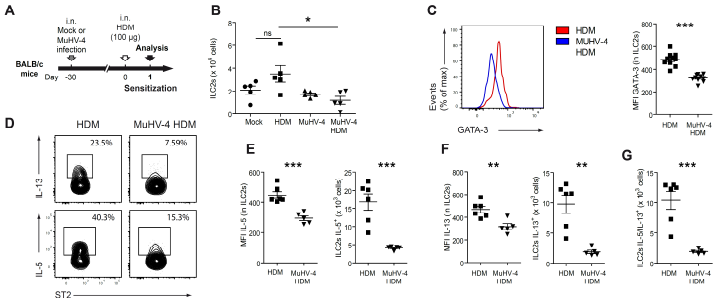
1. Gammaherpesvirus infection induces persisting changes in the alveolar niche that protect against airway allergy



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.

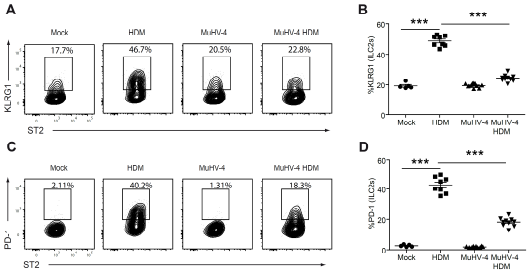
Adapted from Bérengère de Laval & Michael H Sieweke, *Nature Immunology,* News and views, 18(12):1279-1280 (2017).

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



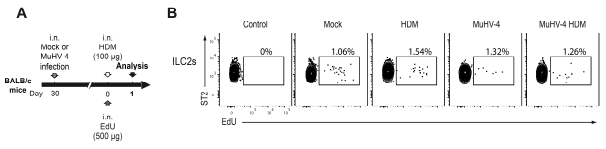
(A) Experimental model of MuHV-4 infection (1.10^6PFU/50µL) and HDM sensitization (100µg/50µL) in 8-week-old BALB/c mice (n = 6 to 10 in each group). **(B)** Quantification of lung ILC2s (live Lin⁻CD45⁺CD127⁺CD90.2⁺ST2⁺CD25⁺) cells, after HDM sensitization (lineage markers : B220, CD11c, CD3, CD4, CD49b, 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. Data were analyzed by 1way-ANOVA and Bonferroni posttests or two-tailed Student's t-test * p<0.05; ** p<0.01; *** p<0.001. Error bars represent SEM.

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



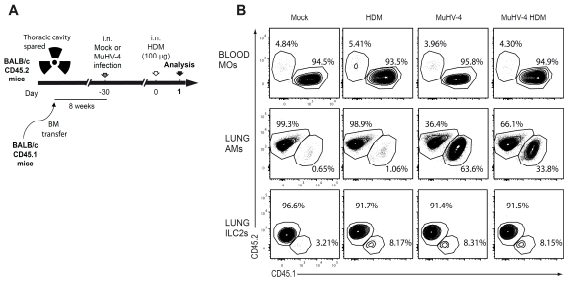
BALB/c mice (n= 5 to 10 in each group) were infected or not with MuHV-4 and submitted 30 days after infection to HDM sensitization (100µg/50µl) **(A)** Representative contour plots of KLRG1 expression on lung ILC2s from the different groups. Numbers in outlined area indicate percent KLRG1+ILC2s. **(B)** 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. Data were analyzed by 1way-ANOVA and Bonferroni posttests *** p<0.001. Error bars represent SEM.

4. MuHV-4 infection does not modify lung ILC2s expansion after HDM sensitization



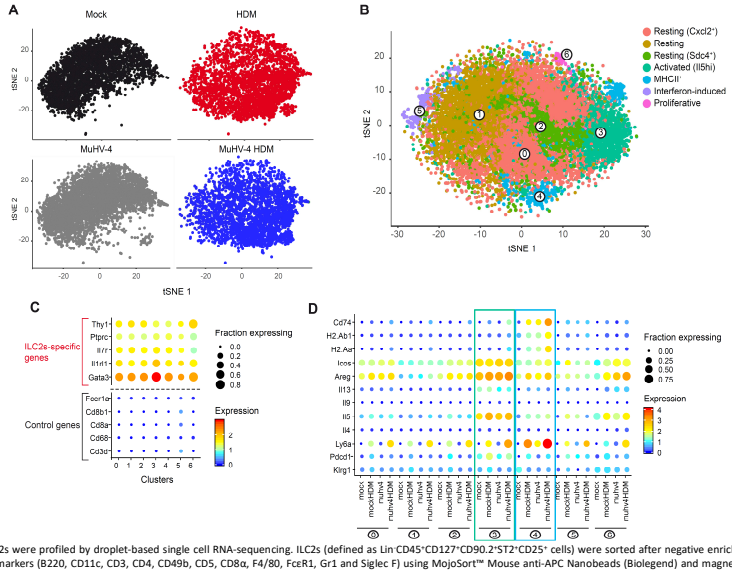
(A) Experimental model of MuHV-4 infection and HDM sensitization in 8-week-old BALB/c mice (n = 5 in each group), submitted to one intranasal instillation of EdU (500µg/50µL) **(B)** Representative contour plots of EdU staining in lung ILC2s from the different groups (control mice did not receive any EdU). Numbers in outlined area indicate percent EdU⁺ILC2s.

5. HDM sensitization initiates recruitment of ILC2s that is not impacted by MuHV-4 infection



(A) Experimental model of bone marrow chimeras : CD45.2⁺ BALB/c mice were exposed to lethal irradiation protocol (7Gy) that preserves the thoracic cavity. These recipient mice were then given 5 x 10^6 BM cells isolated from femur and tibia of CD45.1⁺ congenic donors. 8 weeks after BM transfer, those mice were submitted to MuHV-4 infection and one month later to HDM sensitization protocol. **(B)** Representative contour plots for the evaluation of chimerism between recipient (CD45.2⁺) and donor (CD45.1⁺) cells in different cell populations. Blood monocytes (MOs) were described as live Ly6C⁺ CD11b⁺ cells. AMs were described as live autofluorescent CD11c⁺ cells. Lung ILC2s were described as live Lin⁻CD45⁺CD127⁺CD90.2⁺ST2⁺CD25⁺ cells. Numbers adjacent to the outlined areas (top left and bottom right) indicate percent CD45.2⁺ (host) cells (top area) or CD45.1⁺ (donor) cells (bottom area).

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



Lung ILC2s 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). **(A)** *t*-Distributed stochastic neighbor embedding (t-SNE) plots show 15,846 ILC2s in a nonlinear representation of the top 20 principal components (PCs) from mice infected or not with MuHV-4 (1.10^4PFU/ 50µL) and submitted or not to one instillation of HDM (100µg/ 50µL) 30 days after the infection (n = 6 in each group). **(B)** t-SNE 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)** 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 ILC2s following HDM treatment and reduces their capacity to produce type 2 cytokines IL-13 and IL-5. The increased expression of ILC2s activation markers PD-1 and KLRG1 following HDM treatment was blocked by the infection while proliferation and recruitment of ILC2s were unchanged. 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, ICOS and Ly6a 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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