Study of the interplay between a gammaherpesvirus infection and innate lymphoid cells in the context of type 2 immunity

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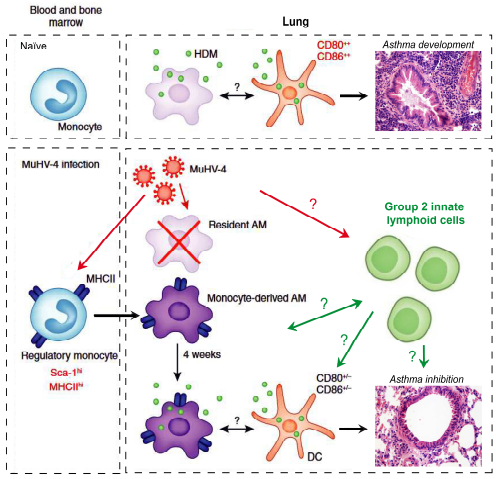
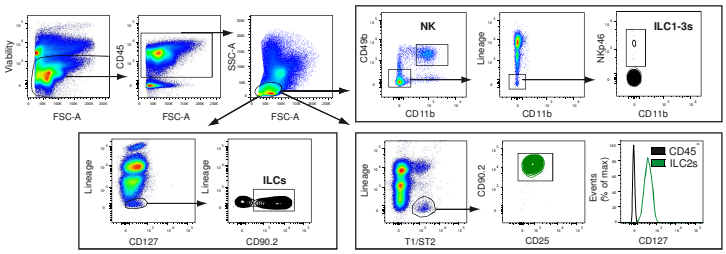
Persistent viruses, such as gammaherpesviruses, profoundly imprint on the immune system of their hosts. Accordingly, we recently showed that infection by Murid herpesvirus 4 (MuHV-4), a gammaherpesvirus infecting mice, inhibits the development of House Dust Mites (HDM)-induced airway allergy. Group 2 innate lymphoid cells (ILC2s) play a major role in the initiation, maintenance and memory of type 2 immune responses. Activation of these cells can be triggered by allergens but also by viruses such as influenza, rhinovirus and respiratory syncytial virus. Here, we therefore investigated whether, and by which potential mechanisms, MuHV-4 infection affects the lung ILC2 compartment.

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

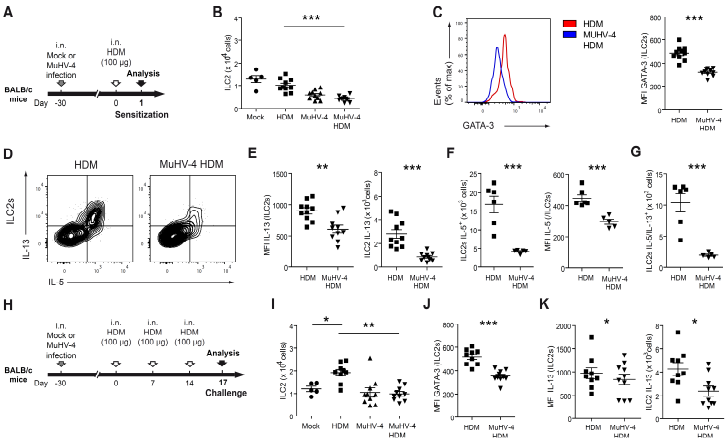


2. Identification of lung NK and ILC subpopulations by flow cytometry strategy



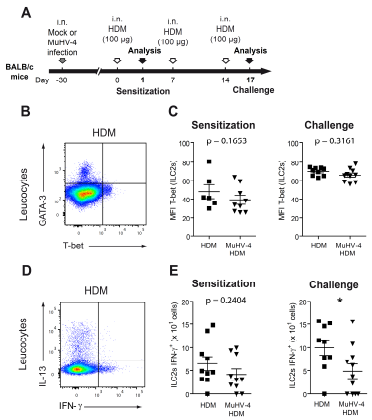
ILCs were described as live, Lin$^-$CD45$^+$CD127$^+$CD90.2$^+$ cells, ILC2s as live, Lin$^-$CD45$^+$CD127$^+$CD90.2$^+$ST2$^+$CD25$^+$ cells, ILC1-3 as live, CD45$^+$Lin$^-$ NKp46$^+$ cells and NK cells as live, CD45$^+$CD11b$^+$CD49b$^+$Nkp46$^+$ cells.

