UNIVERSITY OF LIEGE
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF INFECTIOUS DISEASES
LABORATORY OF IMMUNOLOGY-VACCINOLOGY

Modulation des cellules lymphoïdes innées de type 2 par les gammaherpèsvirus dans le contexte de l'asthme allergique.

Modulation of type 2 innate lymphoid cells by gammaherpesviruses in the context of allergic asthma.

Pauline LOOS

THESIS PRESENTED IN ORDER TO OBTAIN THE DEGREE OF
Doctor of Veterinary Sciences

ACADEMIC YEAR 2021-2022

Abbreviations

α-CGRP	α-calcitonin gene-related peptide
αLP	α-lymphoid precursor
γHV	Gammaherpesvirus
AEC	Airway epithelial cell
AHR	Airway hyperresponsiveness
AM	Alveolar macrophages
APC	Antigen presenting cell
Areg	Amphiregulin
ATAC-seq	Assay for Transposase-Accessible Chromatin using sequencing
BAC	Bacterial artificial chromosome
BALF	Bronchoalveolar lavage fluids
BATF	Basic leucine-zipper transcription factor
BM	Bone marrow
BV	Blood vessel
CHILP	Common helper-like ILC
CILCP	Common ILC progenitor
CLP	Common lymphoid precursor
COPD	Chronic obstructive pulmonary disease
CysLTs	Cysteinil leukotrienes
DC	Dentritic cell
DNA	Deoxyribonucleic acid
EBNA	Epstein-Barr virus nuclear antigen 1
EBV	Epstein-Barr virus
EET	Eosinophil extracellular traps
ECAR	Extracellular acidification rate
EILCP	Early innate lymphoid cell progenitor
EToP	Early tonsillar progenitors
GAG	Glycocaminoglycans
GATA	Gata-binding protein
GCM	Goblet cell metaplasia
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Genome maintenance protein
HCMV	Human cytomegalovirus
HDM	House dust mite
HMGB1	High mobility group 1
HSV	Herpes simplex virus
HV	Herpesvirus
ICAM	Intercellular cell adhesion molecule
ICOS	Inducible T-cell costimulator
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cell
ILCP	Innate lymphoid cell progenitor
IRF	Interferon regulatory factors
KSHV	Kaposi’s sarcoma-associated herpesvirus
LANA	Latency-associated nuclear antigen
LTD4	Leukotriene D4

Lti	Lymphoid tissue inducer
M-CSF	Macrophage colony stimulating factor
MHC	Major histocompatibility complex
Mo	Monocyte
MuHV-4	*Murid gammaherpesvirus 4*
NET	Neutrophil extracellular trap
NK	Natural Killer cell
NKP	Natural Killer cell precursor
NPC	Nuclear pore complex
OCR	Oxidation consumption rate
ORF	Open reading frame
OXPHOS	Oxidative phosphorylation system
OVA	Ovalbumin
NMU	Neuromedin-U
PAMPs	Pathogen associated molecular patterns
PRR	Pattern recogition receptor
qCLASH	Quick crosslinking, ligation and sequencing of hybrids
RAG	Recombination-activating genes
RCA	Regulators of complement activation
RNA	Ribonucleic acid
ROR	Retinoid-related orphan receptor
RSV	Respiratory syncitial virus
RV	Rhinovirus
scRNA-seq	Single-cell RNA-sequencing
SMH	Smooth muscle hypertrophia
TAP	Transporter associated with antigen processing
TF	Transcription factor
TGF	Transforming growth factor
TH	T helper
TBM	Thickening of basement membrane
Trm	Resident memory T cell
TSLP	Thymic stromal lymphopoietin
VCAM	Vascular cell adhesion molecule
VIP	Vasoactive intestinale peptide
WT	Wild-type

Résumé - Summary

Résumé

Selon l'hypothèse de l'hygiène, l'exposition durant l'enfance à certains micro-organismes protège du développement de maladies allergiques telles que l'asthme. Les gammaherpèsvirus (γHVs) font partie des virus humains les plus répandus. L'infection par ces virus survient dès le jeune âge et modifie profondément le système immunitaire de leurs hôtes. En utilisant le *Gammaherpèsvirus murin 4* (MuHV-4), modèle murin pour le virus humain d'Epstein-Barr, notre laboratoire a montré que l'infection par un γHV peut inhiber le développement de l'asthme allergique. Cependant, les mécanismes par lesquels l'allergie des voies aériennes est contrôlée dans ce contexte sont encore mal définis. Les cellules lymphoïdes innées de type 2 (ILC2s) jouent un rôle majeur dans l'asthme et peuvent être modulées par des infections virales respiratoires, nous avons donc cherché à comprendre si et comment l'infection par le MuHV-4 affecte le compartiment pulmonaire des ILC2s.

Dans cette thèse, nous montrons que l'infection par le MuHV-4 réduit considérablement la capacité des ILC2s pulmonaires, résidentes ou recrutées à partir de la moelle osseuse, à s'épandre et à produire des cytokines de type 2 en réponse aux allergènes d'acariens, ce qui a un effet direct sur le développement de l'asthme allergique. Ces modifications dépendent du microenvironnement et surtout de la production d'IFN-γ après l'infection virale. Contrairement à ce qui a été rapporté pour d'autres infections, les ILC2s pulmonaires de souris infectées par le MuHV-4 ne présentent aucune caractéristique de plasticité vers un phénotype ILC1 mais affichent une expression réduite du facteur de transcription pro-T helper 2 (TH2), GATA 3. De manière importante, nous avons mis en évidence un crosstalk étroit entre ILC2s et macrophages alvéolaires déterminant les propriétés fonctionnelles de type 2 de ces derniers. L'infection par le MuHV-4 induisant une déplétion des macrophages alvéolaires embryonnaires résidents, nous avons identifié les ILC2s pulmonaires comme des acteurs essentiels au sein de la niche pour conférer une identité spécifique aux macrophages alvéolaires dérivés des monocytes après infection par le MuHV-4. Ces résultats révèlent que l'infection persistante par le MuHV-4 façonne le paysage alvéolaire bien au-delà de l'infection aiguë initiale en réprimant sur le long terme la fonctionnalité des cellules de la niche alvéolaire telles que les ILC2s.

Summary

The hygiene hypothesis states that early childhood exposure to particular microorganisms protects against allergic diseases such as asthma. Gammaherpesviruses (γHVs) are among the most prevalent human viruses. The infection by these viruses occurs at early age and profoundly imprints the immune system of their hosts. Using *Murid gammaherpesvirus 4* (MuHV-4), a mouse model of human γHV infections, our laboratory showed that a γHV infection can inhibit the development of allergic asthma. However, the mechanisms by which airway allergy is controlled in that context are still ill-defined. As type 2 innate lymphoid cells (ILC2s) play a major role in asthma and can be modulated by respiratory virus infections, we investigated whether MuHV-4 infection affects the lung ILC2s compartment.

Here, we showed that γHV infection substantially reduces the capacity of both lung resident and bone marrow-derived type 2 innate lymphoid cells to expand and to produce type 2 cytokines in response to house dust mite allergens. These modifications appeared to be related to the microenvironment and especially to production of IFN-γ. In contrast with reports from other viral infections, ILC2s from MuHV-4 infected mice did not exhibit any characteristic of plasticity towards an ILC1 phenotype but displayed decreased expression of the canonical TH2 transcription factor GATA 3. Importantly, we demonstrated a tight crosstalk between ILC2s and alveolar macrophages determining the type 2 functional properties of these cells. As MuHV-4 infection induces a profound depletion of embryonic resident alveolar macrophages, we identified pulmonary ILC2s as key players within the niche to confer a specific identity to monocyte-derived alveolar macrophages after MuHV-4 infection. These results reveal that persistent MuHV-4 infection shapes the alveolar landscape much beyond the initial acute infection by repressing the functionality of alveolar niche cells such as ILC2s over the long term.

Preamble

Preamble

This PhD work focused on deciphering the impact of a γHV infection on lung ILC2s and the mechanisms underlying the protection conferred by these viruses against HDM-induced airway allergy.

We investigated the influence of MuHV-4 infection on subsequent HDM-induced airway allergy by studying its impact on pulmonary ILC2s. During type 2 immunity, ILC2s produce important amount of type 2 cytokines leading to the three main symptoms of asthma, namely mucus hyperproduction, eosinophilic inflammation and airway hyperresponsiveness. After respiratory viral infection, ILC2s can play two roles: on one hand, they can exacerbate the lung inflammation and, on the other hand, they can maintain pulmonary homeostasis. Although ILC2s have been extensively studied in the context of asthma or viral infections exacerbating asthma, their exact role following a persistent immunomodulatory viral infection has remained unclear.

The purpose of the following introduction is to provide an overview of various theoretical concepts and recent findings that may help in understanding this work. To this end, chapter 1 describes asthma both in a general epidemiological way and more specifically from an immunological point of view. The second chapter is devoted to γHVs and how they evade immune responses. The final chapter focuses on ILC2s, from their discovery and critical role in asthma pathology to a comprehensive review of the latest findings about their modulation by viral infections. After the introduction, the experimental section consists in a study entitled "*A persistent gammaherpesvirus infection reprograms the alveolar macrophage niche for the long-term by dampening type 2 properties of group 2 innate lymphoid cells*". This manuscript concludes by a discussion including the summary of the major results obtained and the perspectives and questions raised by this work.

Introduction

1 Asthma

1.1 General information

Asthma is one of the most common chronic, non-communicable, respiratory disease in the world. It is characterized by episodes of wheezing, coughing, chest tightness, and shortness of breath. It reflects more a syndrome than a single disease and the global prevalence is not easy to assess as the diagnosis is primarily based on symptoms. Asthma rates around the world have been steadily increasing from 1960 to the present (Anandan *et al.*, 2010; Loftus and Wise, 2016). In 2017, prevalence varied between 1 and 14% depending on the countries (**Figure 1**). The last report showed that over 250 million people were affected and one thousand died every day from the disease in 2019 (Vos *et al.*, 2020).

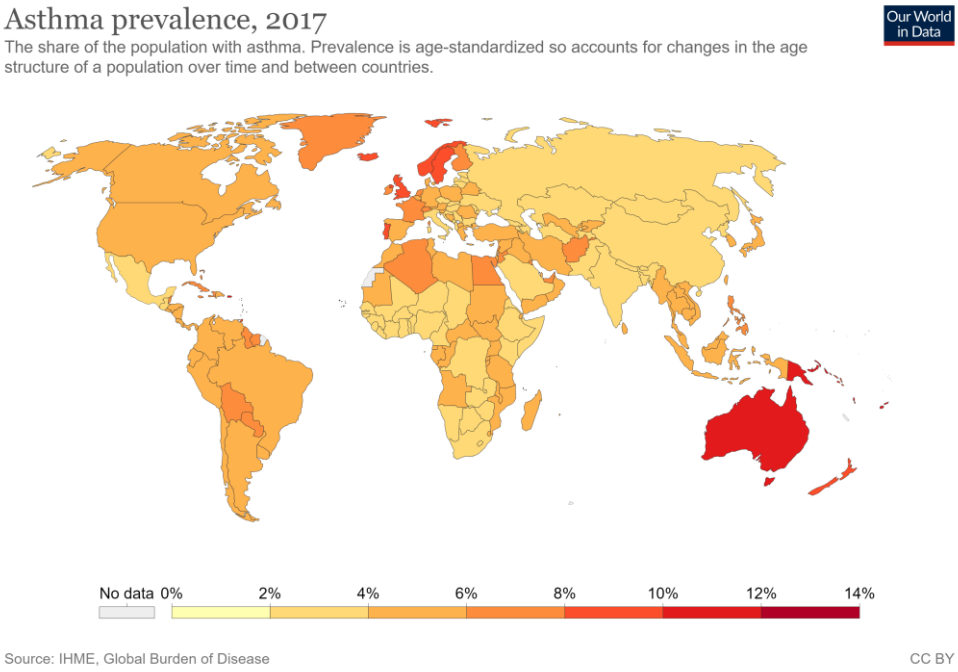


Figure 1. Asthma wordwide prevalence in 2017.

Distribution of asthma cases according to geographical area. Lower rates are observed in Asia, Eastern Europe and Africa. The prevalence varies from 0 to 14% (see color scale). Data are not available for uncolored areas. (https://ourworldindata.org/grapher/asthma-prevalence).

1.2 Pathogenesis

Asthma usually starts in childhood (early-onset) and mainly affects children but it can also develop later in life (late-onset). Early-onset asthma is usually associated with allergy and less severe disorders than late-onset asthma occurring in adulthood (Hammad and Lambrecht, 2021). Symptoms fluctuate over time and respiratory failure can be precipitated by respiratory viral infection or exposure to aeroallergens and air pollutants. An inadequate treatment may lead to substantial morbidity and

mortality. However, in most cases, proper management can control the triggers and reduce the severity of asthma, which can allow people to enjoy a normal quality of life (Global Asthma Report, 2020). The mainstay of asthma therapy remains on the recurrent inhalation of corticosteroids and a short- or long-acting β2 adrenergic agonist (Global Asthma Report, 2020). Nevertheless, 5 to 10% of patients are refractory to corticosteroid treatment (Lambrecht and Hammad, 2015). Medication is the major contributor to the overall cost of asthma (Dierick *et al.*, 2020). Undoubtedly, asthma remains a global health and socio-economic burden.

This chronic pulmonary pathology is associated with airway remodeling and narrowing, mucus hypersecretion and airway hyperresponsiveness/hyperreactivity (AHR) (**Figure 2**). Most of time AHR, which is a bronchospasm easily triggered by non-specific stimuli, is reversible with the use of bronchodilators but, unfortunately, in some severe forms of asthma, the resulting airway remodeling remains permanent. As a matter of fact, smooth muscle hyperplasia, goblet cell metaplasia, excessive subepithelial collagen deposition and mucus plugs can lead to deleterious airway obstruction (Dunican *et al.*, 2018).

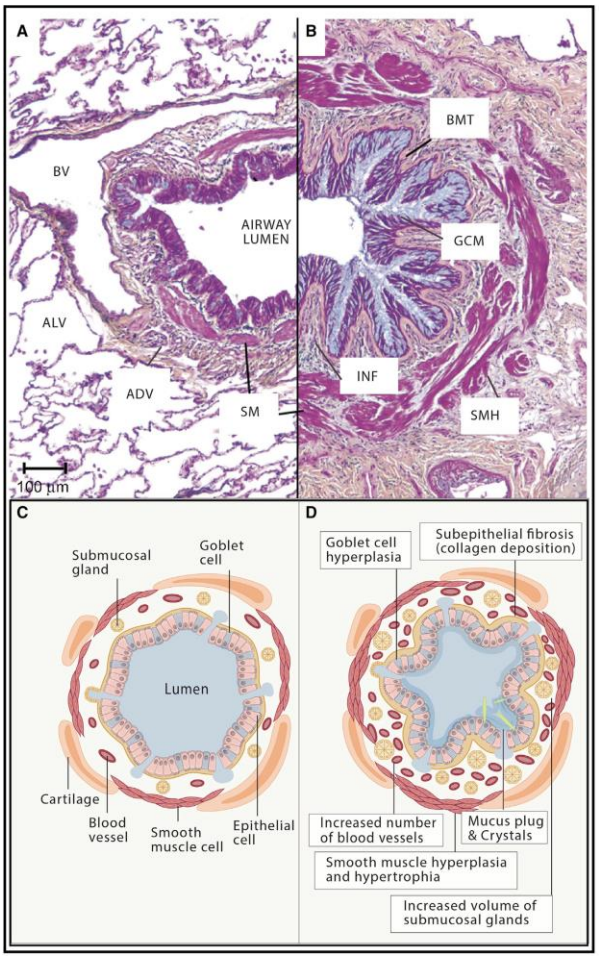


Figure 2. Asthma features.

Histology and schematic representation of healthy control lung (A & C) and lung of a patient with severe asthma (B & D). Asthma is associated with airway inflammation (INF), airway remodeling such as tickening of the basement membrane (TBM), smooth muscle hypertrophia (SMH) and goblet cell metaplasia (GCM) (Lambrecht, Hammad and Fahy, 2019). Abbreviations: ALV: alveoli; ADV: adventitia; BV: blood vessels; SM: smoot muscle.

Historically, two main types of asthma have been described: allergic (childhood-onset) and non-allergic asthma (late-onset) (Romanet-Manent *et al.*, 2002). Allergic asthma, which mainly affects children and approximatively 50% of adults, is characterized by a type 2 - allergic immune response (described in detail later) (Lambrecht and Hammad, 2015). The childhood-onset disease is associated to allergy and most often begins with the "atopic march": a progressive increase in symptoms starting with allergic sensitization, often accompanied by eczema and later by allergic rhinitis. Asthma is highly heritable with genetic contribution estimated to be greater than 50% for early-onset asthma (Thomsen *et al.*, 2011). Unlike the allergic form, non-allergic asthma (late-onset) is not associated to any response to allergens and is precipitated by unnocious stimuli such as air pollution, cold, medications and exercise. It is sometimes resistant to glucocorticoid treatment and mostly observed in women and often associated with obesity, aging and smoking (Pakkasela *et al.*, 2020).

However, the allergic and non-allergic classification turned to be an oversimplification for clinical purposes and, in fact, asthma can be divided into several phenotypes or endotypes (Lötvall *et al.*, 2011; Wu *et al.*, 2014). Phenotypes are relied on distinct visible properties of asthma (Wu *et al.*, 2014) and endotypes are based on the interplay of wide range of components, including genetic susceptibility, environmental risk factors, age of onset, clinical presentation and response to standard and novel therapies (Lötvall *et al.*, 2011). Endotypes shape better diagnostic and therapeutic tools in support of stratified/personalized interventions based on recognition of differences in responsiveness to various therapeutic interventions (Cevhertas *et al.*, 2020). In summary, there are two mains types of endotypes: type 2-high and type 2-low asthma (**Figure 3**).

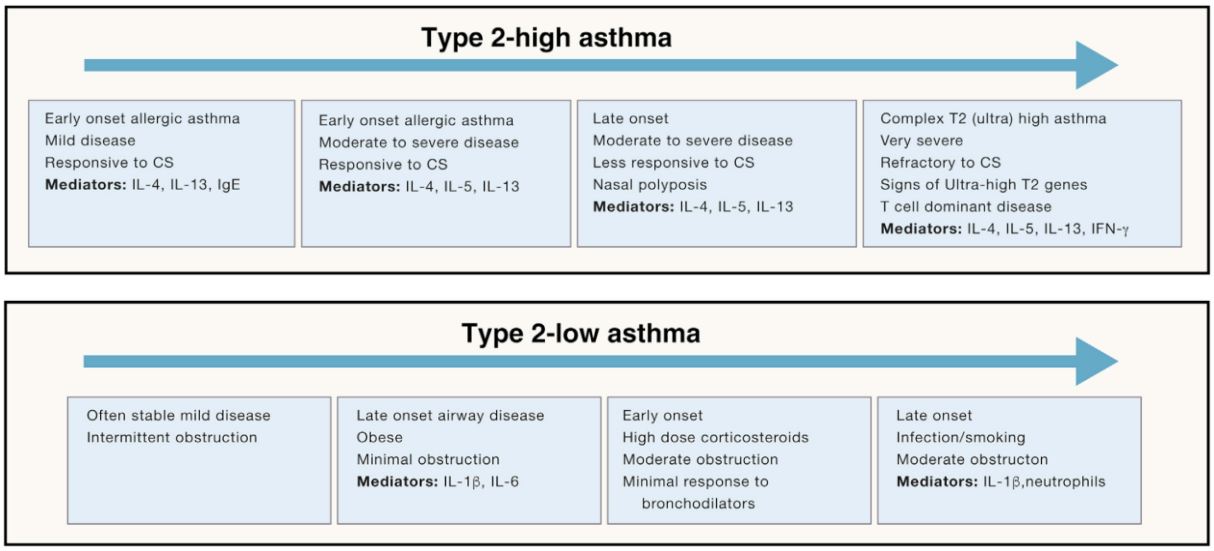


Figure 3. Endotypes of asthma.

Type 2-high/ultra-high asthma: mainly eosinophilic (blood and airway), orchestrated by T helper (TH)2-associated cytokines such as interleukin (IL)-4, IL-5 and IL-13, with ultra-type 2-high asthma reflecting a more severe form of the disease.

Type 2-low asthma: non-eosinophilic and usually neutrophilic. Without clear definition, it includes most of patients without any sign of type 2-high inflammation (Hammad and Lambrecht, 2021).

The diagnosis of asthma is typically based on the symptoms and response to treatment combined with the measurement of expiratory airflow. Serum immunoglobulin (Ig) E antibodies assay and positive skin-prick test to the proteins of common inhaled allergens help to confirm the allergic form. It is interesting to note that serum IgE titers are ultimately poor predictors of a type 2 immune response signature and other more accurate biomarkers could be used to assess the level of tissue eosinophilia such as level of IL-25 in serum, although they are not commonly used (Cheng *et al.*, 2014). In the end, only 50% of asthmatics show clear evidence of type 2 immunity in the airways, the canonical marker of allergic-asthma (Fahy, 2014).

Finally, asthma can also be classified according to its immunological aspects on sputum/bronchoalveolar lavages analysis (Simpson *et al.*, 2006; Schleich *et al.*, 2013):

- Eosinophilic
- Non-eosinophilic (neutrophilic type 1 and type 17 and paucigranulocytic)
- Mixed granulocytic

1.3 Immunological mechanisms

This thesis focuses on understanding allergic asthma and for this purpose we used, in the experimental part, the allergic airway model induced by instillations of a common allergen, house dust mite (HDM, *Dermatophagoides farinae)*. Therefore, this chapter will mainly describe the development of the corresponding eosinophilic – allergic - type 2-high asthma (Hammad *et al.*, 2009). It is the result of an aberrant TH2 immune response to one or more common allergens – innocuous environmental proteins - like HDM (*Dermatophagoides farinae-pteronyssinus*), pollen, cockroach (*Blatella germanica*), fungi (*Aspergillus fumigatus, Alternaria alternate*) or animal dander (Chitin) (Haspeslagh *et al.*, 2017).

1.3.1 Allergic sensitization

The first encounter with an allergen leads to the development of the innate immune response corresponding to allergic sensitization (**Figure 4**). From an immunological point of view, upon entering the lung, allergens first face the epithelial cells barrier, mediator of the allergic response. Through proteolytic activity, some allergens such as HDM or cockroach allergens have the ability to disrupt intercellular junctions (Wan *et al.*, 1999). The respiratory epithelial cells are stimulated by allergens through pattern recognition receptors (PRRs) binding pathogen associated molecular patterns (PAMPs) (Harker and Lloyd, 2021). Activation of PRRs leads to almost immediate production of chemokines and cytokines by epithelial cells. In murine models of eosinophilic asthma, the alarmins IL-33, IL-25 and Thymic Stromal Lymphopoietin (TLSP) are produced within minutes to hours, in a TLR4/Myd88-dependent manner following allergens encounter (Hammad *et al.*, 2009; Willart *et al.*, 2012; Cayrol *et*

al., 2018). Various components of HDM stimulate the same pathways in human epithelial cells *ex vivo* (Smole *et al.*, 2020). The severity of human asthma is related to epithelial cell damage and high levels of IL-33, IL-25 and TSLP (Cheng *et al.*, 2014; Li *et al.*, 2016). Their roles is undeniable as isolated blockade of IL-25, IL-33 and TSLP as well as a combined blockade has been shown to reduce airways inflammation and AHR in murine asthma model (An *et al.*, 2020). Production of granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF) and transforming growth factor β (TGF-β) have also been described in mouse model of airway inflammation but their roles in humans are currently not clearly defined (Willart *et al.*, 2012; Denney *et al.*, 2015; Moon *et al.*, 2018).

Lung ILC2s are activated by epithelial-derived alarmins and, in response, produce canonical type 2 cytokines such as IL-5, IL-9 and IL-13 that directly control asthma features such as eosinophil recruitment, goblet cell metaplasia and AHR (Morita, Moro and Koyasu, 2016). As ILC2s are central components of this study, Chapter 3 (See: 3. Type 2 innate lymphoids cells) will be devoted entirely to them. The combination of epithelial-derived cytokines and IL-13 produced by ILC2s provides the stimuli to prime conventional type 2 dendritic cells (cDC2s) and induce their migration to the draining lymph node where they direct a TH2 cell response (Hammad and Lambrecht, 2008; Halim *et al.*, 2015). A subtype of conventional DCs-cDC1 that promotes airway tolerance (Khare *et al.*, 2013) is also found in type 2-high patients and their expression of the IgE receptor – FcεRI - suggests a potential role in TH2 airway inflammation (Dutertre *et al.*, 2019; Naessens *et al.*, 2020). The depletion of $CD11c^{+}$ DCs in murine asthma model suppressed all features of the disease, highlighting their essential role in the mechanism of asthma (Van Rijt *et al.*, 2005).

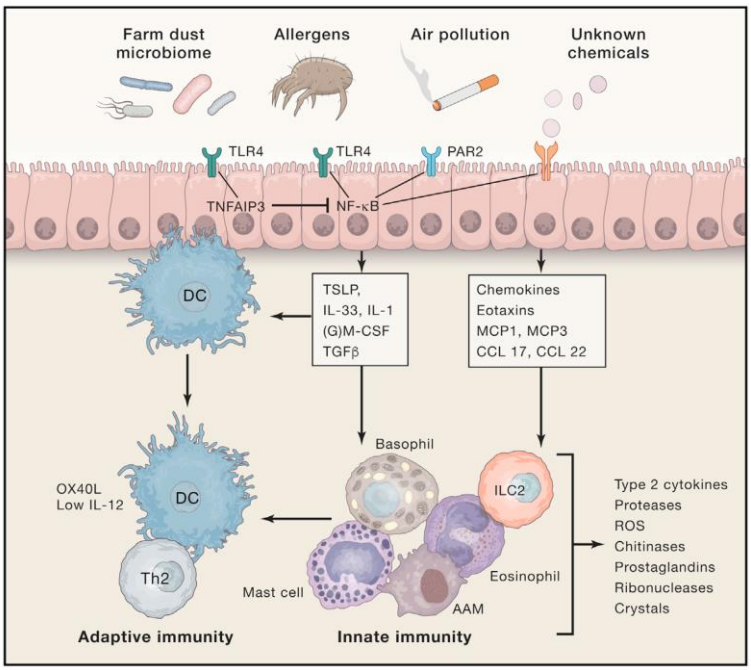


Figure 4. Sensitization.