3. MuHV-4 infection affects number and function of lung ILC2s after HDM treatment



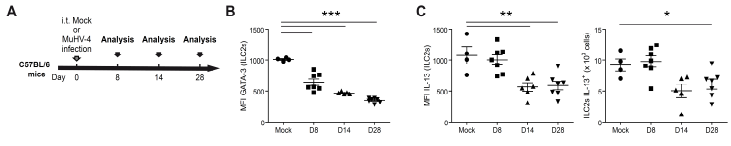
(A) Experimental model of MuHV-4 infection and HDM sensitization in 8-week-old BALB/c mice (n = 5 in mock group and 10 in other groups). **(B)** Quantification of lung ILC2s after HDM sensitization. **(C)** Representative histograms and mean fluorescence intensity (MFI) of GATA-3 staining in ILC2s of 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, control was not restimulated. **(E)** MFI and production of IL-13 and **(F)** IL-5 in lung ILC2s after HDM sensitization. **(G)** IL-5/IL-13 producing ILC2s after HDM sensitization. **(H)** Experimental model of MuHV-4 infection and HDM challenge in 8-week-old BALB/c mice (n = 5 in mock group and 10 in other groups). **(I)** Quantification of lung ILC2s after HDM challenge. **(J)** Representative histograms and MFI of GATA-3 staining in ILC2s of mice infected or not with MuHV-4 and treated with HDM. **(K)** MFI and production of IL-13 in lung ILC2s after HDM challenge. 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.

4. MuHV-4 infection does not induce ILC2 plasticity towards an ILC1 phenotype



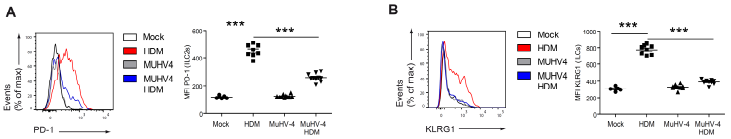
(A) Experimental protocol for MuHV-4 infection (10^4 PFU administered intranasally under anesthesia) and HDM sensitization or challenge in 8-week-old female BALB/c mice (n = 5 in mock group and 10 in each other groups). **(B)** Representative dot plots for the intracellular staining of GATA-3 and T-bet in leucocytes from mice of the HDM group. **(C)** MFI of T-bet in ILC2s after HDM sensitization or challenge. **(D)** Representative dot plots of the intracellular stainings of IL-13 and IFN-γ in leucocytes isolated from lung of HDM mouse. **(E)** IFN-γ production in ILC2s after HDM sensitization or challenge. Data were analyzed by two-tailed Student's t-test * p<0.05. Error bars represent SEM.

5. MuHV-4 infection affects ILC2s activation at early time points



(A) Experimental model of MuHV-4 infection on C57BL/6 mice of 8-week-old (n = 4 in mock and 7 in other groups). **(B)** MFI of GATA-3 staining in lung ILC2s. **(C)** MFI of IL-13 staining in lung ILC2s and number of ILC2s producing IL-13. 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.

6. MuHV-4 infection blocks the increase of PD1/KLRG1 expression in ILC2s after HDM sensitization



(A) Representative histograms and MFI of PD-1 in ILC2s. **(B)** Representative histograms and MFI of KLRG1 in ILCs. 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.

Our results showed that MuHV-4 respiratory infection profoundly imprints the number and function of ILC2s following HDM treatment :

- ✓ by reducing their capacity to produce type 2 cytokines IL-13 and IL-5 after HDM sensitization or challenge
- ✓ by decreasing their PD-1 and KLRG1 surface expression which have been associated with ILC2s activation
- ✓ as early as 8 days post-infection

Overall, these results show that MuHV-4 infection significantly and sustainably affects the lung ILC2s population. This may have a determining role in the subsequent development of immune responses against respiratory allergens.

In the future, we want to test the effect of MuHV-4 infection on both resident and inflammatory ILC2s and to identify the mechanisms triggering these differences.

We thank L. Dams, C. Delforge, E. Deglaire, A. Vanderlinden and C. Espert for excellent technical and secretary assistance. This work was supported by the following grants VIR-IMPRINT ARC of the University of Liège, Institut MERIEUX starting grant and "credit de recherche" n° J007515F and "projet de recherche" n° T.0195.16 of the "Fonds de la Recherche Scientifique - Fonds National Belge de la Recherche Scientifique" (F.R.S. - FNRS). P.L. is a research fellow of the F.R.S./FNRS.