The early innate immune response leading to asthma is driven by alarmins released from the damaged epithelium. Those cytokines activate adaptive as well as innate immune cells to produce numerous mediators that contribute to airway inflammation (Hammad and Lambrecht, 2021).

Epithelial damage, DC migration and TH2 priming trigger production of IL-4 that stimulates B cells. The activation of B cells results to class switching of IgG to IgE. The role of IgE in asthma is complex. They can bind to the high-affinity receptor FcεRI which is expressed on basophils, mast cells, eosinophils, and DCs but also on airway smooth muscle cells, endothelial cells and epithelial cells (Redhu and Gounni, 2013). Interaction between IgE and mast cells induces the degranulation process resulting in the release of inflammatory mediators (preformed biogenic amines (histamine), enzymes and proteoglycans) followed by the release of newly synthesized lipid mediators (prostaglandin D2, leukotrienes and platelet-activating factor) which all reinforce the ongoing pro-inflammatory TH2 environment. Activated mast cells trigger mucous hyperproduction by goblet cells, increase vascular permeability and contribute to smooth muscle hypertrophy and establishment of AHR (Elieh Ali Komi and Bjermer, 2019). This leads to chemotaxis of more inflammatory cells producing cytokines (TNF, IL-1, IL-4, IL-5, IL-6, IL-13, CCL3, CCL4) that mediate late-phase inflammatory reactions. In addition to their role in mast cell activation, IgE promote AHR by directly activating, proliferating and contracting airway smooth muscle cells (Ferreira *et al.*, 2018). Crosslinking between IgE and DCs can also facilitate allergen presentation to memory TH2 lymphocytes (**Figure 5**).

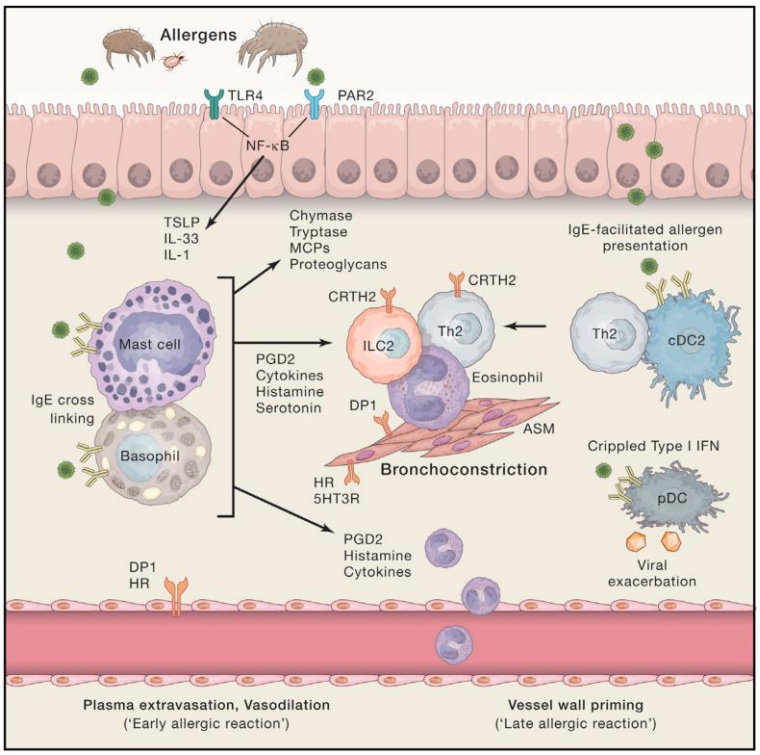


Figure 5. Complex role of IgE in asthma.

Crosslinking between IgE and mast cells induces degranulation process that leads to the release of inflammatory mediators which enhance the existing pro-inflammatory TH2 environment. IgE crosslinking on antigen-presenting cells (APC) facilitates allergen presentation and contributes to the late allergic response. Similarly, when allergens crosslink IgE on APC, this may decrease antiviral properties, making the asthmatic person more susceptible to developing a viral infection (Hammad and Lambrecht, 2021).

In parallel with mast cell activation, eosinophils are activated by IL-5, which promotes their maturation and egress from bone marrow (BM). Eosinophils produce a wide range of proteins such as cytotoxic granules, acute proinflammatory cytokines, chemokines and lipid mediators all contributing to pathogenic mechanisms of asthma. In some specific cases, eosinophils can form extracellular deoxyribonucleic acid (DNA) fibers grouped under the name of eosinophil extracellular traps (EETs) for which new roles in asthma seem to be described through ILC2s activation (Choi *et al.*, 2020). Persistent production of eosinophils-derived exosomes (extracellular nanovesicles containing multiple molecules, proteins and lipid mediators) can also lead to structural damage in the lung (Cañas *et al.*, 2018; Kanda *et al.*, 2020).

Basophils are recruited to the lung after inhalation of allergens and produce IL-4 and IL-13 (Hammad *et al.*, 2010; Motomura *et al.*, 2014). Recently described in asthma, neutrophils are recruited to the lungs of symptomatic allergic asthma patients and may play a deleterious pro-inflammatory role, although an immunoregulatory role is also suggested. (Radermecker *et al.*, 2018). In some cases of severe asthma, the formation of neutrophil extracellular traps (NETs) may induce epithelial cell damage and contribute to increased mucus production (Uddin *et al.*, 2019).

1.3.2 Allergic challenge

Subsequent encounters with allergens constitute the allergic challenge. Briefly, after re-exposure to the allergen, previously activated TH2 cells secrete the cytokines IL-4, IL-5, IL-9 and IL-13. TH2 resident memory T cells (Trm) reside in the lung very long after exposure to allergens and are able to quickly respond and produce more TH2 cytokines after re-exposure (Hondowicz *et al.*, 2016).

- IL-4 induces differentiation of naive helper T cells to TH2 cells. It also stimulates B cells to secrete IgE and IgG1 and promotes the extravasation of eosinophils to the inflamed airways by the expression of ICAM-1 (intercellular cell adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule) (Cohn *et al.*, 1997; Godar *et al.*, 2018). IL-4 is produced primarily by mast cells, TH2 cells, eosinophils and basophils (Gadani *et al.*, 2012).
- IL-13 induces mucus production, goblet cell metaplasia and AHR and is mainly produced by the TH2 cells and ILC2s (Liang *et al.*, 2012; Wolterink *et al.*, 2012). IL-13 signaling begins with a multi-subunit receptor shared with IL-4, the type II IL-4 receptor: a heterodimeric complex composed of IL-4 receptor alpha (IL-4Rα) and IL-13 receptor alpha (IL-13Rα1) (Nelms *et al.*, 1999). The type I IL-4 receptor is formed by IL-4/IL-4Rα with γc (Nelms *et al.*, 1999).
- IL-5 drives the recruitment, activation, proliferation and survival of eosinophils in blood and tissue (Kung *et al.*, 1995). It is produced by TH2 cells and ILC2s (Wolterink *et al.*, 2012).

- IL-9 can be produced by TH2 cells, TH9 cells, eosinophils and neutrophils as well as ILC2s (Gounni *et al.*, 2000; Veldhoen *et al.*, 2008; Sun *et al.*, 2018; Seumois *et al.*, 2020). Its precise role in type-2-high asthma remains undefined but it seems to promote mast cell survival, mucus cell metaplasia and airway wall remodeling (Kearley *et al.*, 2011).

Monocytes-derived DC (MoDCs) enhance tissue TH2 responses by restimulating the effector functions of resident pulmonary lymphocytes and recruiting additional effector TH2 lymphocytes through the release of chemokines CCL-17 and CCL-22 (Lambrecht and Hammad, 2015). Recognition of IgE-allergen complexes by FcεRI receptors present on mast cells, MoDCs and some macrophages also amplifies the asthmatic TH2 response (Khan and Grayson, 2010; Lambrecht and Hammad, 2015).

All previous factors lead to the characteristics of allergic asthma including airway obstruction, AHR, hypertrophy of bronchial smooth muscle cells, airway eosinophilia, mucus overproduction and IgE synthesis. As demonstrated, asthma cannot be reduced to eosinophils recruitment and mast cells degranulation; but rather results in a complex communication network between structural and innate or adaptative immune cells.

1.4 Asthma and viral infections

1.4.1 Exacerbation of asthma pathogenesis

Asthma can be hastened by different stimuli such as allergens, pollution, cold air and infectious agents. Some respiratory viruses are strongly associated with asthma exacerbations or can even promote its development (Denlinger *et al.*, 2017). Actually, these infections can conduct to early acute bronchiolitis or severe wheezing leading to subsequent asthma that may persist into adulthood (Bergroth *et al.*, 2020).

Early-life wheezing-associated respiratory tract infection by human rhinovirus (RV) and respiratory syncitial virus (RSV) are considered main risk factors for asthma development (Rubner *et al.*, 2017). The widespread RV, particularly the subtypes A and C among the many different strains, is the most frequent trigger for exacerbation of allergic asthma (Rajput *et al.*, 2020). Typically, it prones a strong type 2 immune response in the lung, with epithelial cells damages, which may aggravate previous asthma or even promote its development (Kotaniemi-Syrjänen *et al.*, 2003; Jackson *et al.*, 2008). The second virus responsible for asthma exacerbation is human RSV, which is the main agent associated with bronchiolitis in 10-30% of children (Kusel *et al.*, 2007). The infection can drive severe lung disease in infants that may result in persistent airway inflammation leading to recurrent wheezing and asthma development (Kusel *et al.*, 2007). RSV-dependent bronchiolitis provokes airway epithelial cell (AEC) necroptosis (Simpson *et al.*, 2020).

Influenza virus can also promote asthma (Mahut *et al.*, 2011), and again, likely through epithelial-derived alarmins release. Ravanetti et *al.* showed that production of IL-33 following influenza infection increased airway inflammation by instructing epithelial cells and DCs, from HDM-sensitized mice, to dampen interferon (IFN)-β expression and prevent the TH1-promoting DC phenotype (Ravanetti *et al.*, 2019).

Other viruses have also been reported to exacerbate asthma: enteroviruses (Jartti *et al.*, 2004), metapneumoviruses, bocaviruses, parainfluenza virus (Coverstone, Wang and Sumino, 2019), adenoviruses and coronaviruses (Jartti and Gern, 2017).

The main mechanism explaining the aggravation of asthma following a viral infection seems to lie on the alteration of the epithelium and the ensuing inflammation that impairs the healing capacity of airway epithelial and immune cells. Persistent neutrophilia could lead to asthma development and important amount of NETs are reported in RV lung diseases (Uddin *et al.*, 2019), as well as neutrophilic inflammation in children with both mild and severe RSV-diseases (Sebina and Phipps, 2020). Moreover, TH17 differentiation and EETs are recently discovered mechanisms in RSV immunopathology that are related to severe asthma (Newcomb and Peebles, 2013; Qin *et al.*, 2019; Silveira *et al.*, 2019). A bold hypothesis suggests a potential role of IgE binding. Indeed, during viral infections, bona fide inflammatory cDC2s acquired characteristics traditionally defining cDC1 and macrophages in a type I IFN dependent manner. cDC2s induced by type I IFN ($IFNAR^+$) express high levels of Fc receptors and are able to mount specific antiviral responses through CD4 and CD8 T cells activation (Bosteels *et al.*, 2020). It is then postulated that, in a TH2-prone environment, such as type 2-high asthma, the low levels of type I IFN present in the lung impact these $IFNAR^+$ cDC2s, resulting in less virus-induced migration to the draining lymph nodes (**Figure 4**) (Hammad and Lambrecht, 2021). Therefore, this may contribute to the increased susceptibility of asthmatics to respiratory viral infections that cause asthma exacerbation. Overall, respiratory viruses primarily infect lung epithelial cells, modulate the airway epithelial barrier, and subsequently lead to altered immunity to allergic sensitization (Michi, Love and Proud, 2020; Veerati *et al.*, 2020).

Furthermore, the weakening of asthmatic airways can also cause a viral infection to worsen. In response to virus, healthy lung epithelial cells promote the production of antiviral factors leading to viral clearance, which is impaired in chronic airway inflammation, resulting in sustained inflammation and exacerbation of symptoms (Busse, Lemanske and Gern, 2010). Also, IFN signaling, a key antiviral weapon, is commonly impaired in asthmatic patients and may therefore render them more vulnerable to infections. IFN was originally thought to be an asthma inhibitor but surprisingly numerous IFN-γ-producing TH1 cells are found in mild to moderate asthma (Raundhal *et al.*, 2015). Some studies focused on the potential role of IFN cytokines in the pathogenesis of asthma, as dysregulation of IFNs pathway

is commonly observed in asthmatics patients (Rich *et al.*, 2020). Altered IFN pathways allow the virus to spread easily and to induce more damages and inflammation, leading to asthma exacerbation (Altman *et al.*, 2019). Other explanation may be related to reduced number of classical activated M1-Alveolar macrophages (AMs), which are known to have a major roles in host defense against various microbial pathogens, observed in bronchoalveolar lavage fluids (BALF) analysis of patients with RV-induced asthma exacerbations, supporting the fact that allergic patients have deficient antiviral immunity compared with healthy ones (Nikonova *et al.*, 2020).

Asthma is not an aggravating factor for all respiratory viral infections. Indeed, patients with type 2-asthma were not overrepresented among those with severe COVID-19, the ongoing pandemic that began in 2019 and is caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) (Skevaki *et al.*, 2021). There are no reports of asthma or recurrent wheezing as a comorbidity or risk factor for COVID-19. One reason advanced is based on the recurrent use of immunosuppressive drugs such as glucocorticoids to control asthma, which could prevent the deadly cytokine storm caused by SARS-CoV-2 (Skevaki *et al.*, 2021).

Respiratory viruses may be causative agents of asthma or simply secondary to an underlying condition. Ultimately, the combination of viral virulence properties, personal risk factors and environmental exposures precipitates respiratory disorders such as asthma (Denlinger *et al.*, 2017; Jartti and Gern, 2017).

1.4.2 Protection against asthma development

It is generally held that the important increase in allergic diseases observed in Western countries is partly the consequence of a lifestyle based on a hygienic/aseptic environment resulting in reduced exposure to microbes during childhood. This is known as the “hygiene hypothesis” (**Figure 6**) (See: Box 1: Is Hygiene hypothesis the only key to explain asthma development?).

In this context, some viruses might profoundly remodel the lung immune system. Some common herpesvirus infections, including infections by human herpesvirus 6 and Epstein-Barr virus, have been linked to a reduced incidence of IgE sensitization and development of allergic diseases (Nilsson *et al.*, 2005; Nordström *et al.*, 2010; Svensson *et al.*, 2010). Both infections induce a TH1 driven immune response that may unbalance the pro-TH2 environment of allergic asthma. Surprisingly, influenza A infection of mice has also been shown to diminish the allergic response in mice, by shifting the TH2 response towards a TH1 response and by blocking TH2 cell infiltration to the lung (Wohlleben *et al.*, 2003). However, influenza infection remains mainly described as an aggravating factor in asthma. Infection with orofecal and foodborne microbes, such as Hepatitis A, Toxoplasma gondii or Helicobacter pylori, confirmed that respiratory allergy is less frequent in people heavily exposed

microbes (Matricardi *et al.*, 2000). Finally, our laboratory described protection against allergic asthma in a murine model following a γHV infection (Machiels *et al.*, 2017). γHVs show multiple immunoregulatory properties and will be further described in the next chapter.

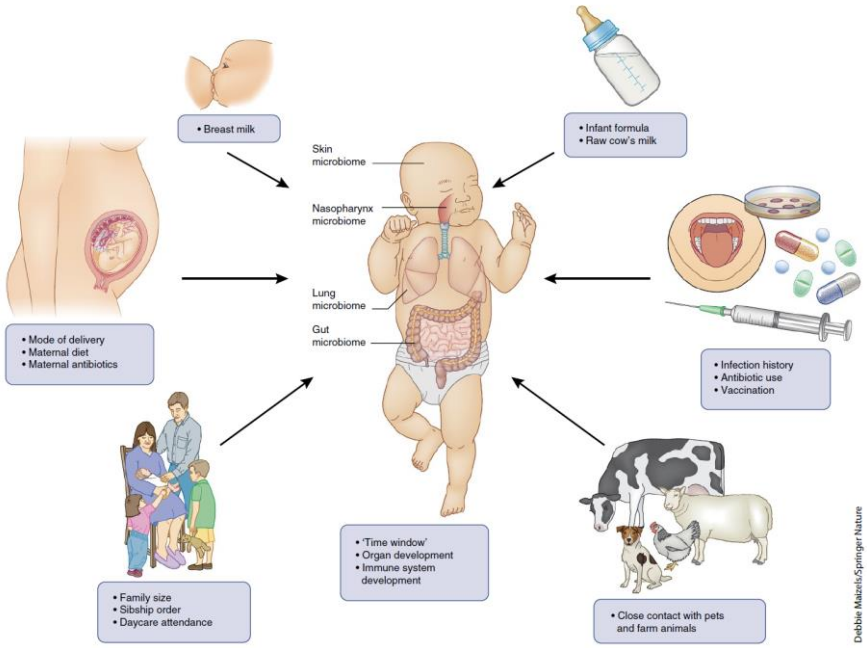


Figure 6. The critical window for the development of allergy in early childhood.

The microbiome of newborns is affected by many factors often modified by the Western lifestyle (infant feeding, maternal and infant dietary fibre content, mode of delivery, order of siblings in a family and the presence of pets and/or farm animals) that may lead to the development of allergic diseases (Lambrecht and Hammad, 2017).

Box 1: Is Hygiene hypothesis the only key to explain asthma development?

The idea emerged in the 1970s following the observation of a higher prevalence of allergic diseases in different social and environmental classes (rural versus urban). It was then suggested that "allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally from a mother infected by contact with her older children" (Strachan, 1989). Mouse models of allergy have provided compelling evidence that various infections can profoundly affect the development of disease. Some epidemiological studies in humans have corroborated these observations, but they remain difficult to conduct because of the highly dynamic behavior of humans, who adapt rapidly to changing socio-economic environments.

While some infections may have adverse effects on the development of allergies, the hypothesis, short of demonstrating a decrease in infections, has instead been correlated to a loss of contact with symbiotic pathogens that have beneficial properties. The Western lifestyle is linked to a loss of physical activity and an increase in time spent indoors, which exposes us less to the various pathogens normally encountered. Changes in living environment, diet, lifestyle and weight strongly influence the composition and diversity of the body's microbiome with subsequent effects on the development of allergic diseases. Lambrecht and Hammad proposed the concept that barrier tissues such as lung epithelial cells, and probably also skin and gut epithelial cells, are strongly influenced by the environment and microbiome diversity during a vulnerable period of early life and act as important rheostats for immunoregulation in response to allergens (Lambrecht and Hammad, 2017).

It is interesting to note that type 2-asthma undoubtedly fits to hygiene hypothesis as observed through many studies using murine models of allergic asthma (Smits *et al.*, 2016), however, it is unclear for type-2 low asthma which seems more related to comorbidities.

2 Gammaherpesviruses

2.1 Nomenclature and classification

The *Herpesvirales* is an order of double-stranded DNA viruses infecting a wide range of species. This order is divided in three families: *Alloherpesviridae* that infect fishes and amphibians; *Malacoherpesviridae*, whose hosts are molluscs; and finally the largest family, the *Herpesviridae*, which infect mammals, birds and reptiles (Davison, 2010). The *Herpesviridae* have been divided into three subfamilies: the *Alpha-*, *Beta-* and *Gammaherpesvirinae*. Gammaherpesviruses (γHV) preferentially establish a latent infection, with lytic infection involving only a small proportion of infected cells (See: 2.5. Viral life cycle). They have a limited host range *in vivo* and *in vitro*, and their cycle length is variable. The majority of them set up their latency primarily in B cells (Barton, Mandal and Speck, 2011). The subfamily *Gammaherpesvirinae* includes 7 genera (*Bossavirus*, *Lymphocryptovirus*, *Macavirus*, *Manticavirus*, *Patagivirus*, *Percavirus* and *Rhadinovirus*) and 43 species (International Committee on Taxonomy of Viruses *http://ictvonline.org*).

2.2 Morphology

γHVs are large spherical viruses measuring 150 to 200 nm. (**Figure 7**). They contain a double-stranded linear DNA, measuring between 125 and 295 kpb depending on the species. This DNA is anchored in an icosahedral capsid composed of 162 capsomeres (150 hexons and 12 pentons), which is itself surrounded by a protein matrix called tegument. Finally, a lipid membrane, derived from the cell membrane during the maturation of the virion, completes the particle. Various viral proteins are anchored to it, most of them are glycolysed (Prasad and Schmid, 2012).

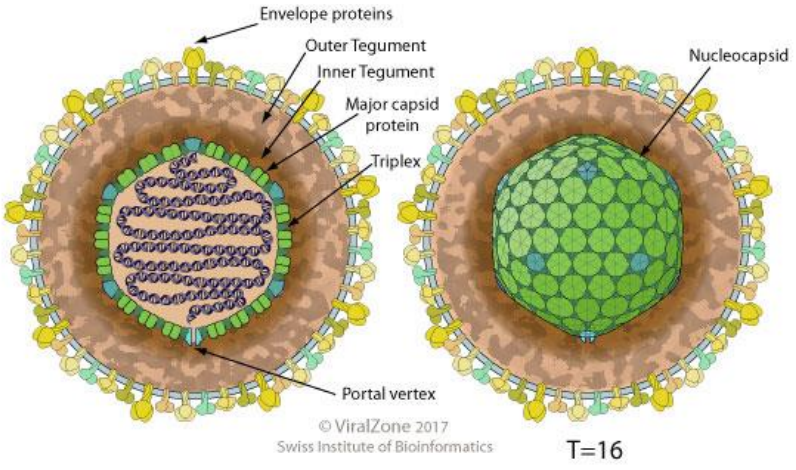


Figure 7. Morphology of *Gammaherpesvirinae.*

The viral particle is composed of a linear double-stranded DNA, contained in a protein capsid. This capsid, icoshedral in shape, is composed of 162 capsomers: 150 hexons (green) and 12 pentons (blue), the pentons being placed at the tips of the structure. The portal proteins (in light blue) allow the encapsidation of the DNA. The capsid is surrounded by a protein matrix called tegument. Finally, a membrane of cellular origin, in which different viral glycoproteins are anchored, envelops the whole particle (https://viralzone.expasy.org/18?outline=all_by_species).

2.3 Human gammaherpesviruses

γHV infections are mainly asymptomatic or associated with few clinical signs. Two human γHVs have been identified so far: the Epstein-Barr virus (EBV – HHV-4) and Kaposi's sarcoma-associated herpesvirus (KSHV – HHV-8). These viruses are important to public health because of their high prevalence and, like all γHVs, they have oncogenic potential. Both establish lifelong persistence that remains, most of the time, asymptomatic except in immunocompromised people.

- Epstein-Barr virus (*Human gammaherpesvirus 4*): belongs to the genus *Lymphocryptovirus* (composed of 9 species affecting humans and primates). EBV is commonly transmitted through infected saliva, earning it the nickname "kissing disease". Widespread in approximatively 90% of the population, the first infection is mostly asymptomatic but can cause infectious mononucleosis, a self-limiting lymphoproliferative disease (Kurth *et al.*, 2000). Besides, it is linked to various malignant lymphoproliferative diseases and tumors, being responsible for some 200,000 new cases of cancer arising worldwide each year (Rochford *et al.*, 2019). The age of first infection varies considerably around the world, correlating with socioeconomic factors: in densely populated countries, EBV is acquired at a young age and is usually asymptomatic. In contrast, in Western countries, the infection usually occurs in adolescence, resulting in 25-75% of infectious mononucleosis in late-infected individuals (Chabay *et al.*, 2020).

- Kaposi's sarcoma-associated herpesvirus (*Human gammaherpesvirus 8*): belongs to the *Rhadinovirus* genus (composed of 12 species affecting humans and other mammals). As its name implies, it is responsible for the development of Kaposi's sarcoma, a cancer that frequently occurs in immunocompromised patients, as well as primary effusion lymphoma, HHV-8-associated multicentric Castleman's diseases and KSHV inflammatory cytokine syndrome (Giffin and Damania, 2014). In general, KSHV appears to be transmitted primarily in childhood through infected saliva. Infections have also been reported through sexual transmission or blood contamination (Minhas and Wood, 2014). It is one of the few pathogens recognized as direct carcinogen, causing over 40,000 cases of cancer each year (Bray *et al.*, 2018). Unlike other herpesviruses that are ubiquitous, KSHV seroprevalence varies geographically: endemic areas in Africa and the Middle East have a seroprevalence ranging from 14% to 86%, while in USA and Northern Europe it is estimated to be around 6% (Mesri, Cesarman and Boshoff, 2010).

2.4 Murine model

γHVs are extremely host specific which makes it challenging to study EBV or KSHV in species other than humans. In addition to that, they also show limited growth *in vitro*. *Murid gammaherpesvirus 4* (MuHV-4) belongs to the genus *Rhadinovirus*, like KSHV. First discovered as a wild rodent pathogen (Blaskovic *et al.*, 1980), it has been rapidly used in laboratory mice. MuHV-4 cycle is extremely well described in laboratory mice: the lytic cycle, latency and associated reactivation events are reproducibly initiated in the host (François *et al.*, 2010). Moreover, MuHV-4 has also a genome that is easily accessible and editable due to cloning as bacterial artificial chromosome (BAC) which allows easy manipulations in prokaryotic systems (Adler *et al.*, 2000). Remarkably, MuHV-4 shares genetic homology with EBV and KSHV (Barton, Mandal and Speck, 2011). For all these reasons, it represents a robust model for studying human γHV pathogenesis (See: Box 2: Oncogenic properties of MuHV-4).

The natural route of MuHV-4 infection is not known, but the most likely route is respiratory infection. In laboratory, intranasal infection under anesthesia induces pulmonary infection (Lawler *et al.*, 2015), whereas without anesthesia, primary replication is limited to the nose (Milho *et al.*, 2009). After intranasal inoculation, the virus infects the mucosa, where lytic replication occurs in the neuroepithelium (Milho *et al.*, 2012) (**Figure 8**). Indeed, the virus targets the neuronal cilia on the surface of the mucus. This allows it to access the olfactory neuron under the mucus, probably via retrograde transport along the cilium. Once in the epithelium, the virus infects the sustentacular cells which rapidly become the main source of infection. Upon pulmonary infection, the virus exploits the glycocaminoglycans (GAGs) present on the surface of the alveolar epithelial cells in order to be presented to AMs. In a second step, the infection is transferred to the alveolar epithelial cells (Lawler *et al.*, 2015). Viral replication in the lungs induces interstitial pneumonia with perivascular and peribronchiolar leukocyte infiltration. The primary infection is controlled in 10 to 14 days (Sunil-Chandra *et al.*, 1992). Concurrently, the virus colonizes the draining lymph nodes and the spleen and reaches its preferred site of latency, the B lymphocyte. For this purpose, MuHV-4 exploits the $CD11c^+$ myeloid cells present in the mucosa, which causes the migration of infected $CD11c^+$ myeloid cells to the draining lymph node (Gaspar *et al.*, 2011). The passage through $CD11c^+$ myeloid cells thus favors the transfer of the infection to B cells, some of which form a latency reservoir. Latency can also be established in macrophages and DCs (Flaño *et al.*, 2000).

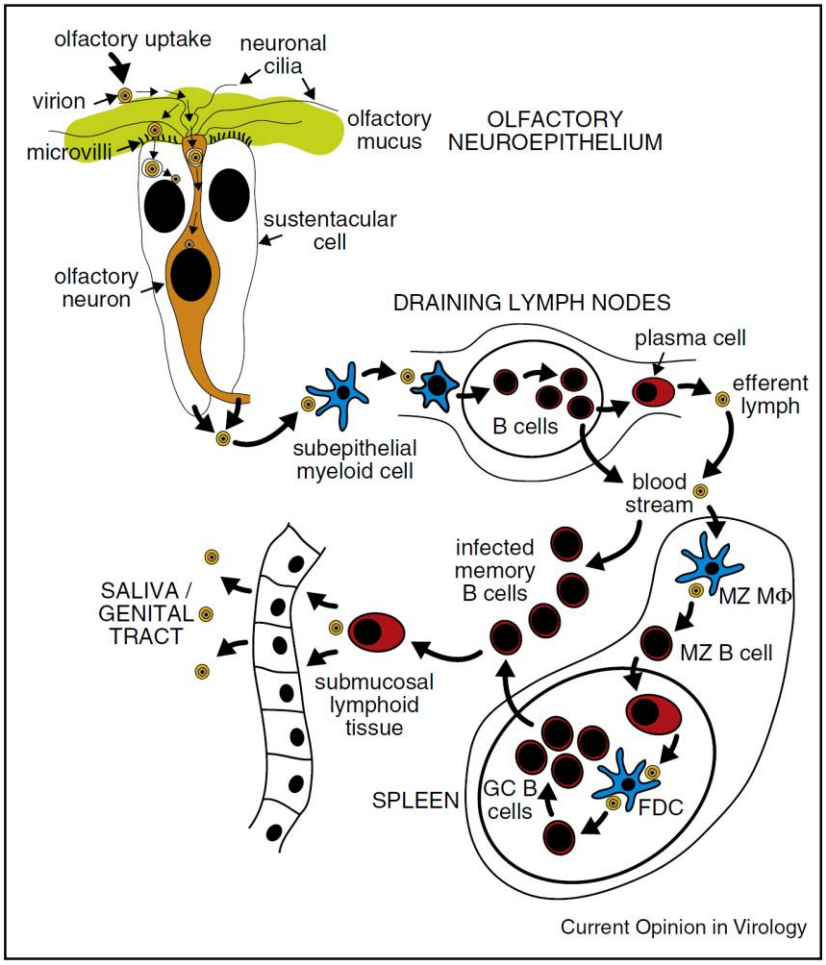


Figure 8. Schematic outline of MuHV-4 traffic through its murine host.

Virions acquired by olfactory sampling bin to neuronal cilia embedded in mucus. The cilia provide a bridge across the mucus to olfactory neurons and sustentacular cells. Virus released basolaterally encounters sub-epithelial myeloid cells. Infection of $CD11c^{+}$ myeloid cells allows MuHV-4 to reach B cells in draining lymph nodes. After proliferating in germinal centres, some infected B cells differentiate into plasma cells in lymph node medullary cords and release virus into the efferent lymph. From here it passes to the spleen via the blood and is taken up by marginal zone macrophages. They pass infection to marginal zone B cells, which migrate into the white pulp and differentiate into plasma cells to release more virions, which follicular dendritic cells transfer to follicular B cells. These then emerge from germinal centres as memory B cells. This allows virus transport to distant sites (Gillet, Frederico and Stevenson, 2015).

Box 2: Oncogenic properties of MuHV-4

As observed in humans, young immunocompetent mice do not develop tumors secondary to MuHV-4 infection. Following intranasal infection, mice develop a transient acute pulmonary infection followed by the establishment of latency in the spleen resulting in splenomegaly. However, chronically infected older (0,75-3 years of age) BALB/c mice may develop lymphoproliferative diseases, including high-grade lymphomas mirroring centroblastic or plasmoblastic non-Hodgkin lymphomas seen in humans (Sunil-Chandra *et al.*, 1994). In addition, infection of IFNγR$^{-/-}$ mice induces the development of pulmonary B-cell lymphomas mirroring EBV-associated lymphomatoid granulomatosis in humans (Lee *et al.*, 2009).

Interestingly, the opposite effect has also been reported. Indeed infection with MuHV-4 of DR4 mice – a mouse strain that spontaneously develops hematological tumors - completely prevents tumor formation (Raffegerst *et al.*, 2015). No underlying mechanism is currently defined, although it appears to be related to latency, as latency-deficient mutant failed to reproduce the same effect. In addition, treatment with MuHV-4 related growth factor suppressed tumor xenografts development *ex vivo* (Šupolíková *et al.*, 2018). Therefore, MuHV-4 seems to represent both a pro- and anti-tumor model with a promising potential for modulating tumor growth.

2.5 Viral life cycle

The herpesviruses life cycle is divided into two distinct parts, differentiated by the set of expressed genes: the lytic cycle and the latent cycle.

2.5.1 Lytic cycle

Although the pathogenesis varies depending on the herpesvirus, the multiplication cycle remains similar (**Figure 9**). The first step, which consists of the binding of the virion to the cell membrane, involves different viral glycoproteins. Briefly, two main mechanisms are described: either the viral envelope fuses directly with the plasma membrane, or the virion first enters the cell by endocytosis and then fuses its viral envelope with the endosome membrane in a second step (Peng *et al.*, 2010). Once the capsid is released into the cytoplasm, it is directed to the nucleopore through the cytoskeleton. The capsid is actively transported along microtubules and actin filaments (Radtke *et al.*, 2010), promoted by the "dynein/dynactin" motor protein complex (Döhner and Sodeik, 2005). In the vicinity of the nucleus, the capsid interacts with the proteins of the nuclear pore complex (NPC), resulting in the opening of the capsid and the release of its content into the nucleus (Kobiler *et al.*, 2012). The viral DNA is then rapidly circularized, even before any production of viral proteins (Lieberman, 2013). Gene transcription then begins, in successive waves, regulating the different phases of virus replication: immediate early, early

or late. While the encapsidation of the genome takes place in the nucleus, the rest of the maturation of the virion (tegumentation and envelopment) takes place in the cytosol. The main hypothesis for the exit of the nucleocapsid from the nucleus is based on the envelopment – deenvelopment – reenvelopment pathway (Skepper *et al.*, 2001): the virion acquires a first envelope by budding at the inner nuclear membrane, and is found in the perinuclear space. This first envelope then fuses with the outer nuclear membrane, releasing the naked nucleocapsid into the cytoplasm. The particle then acquires its secondary viral envelope by budding into vesicles of the *trans*-Golgi network. Finally, the virus is released by exocytosis (Bigalke and Heldwein, 2016).

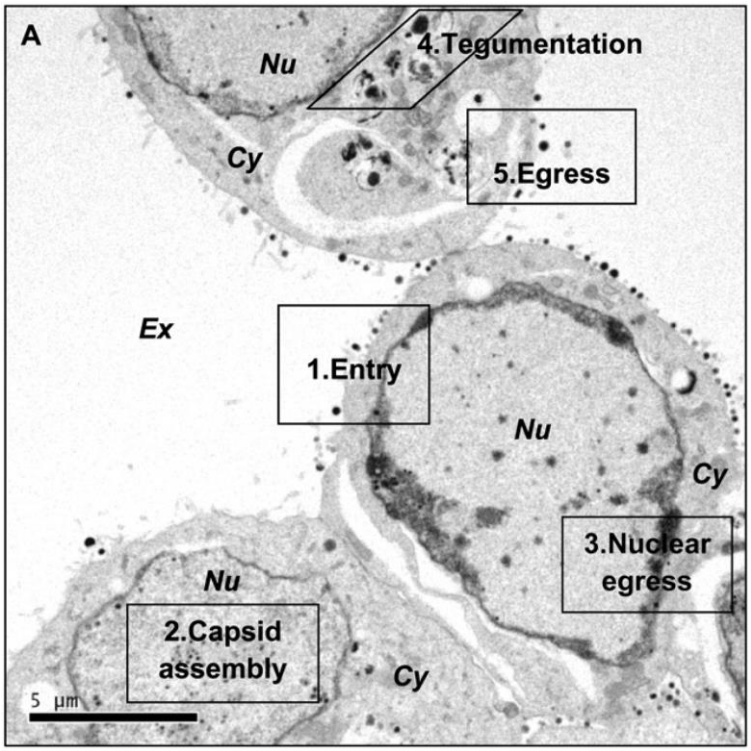


Figure 9. Life cycle of MuHV-4.

Successively (1) the entry of infectious virions by endocytosis and transport into endosomes (2) injection of viral DNA into the cell nucleus and assembly of pro-capsids and encapsidation of viral DNA (3) nuclear membrane invaginations (4) tegumentation (5) secondary envelopment and viral exit by exocytosis (Peng *et al.,* 2010).

Some γHVs are able to undergo lytic replication in epithelial or fibroblastic cells *in vitro*. Finally, endemic infections are maintained mainly by salivary transmission with carriers excreting the virus in their saliva. Sexual transmission can occur in low-prevalence population, and, in the case of MuHV-4, is dependent on the major virion envelope glycoprotein gp150 (Zeippen *et al.*, 2017).

2.5.2 Latent cycle

The key stage of the γHVs life cycle is, obviously, the establishment of the latency that allows the virus to remain in the infected host. During latency, the viral genome persists in the nucleus of

infected cells as a circular episomal element (viral DNA is then not integrated in chromosomes) while the viral gene expression program is restricted to non-coding ribonucleic acid (RNA)s and a few latency proteins. Latency is a necessary step for the biological cycle of herpesviruses. Showing lymphotropism, γHVs mostly establish latency in subpopulations of B or certain T lymphocytes (Speck and Ganem, 2010). In order to ensure transmission of the genome within the generations of dividing cells, one open reading frame (ORF) encodes the genome maintenance protein (GMP) that maintains viral episome, represses expression of lytic genes and inhibits cell apoptosis based on the p53 protein (Friborg *et al.*, 1999; Blake, 2010). This protein corresponds to EBNA-1 (Epstein–Barr virus nuclear antigen 1) in EBV (Leight and Sugden, 2000), LANA (latency-associated nuclear antigen) in KSHV (Uppal *et al.*, 2014) and proteins ORF73 in MuHV-4 (Fowler *et al.*, 2003). ORF73 from MuHV-4 is the homolog of LANA in KSHV.

The virus can reactivate under various stimuli, interrupting the latent state, and begins to produce a large amount of viral progenies. Reactivation corresponds then to a lytic phase of replication and the release of new viral progeny particles. In γHVs, reactivation is primarily dependent on gene expression BRLF1 (EBV) (Zalani, Holley-Guthrie and Kenney, 1996) or ORF50 (KSHV and MuHV-4) (Lukac, Kirshner and Ganem, 1999; Pavlova, Virgin Iv and Speck, 2003).

The establishment and maintenance of infection will depend on viral immunoevasion strategies and the control of the immune response. The next sub-section focuses on the deeply characterized immune evasion strategies described mainly for MuHV-4.

2.6 Immune evasion strategies

Herpesviruses have coevolved with their hosts for over 100 million years, long before human speciation. Therfore, they have evolved to elicit immunoregulatory responses to coexist with their hosts in a symbiotic relationship (McGeoch, Rixon and Davison, 2006).

2.6.1 Manipulation and evasion of the host immune system; beneficial effects for the virus life cycle

Herpesviruses are known for their large number of proteins specifically dedicated to the manipulation and evasion of the host immune system. This sub-chapter lists a non-exhaustive summary of the immune evasion techniques used by MuHV-4.

Many viruses, including γHVs, code for viral homologues of cellular cytokines and their receptors or secrete cellular chemokine-binding proteins (Heidarieh, Hernáez and Alcamí, 2015). Three are described in MuHV-4:

- M1 is a secreted protein that controls virus reactivation from latently infected macrophages. It induces the expansion of IFN-γ producing CD8 T cells which suppress the viral reactivation in latently infected macrophages (O'Flaherty *et al.*, 2014) .
- M2 protein plays pleotropic roles such as avoiding apoptosis by DNA damages, stimulating the production of IL-10 and modulating splenic B cell activation and differentiation (Liang *et al.*, 2009; Terrell and Speck, 2017). M2 deletion in MuHV-4 impairs host colonization and viral reactivation (Owens *et al.*, 2020).
- M3, a chemokine binding protein, exhibits a high-affinity for a broad spectrum of chemokines and subsequently blocks the recruitment of immune cells such as cytotoxic T cells (Hughes *et al.*, 2011; Šebová *et al.*, 2019).

γHVs code for viral(v)-IFN regulatory factors (IRF) homologous of IRF transcription factor of IFN signaling. MuHV-4 dysregulates IFN signal transduction by targeting the type I IFN receptor, possibly through ORF54 (Lopušná *et al.*, 2016). It also blocks IFN-β production through ORF11 (Kang *et al.*, 2014) that also blocks the phosphorylation and activation of the transcription factor IRF3 (Stempel, Chan and Brinkmann, 2019). Finally, it can induce the expression of SOCS1 that counteracts the antiviral effect of IFN-γ during infection (Shen *et al.*, 2018).

The protein ORF4 of MuHV-4 encodes homologs of regulators of complement activation (RCA) proteins, which inhibit complement activation at the level of C3 and C4 deposition. MuHV-4 RCA protein effectively prevents the deposition of murine C3 on activating particles like zymosan by regulating the function of both the classical and alternative pathway C3 convertases. The viral-RCA encoded by MuHV-4 shares sequence homology with other viral and host regulators of complement activation (Tarakanova *et al.*, 2010).

γHVs have the ability to hijack the humoral response by changing some of their antigenic conformation of envelope glycoproteins during the entry process. This mechanism prevents the recognition of the active form of the glycoproteins and consequently their neutralization by antibodies and the blocking of membrane fusion (Gillet *et al.*, 2009). Gp150 acts as an immunogenic decoy to limit virion neutralization and, in addition, promotes the infection of cells expressing Fc receptor (Gillet *et al.*, 2007). Glycoprotein B is the most conserved component of the HV entry mechanism and its N terminus (gB-NT) is a common neutralization target. Finally, specific glycosylation of glycoprotein epitopes is also a protective mechanism against the production of neutralizing antibodies (Gillet and Stevenson, 2007).

Moreover, γHVs can affect antigenic presentation. The MK3 protein binds to major histocompatibility complex (MHC)-I molecules and causes their degradation in lysosomes after

endocytosis. It also induces resistance to IFN-γ-induced surface overexpression of MHC-I molecules by increasing the degradation of TAP (transporter associated with antigen processing) proteins (Stevenson, 2004). Finally, GMP is a stable protein that regulates the maintenance of viral episomes in actively dividing lymphocytes through tethering the viral genome to cellular chromosomes. GMPs have acquired the capacity to evade the cytotoxic T cell response through self-limitation of MHC class I-restricted antigen presentation, further ensuring virus persistence in infected host (Blake, 2010; Sorel and Dewals, 2019) (**Figure 10**).

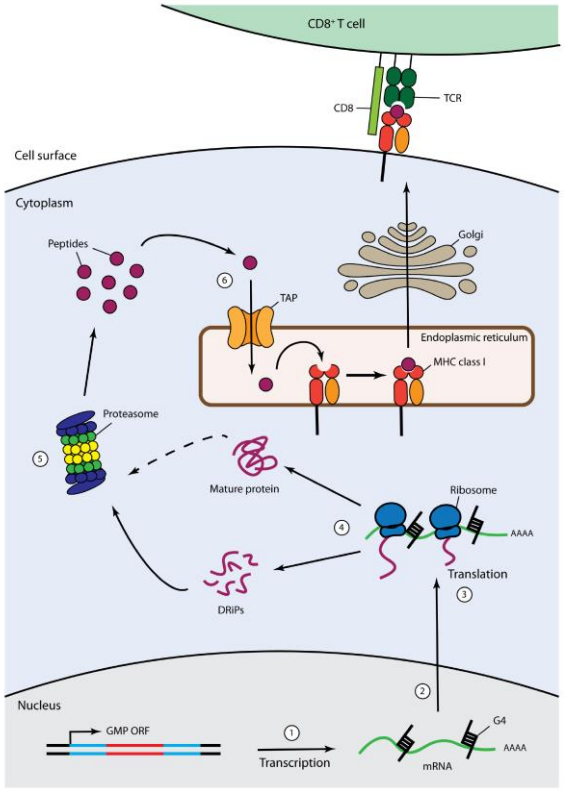


Figure 10. Role of GMP in immune evasion during γHV latency.

GMP are able to inhibit the process of antigenic peptides presentation by MHC class I molecules for recognition by $CD8^+$ cytotoxic lymphocytes through various mechanisms:

(1) decreasing transcription levels (2) inhibiting pre mRNA processing (3) regulating self-translation (4) delaying self-translation (5) avoiding proteasome degradation (6) inhibitor effect prior to the translation into the endoplasmic reticulum (examples for LANA and EBNA from KSHV and EBV) (Sorel and Dewals, 2019)

miRNAs are small non-coding RNA (containing about 22 nucleotides) that modulate gene expression at a post-transcriptional level by targeting messenger RNA. They are mostly expressed during latency (12 are reported in MuHV-4). *In vitro*, they exhibit a wide range of properties such as modulation of angiogenesis, anti-apoptotic and anti-inflammatory pathways, modulation of cell cycle, NF-κB activation, immunoevasion, viral replication and latency maintenance (Feldman *et al.*, 2014). Using a technique known as qCLASH (quick crosslinking, ligation and sequencing of hybrids), Bullard et *al.* identified thousands of direct miRNA-mRNA interactions during MuHV-4 lytic infection, latent infection and reactivation from latency (Bullard *et al.*, 2019). Remarkably, they found 86% similarity between the MuHV-4 miRNA-host targets identified in B cells with EBV and KSHV. A total of 64%

was shared between the three viruses, demonstrating significant conservation of γHV miRNA targeting (Bullard *et al.*, 2019) .

Box 3: Herpesviruses vaccines

An effective vaccine against herpesviruses is particularly challenging due to an armamentarium of immune evasion genes and a latency phase. The Varicella-Zoster vaccine represents the paradigm of a successful herpesvirus vaccine with 95% prevention of moderate disease with a single dose. The results are more debatable against HSV-2 (genital herpes) and HCMV (human cytomegalovirus). No licensed prophylactic vaccines are currently available against EBV or KSHV. Deletion of immune evasion genes could provide a model for an effective vaccine against tumor-associated herpesviruses. In this context, Lawler et *al*. obtained vaccine protection against MuHV-4 when the vaccine virus lacked both episome maintenance and the genomic region encompassing M1, M2, M3, M4 and ORF4 (Lawler, Simas and Stevenson, 2020). Also, Brar et *al*. engineered a recombinant MuHV-4 virus - targeted inactivation of viral antagonists of the type I interferon pathway and deletion of the latency locus - that stimulates robust innate immunity, differentiates virus-specific memory T cells and elicits neutralizing antibodies, thereby offering a framework for effective subsequent vaccination (Brar *et al.*, 2020).

2.6.2 Consequences of immunomodulation by γHVs for the host

As a reminder, γHVs are oncogenic viruses, which have a strong propensity to induce malignant transformation of the infected cell line. The EBV latency in B cells could lead to the development of lymphoproliferative diseases of B cell origin, these include Hodgkin Lymphoma, Burkitt Lyphoma, Diffuse Large B cell Lymphoma and two rarer tumors associated with profound immune impairment, plasmablastic lymphoma and primary effusion lymphoma (Rochford *et al.*, 2019). KSHV is linked to three different types of tumor which arise from vascular endothelium (Kaposi Sarcoma) and from B cells (primary effusion lymphoma and Multicentric Castleman's disease) (Schulz and Cesarman, 2015). In general, infection alone is almost never sufficient to induce cancer development: either there is co-induction of genetic changes in the infected host, or there is a simultaneous immunosuppression in the infected host, allowing aberrant and uncontrolled expression of viral oncogenes (Schulz and Cesarman, 2015). EBV is also potentially associated with the development of inflammatory, mediated or autoimmune diseases such as systemic lupus erythematosus and Sjögren's syndrome, multiple sclerosis or rheumatoid arthritis (Houen and Trier, 2021).

Immunomodulation, or modulation of the immune system, can be defined as any modification of the immune response induced by the infection towards a subsequent antigenic encounter, whatever

its nature (White, Beard and Barton, 2012). Latency infection by γHVs triggers elevated levels of IFN-γ and TNFα that confer heterologous protection against subsequent *Listeria monocytogenes* and *Yersinia pestis* infections (Barton *et al.*, 2007). This resistance is dependent of IFN-γ which is produced by $CD4^+$ and $CD8^+$T cells during latency. Beneficial immune modulation during herpesvirus latency supports the "hygiene hypothesis", in which microbial exposure in early-life protects against the development of allergy. This hypothesis is supported by the observed association between increased age of seroconversion to HV infections in populations with increased atopy and autoimmunity (Takeuchi *et al.*, 2006).

Similarly, MuHV-4 latency attenuates *Mycobacterium tuberculosis* infection through the interventions of IFN-γ^+ producing $CD4^+$ T cells in the draining lymph node (Miller *et al.*, 2019). It also protects the host from pneumovirus-induced immunopathologies through $CD8^+$ T cells activation (Dourcy *et al.*, 2020). As a reminder, MuHV-4 undergoes transient replication in different cell types and establish latency in memory B cells, macrophages and DC *in vivo* (Barton *et al.*, 2007). A potential role of AMs activated by MuHV-4 infection has been suggested to explain that mice latently infected with MuHV-4 showed significantly higher survival to influenza A virus infection than did mock infected mice (Saito *et al.*, 2013). Moreover, as already mentioned, our laboratory showed that infection induces the replacement of AMs by regulatory monocytes with subsequent protection against airway allergy (Machiels *et al.*, 2017). These experiments highlight that in addition to the cross protection provided by the latency of γHV infections, other immunological mechanisms, even at early times, are involved and remain poorly understood.

3 Type 2 innate lymphoid cells

3.1 Discovery and origin

Innate lymphoid cells (ILCs) were discovered in 2010 and firstly described in 'fat-associated lymphoid clusters' in the mouse peritoneal cavity (Moro *et al.*, 2010). Now, they are described throughout the body in various tissues such as fat, lung, intestine, liver and skin but also in the lymph nodes, meninges, joint fluid, islets (Ebbo *et al.*, 2017). Belonging to the innate immune system, they are now recognized as crucial players in inflammation and homeostasis regulation (Ebbo *et al.*, 2017). They are divided in cytotoxic ILCs, with the well-known Natural Killer (NK) cells, and the non-cytotoxic helper ILCs. These latter are divided in three subtypes: innate lymphoid cells type 1 (ILC1s), type 2 (ILC2s) and type 3 (ILC3s) that were named based on their similarities with TH cells due to their common transcription factors (TF) expression and cytokines production (Hepworth and Sonnenberg, 2014) (**Figure 11**). However, unlike adaptive cells, they lack antigen receptor dependent on the recombination-activating genes (RAG) recombinase and do not express unique cell surface markers homologue(Cherrier, Serafini and Di Santo, 2018). Lymphoid Tissue inducer (LTi) cells, identified long before the establishment of the ILC concept, are considered as part of ILC3 group because of many similar characteristics, although they actually have a unique developmental pathway (Zhong, Zheng and Zhu, 2018). This chapter will focus on ILCs predominantly present in the lung, namely ILC2s.

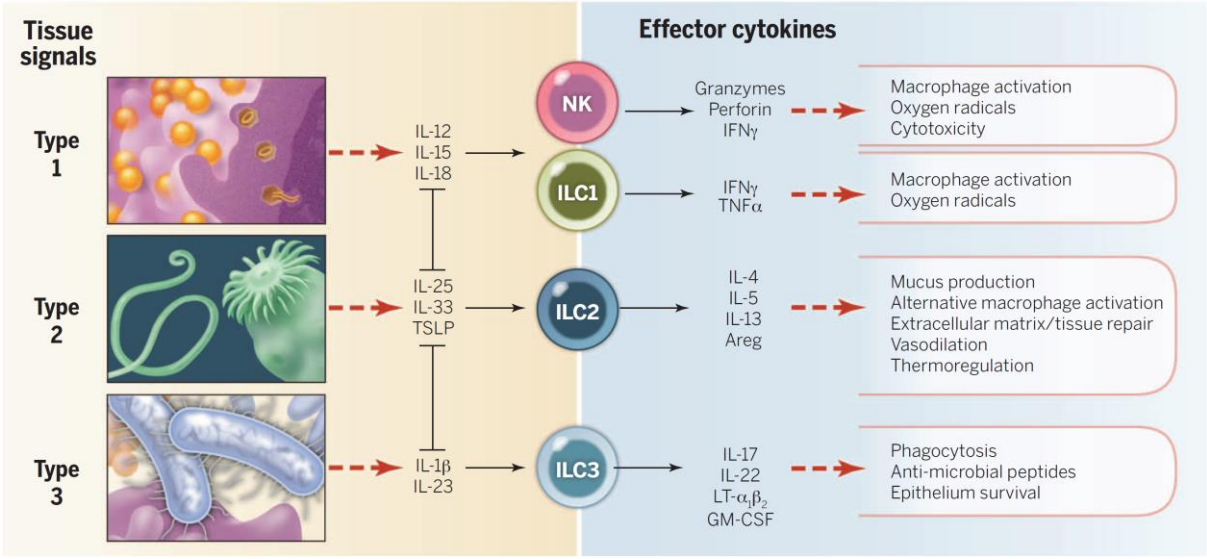


Figure 11. Summary of roles of ILCs.

ILC1s (TF: T-bet) play a role in type 1 immune response targeting tumor cells or intracellular pathogens by the production of IFN-γ and TNFα.

ILC2s (TF: GATA 3) play a role in type 2 immunity which is a whole-tissue response triggered by macroscopic parasites and non-microbial harmful stimuli. ILC2s produce type 2 cytokines IL-4, IL-5, IL-9 and IL-13.

ILC3s (TF: ROR-γT) play a role in type 3 immune response protecting against extracellular bacteria and fungi by the production of IL-17 and IL-22 (Eberl, Di Santo and Vivier, 2015).

3.2 Development

ILCs are initially generated from the fetal liver and seed tissues during fetal development. At birth, a second wave of immigration and a dramatic expansion of tissue ILC pools occurs (**Figure 12**). ILC2s are detectable in peripheral tissues by embryonic day 15,5 in mouse (Bando, Liang and Locksley, 2015). The number of lung ILC2s gradually increases, reaching adult levels by day 7 after birth, and further increases between 10 and 14 days of age when they return to adult equivalent levels (Saluzzo *et al.*, 2017). The role of thymus in ILC development is unclear. Lung ILC2s labeled by BrdU in the neonatal period can be detected in adult lungs (Ghaedi *et al.*, 2016), indicating that neonatal ILC2s persist in the lung of adult mice.

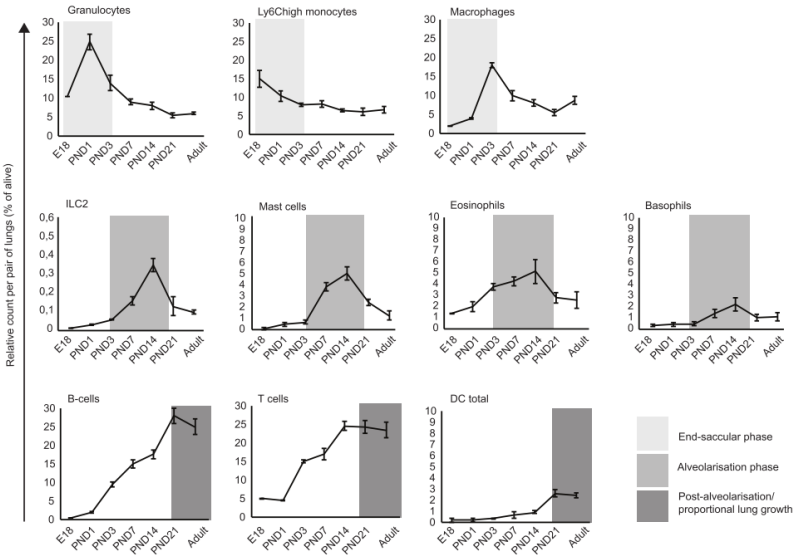


Figure 12. Early kinetics of ILC2s.

The postnatal phase of alveoli formation is characterized by a rapid influx of immune cells involved in type 2 immune responses. Cells of adaptive immune system gradually populate the lung and reach peak numbers once type 2 innate immune cell populations have dropped to post-developmental amounts (de Kleer *et al.,* 2016).

The most active phase of lung development, corresponding to alveoli formation named alveolarization, occurs during the second postnatal week in mice and until 2 to 3 years in humans, which coincides with early-life lung predisposition to a type 2 immune environment. Simultaneously, fetal and perinatally ILCs develop their effector phenotypes under the control of factors coming from tissue, diet and microbiota. They acquire tissue-specific transcriptomics signatures (Ricardo-Gonzalez *et al.*, 2018) (**Figure 13**). ILCs initially originate in the fetal liver and later in the BM. In the BM, they arise from the common lymphoid precursors (CLP) which give rise to common helper-like ILC progenitors (CHILPs), and then, to the more restricted ILC lineage-specific progenitors (ILCPs) that generate helper-like ILC1s, ILC2s and ILC3s (Artis and Spits, 2015) (**Figure 14**). They diverge from B and T cell lineages upon E-box protein antagonism by inhibitor of DNA binding family members, particularly Id2, but also Notch, Nfil3, PLF and Gfi2 (Kim and Van Dyken, 2020). The development of the ILC2 progenitor relies

on the expression of transcription factors GATA 3 (Hoyler *et al.*, 2012; Mjösberg *et al.*, 2012), RORα (Wong *et al.*, 2012) and T-cell factor 1 (Yang *et al.*, 2013) for their specification and maturation. The transcription factor Bcl11b is specifically expressed ILC2s and is also essential for their development (Yu *et al.*, 2015). Single-cell analysis of ILC progenitors also revealed the existence of a direct pulmonary ILC progenitor, present in newborns and adult lung, capable of differentiating into several different ILC populations (Ghaedi *et al.*, 2020).

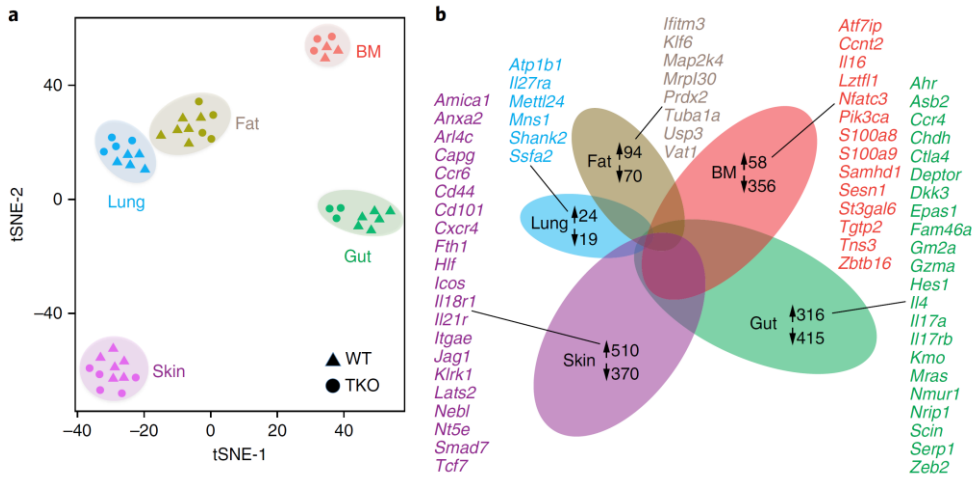


Figure 13. Tissue specificity of ILC2s.

Segregation of ILC2s by tissue with anticipatory function. Lung, fat and skin tissues show enrichment for ILC2s. While most of the ILCs in the gut are ILC3s, ILC2s are also present in the gut (Ricardo-Gonzalez *et al.*, 2018).

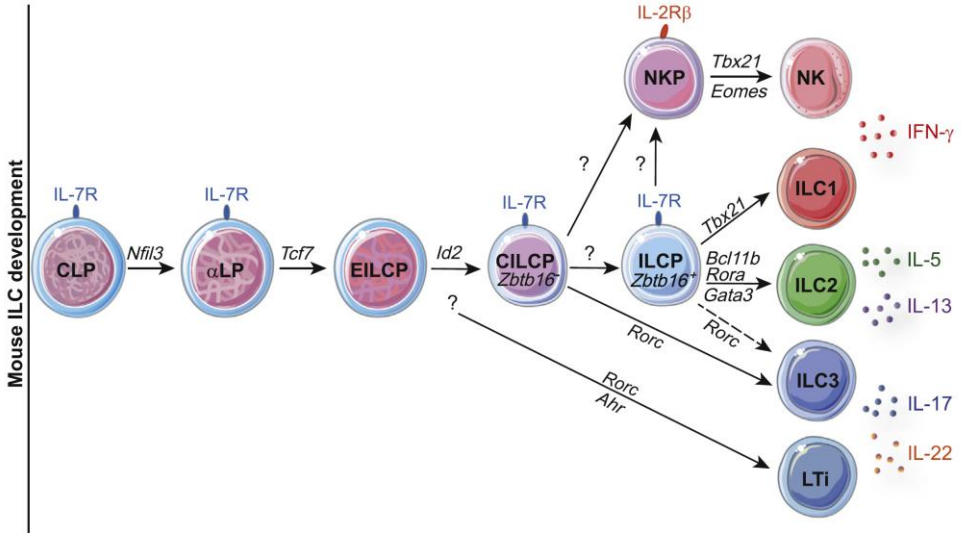


Figure 14. Mouse ILC development.

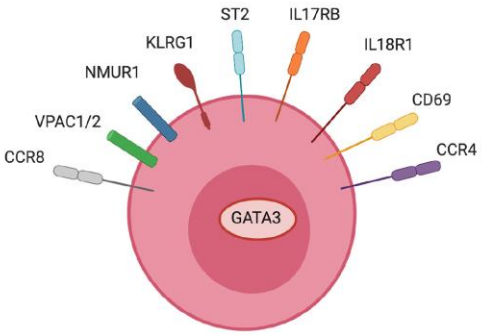
αLP: α-lymphoid precursor - CILCP: common ILC progenitor - CLP: common lymphoid progenitor - EILCP: early innate lymphoid cell progenitor - EToP: early tonsillar progenitors - ILCP: ILC precursor - NK: natural killer - NKP: NK cell precursor - rILCP: restricted ILC precursor (Guia and Narni-Mancinelli, 2020).

There are three distinct stages of ILC2 development during early-life that include:

1) dispersal into tissues
2) expansion and activation of tissue-specific transcriptional programs
3) homeostatic maintenance with differences in local regulation of survival and turnover throughout life

3.3 Localization and recruitment

The unique gene signature of ILCs with tissue-specific functions depends on the tissue microenvironment in mice (Zeis *et al.*, 2020) and in humans (Yudanin *et al.*, 2019). The use of parabiont models highlighted that more than 95% of ILC1s, ILC2s, ILC3s and Lti cells residing in various organs (small lamina propria, salivary gland, lung and liver), were of host origin, demonstrating that ILCs establish tissue-residency in both lymphoid and non-lymphoid organs (Gasteiger *et al.*, 2015). Local tissue niches, rather than progenitor origin, may dominantly imprint ILC phenotypes (Gasteiger *et al.*, 2015). ILC2 signatures, throughout the body, are particularly distinguished by their response to IL-25 and IL-33 via their expression of the corresponding receptors - IL-17 receptor B (IL17RB) and IL-1 receptor-like 1 (IL1RL1/ST2) (**Figure 15**).



	ST2	IL17RB	IL18R1	KLRG1	CD69	NMUR1	VPAC1/2	CCR4	CCR8
Liver	+	+	n.d.	+	+	n.d.	n.d.	n.d.	n.d.
S. intestine	+/–	$+^{hi}$	–	$+^{hi}$	+	+	+	$+^{hi*}$	+/–*
Fat	$+^{hi}$	+	–	+	+	n.d.	n.d.	+*	+*
Spleen	+/–	+	+/–	+	+	n.d.	n.d.	n.d.	n.d.
Lung	$+^{hi}$	+	+/–	+/–	+	+	+	+	+
Skin	$+^{lo}$	$+^{lo}$	$+^{hi}$	+	+	n.d.	n.d.	+*	+*
MLN	+	+	n.d.	+	+	n.d.	n.d.	n.d.	n.d.
Bone marrow	+	+	+/–	–	–	n.d.	n.d.	–*	–*

Trends in Immunology

Figure 15. Identification of mouse ILC2s.

GATA 3 is the defining marker of ILC2s. Within the body, they express variations in markers expression mainly for the IL-33, IL-25 and IL-18 receptors.

$^{+}$: positive - $^{+hi}$: highly expressed - $^{+lo}$: low expression - $^{-}$: negative - $^{+/-}$:heterogeneous - *: RNA expression levels.

(Meininger *et al.*, 2020)

In summary, ILCs expand and differentiate during the first weeks of life, after which they persist primarily as tissue-resident effector cells, where they contribute to homeostasis, immunosurveillance and tissue repair (Gasteiger *et al.*, 2015; Ricardo-Gonzalez *et al.*, 2018). They integrate multiple signals and occupy niches in strategic positions (close to blood vessels and neurons) in order to rapidly initiate local responses (Gasteiger *et al.*, 2015). W. Dahlgren et *al.* identified a dominant adventitial niche of ILC2s around lung bronchi and larger vessels in multiple tissues, where they co-localized with subsets of DC and regulatory T cells (Dahlgren *et al.*, 2019). They showed that ILC2s localize with fibroblast-like adventitial stromal cells, which support ILC2s and TH2s by producing IL-33 and TSLP, and also whose expansion and IL-33 production are also sustained by ILC2s after helminth infection (Dahlgren *et al.*, 2019).

ILC2s are long-lived cells that can survive and proliferate slowly for more than 1 year in response to IL-2 *in vitro* (Moro *et al.*, 2010). Mature ILCs can be found in peripheral organs one month following adoptive transfer of ILC precursor populations (O'Sullivan and Sun, 2018). In naïve adult mice, they self-maintain with a low proliferation rate of 5 to 10% unlike the $CD4^{+}$T cells which present 20 to 30% (Huang *et al.*, 2018). Initially considered to be resident tissue cells, it is becoming increasingly clear that ILC2s could also be recruited. Indeed, ILC progenitors express some chemokines (a4b7, CCR7, CCR9 and/or CXCR6) and homing receptors and have the potential to migrate from the BM to the peripheral tissues (Kim, Hashimoto-Hill and Kim, 2016). ILC2s may also express a number of chemokine receptors, including CCR2, CCR4, CCR5 and CXCR4 under certain conditions (Li *et al.*, 2016). Using parabiotic models, Huang et *al.* revealed that, following IL-25 signaling, intestinal-ILC2s could migrate to the lung in an S1P-dependent manner (Huang *et al.*, 2018). They categorized them as inflammatory ILC2s (iILC2s) expressing IL-17RB and KLRG1 but lacking ST2 marker (one of the specific lung ILC2s marker). It is interesting to note that intranasal instillation of IL-25 did not elicit iILC2s, rather this was only observed after intraperitoneal injection or parasite infection (Huang *et al.*, 2018). iILC2s rely on the basic leucine-zipper transcription factor ATF-like (BATF) and exhibit high expression of the gut-homing CCR9. This reveals that iILC2s arise from resting ILC2s residing in the intestine, and can be recruited to peripheral tissues to contribute to host defense (Miller *et al.*, 2020). Fate-mapping in mice reported that resident ILC2s are not necessarily generated from iILC2s (Ricardo-Gonzalez *et al.*, 2020). Following *N.brasiliensis* infection, tissue-imprinted ILC2s are extruded to the blood not only from the intestine, but also from the lung, to generate a pool of heterogeneous ILC2s that contribute to systemic type 2 immunity (Ricardo-Gonzalez *et al.*, 2020).

3.4 Role in asthma

The key role of ILC2s in asthma pathology has been highlighted by the succesfull induction of lung inflammation in mice lacking the adaptive immune cells T and B lymphocytes ($Rag2^{-/-}$) (Timotheus Y F Halim *et al.*, 2012). In addition, papain injections into ILC2s deficient mice failed to induce strong lung inflammation (Timotheus Y.F. Halim *et al.*, 2012). Ultimately, the transplantation of ILC2s into the ILC2 deficient mice restored the lung inflammation (Timotheus Y.F. Halim *et al.*, 2012). Since then, ILC2s have been extensively described in the context of asthma reaction. They are essential for the initiation of TH2 inflammation (Gold *et al.*, 2014), required for the persistence of asthma (Christianson *et al.*, 2015) and the establishment of memory TH2 responses (Halim *et al.*, 2015; Martinez-Gonzalez *et al.*, 2016). Through the production of type 2 cytokines, ILC2s lead to the characteristic symptoms of allergic asthma (**Figure 16**).

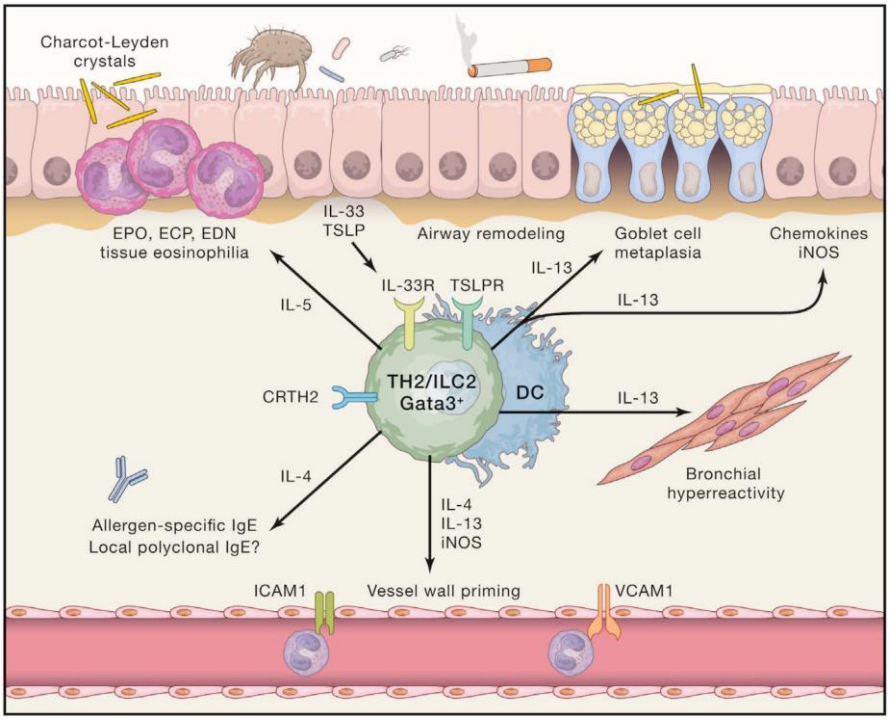


Figure 16. Central role of ILC2s in controlling key features of type 2-high asthma.

ILC2s play a direct role in asthma through the production of type 2 cytokines that provokes airway obstruction, AHR, chronic infiltration and hypertrophy of bronchial smooth muscle cells, airway eosinophilia, mucus overproduction and IgE synthesis (Hammad and Lambrecht, 2021).

Briefly, eosinophilia observed following protease allergen is dependent on the IL-5 produced by activated ILC2s (Boberg *et al.*, 2020a). By this secretion, tissue ILC2s regulate basal eosinophilopoiesis and tissue eosinophil accumulation (Nussbaum *et al.*, 2013). ILC2s produce IL-5 constitutively and are induced to co-express IL-13 during type 2 inflammation leading to AHR and tissue remodeling (Wolterink *et al.*, 2012). IL-13 primes eosinophil egress from vessel by upregulating

expression of adhesion molecules such as VCAM-1 and ICAM-1. It can also disrupt bronchial epithelial barrier integrity by targeting tight junctions, in both *in vivo* murine models and *ex vivo* human co-cultures (Sugita *et al.*, 2018). ILC2-derived IL-9 sustains, through autocrine amplification, IL-5 and IL-13 production (Mohapatra *et al.*, 2016). It can also activate DCs in asthmatic mouse model (Wan *et al.*, 2020). The *in vivo* production of IL-4, driver of class switching of IgE, by ILC2s is controversial. In fact, the IL-4 secretion by ILC2s has only been observed after specific "artificial" restimulation with PMA/ionomycin, cysteinil leukotrienes (CysLTs) or leukotriene D4 (LTD4) (Doherty *et al.*, 2013); no secretion without restimulation has been reported *in vivo*.

In addition to the direct role in establishing airway inflammation, ILC2s can also act very early in the TH2 sensitization process. As already mentioned, they promote DC migration to the draining lymph nodes, via the release of IL-13, essential for effective TH2 cell priming (Halim *et al.*, 2014). However, one study found that the activation of primed TH2 cells can occur independently of ILC2s activation following *N.brasiliensis* infection (well-established model of migratory helminth infection), or HDM-fungal challenge (Van Dyken *et al.*, 2016).

Extensively studied in mouse models, the role of ILC2s is also reported in human asthma. Indeed, asthmatics patients show increase numbers of ILC2s and IL-33 concentrations, in blood and BALF, compared to healthy patients (Zhong *et al.*, 2017; Winkler *et al.*, 2019). Substantial transcriptional changes in human ILC2s are observed following allergens *Dermatophagoides pteronyssinus* or grass mix (Winkler *et al.*, 2019). Finally, a strong correlation has been established between gene regulatory mechanisms in ILC2s and the genetic basis of asthma, supporting a pathogenic role for ILC2 in allergic asthma (Stadhouders *et al.*, 2018).

Box 4: Which model to study ILC2s?

Since they do not express common cell surface markers, temporary or permanent depletion of ILC2s *in vivo* can be a major challenge.

Here is a non-exhaustive summary of the different mouse-tools used to study ILC2s:

- **GATA 3$^{fl/fl}$-Vav-Cre**: inhibition of all ILC subsets, T cells and IL-7Rα^{+}NK1.1^{+} cells (Yagi *et al.*, 2014).
- **IL7$^{-/-}$ or Il7rα$^{-/-}$** : lack or strong reduction of ILC2s, subtype of NK cells and mature T cells (Robinette *et al.*, 2017).
- **Rag2$^{-/-}$Il2rg$^{-/-}$**: deficient mature lymphocytes T and B and all ILCs subsets (Moro *et al.*, 2010).
- **RORα$^{sg/sg}$**: affect all ILC subsets, TH17 and memory T cells. Mice have deficiency of granule cells and Purkinje cells and also neurological disorders (Wong *et al.*, 2012).
- **Mice pre-treated with anti-CD90**: temporary depletion of more than 90% lung resident ILC but also 70% of NK and mesenchymal stem cells (Silver *et al.*, 2016).
- **Icos$^{fl\text{-}DTR\text{-}fl/+}$Cd4$^{Cre/+}$**: temporary depletion of ILC2s using the diphtheria toxin. ICOS is expressed on both T cells and ILC2s. Thus floxed DTx receptor gene inserted into the ICOS locus (resulting in a null allele), allows CD4-cre-mediated excision of the DTR gene only in T cells (Oliphant *et al.*, 2014).
- **RORα$^{fl/sg}$ IL7r^{Cre}**: specific ILC2s deficiency cells (Oliphant *et al.*, 2014).

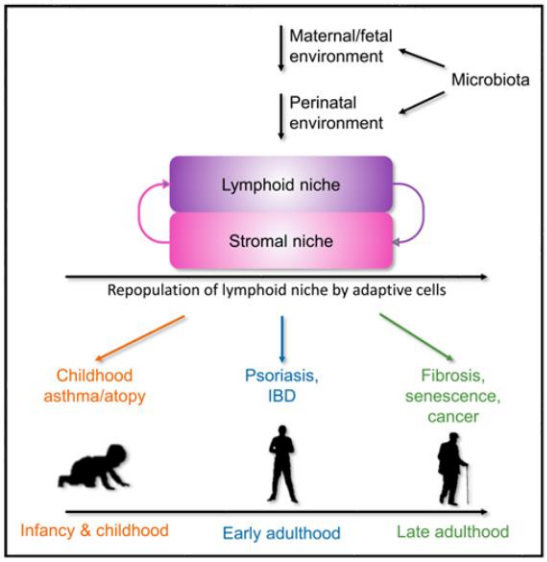
Box 5: ILC2s, the dispensable mirror of TH2 cells?

One interesting question is whether the functions of ILC2s and TH2 cells overlap, given that they produce similar cytokines patterns.

Many genes encoding TF important for ILC2 differentiation and function are similarly expressed in tissue effector TH2 cells (Van Dyken *et al.*, 2016). Despite those similarities, ILC2s and TH2 cells originate from distinct developmental pathways and exhibit unique epigenetic and transcriptional programs (Shih *et al.*, 2016). Moreover, unsupervised hierarchical clustering of murine immune cells clearly segregates ILCs from T lymphocytes (Shih *et al.*, 2016).

In summary:

- ILCs colonize the body long before adaptive immune cells, which are poorly represented during the postnatal period (Kotas and Locksley, 2018).
- ILCs arrive early and occupy "niches" in developing tissues and may affect subsequent repopulation by resident adaptive T cells later in life (Kotas and Locksley, 2018).
- ILCs are tissue- and microenvironment-specifics and then act as early responders to tissue perturbation by producing cytokines within hours of activation, in contrast to the days required for naïve adaptive lymphocytes to be primed, expand, differentiate and enter into tissues.



Selective ILCs deficiencies have been reported in humans and have shown that ILCs may be dispensable under natural conditions, if and only if T and B cells functions are preserved (Vély *et al.*, 2016). However, in chronic asthma the essential role of ILC2s has been demonstrated in contrast to antigen-specific T cells for the persistence of asthma (Christianson *et al.*, 2015). In addition, ILC2-deficient pups show impaired TH2 cell responses compared to WT pups, demonstrating that ILC2s are the drivers of neonatal TH2 cell bias (Wong *et al.*, 2017).

ILCs are preserved among humans, rodents and zebrafish, and associated cytokines or basic transcriptional mechanisms are found in distant ancestors such as bony fish and lampreys. To sum up, ILCs seem to be related to an archaic system, but ultimately, their embryonic shaping may have long-term effects on tissue (Kotas and Locksley, 2018).

3.5 Regulation

3.5.1 Activation

ILC2s are activated by epithelial cytokines such as IL-33, IL-25 and TSLP (Licona-Limón *et al.*, 2013). In the lung, IL-33 is mainly expressed in type 2 pneumocytes and released following cell stretching or death (Cayrol and Girard, 2014). TSLP is produced by a large variety of cells such as epithelial, endothelial, stromal and muscular cells but also by some leukocytes like DCs and mast cells. Its regulation is mostly transcriptional, through pattern recognition receptors, protease-activated receptors and others (Kitajima and Ziegler, 2013). Finally, at steady state, IL-25 is constitutively expressed by tuft cells as well as others cells like TH2 cells, mast cells, AMs, basophils and eosinophils that may secrete it after allergen stimulation or inflammatory triggers (Sharma *et al.*, 2015; Von Moltke *et al.*, 2016).

ILC2s and primed TH2 cells populate the peripheral tissues of mice lacking IL-25, IL-33 and TSLP signaling ($Il25^{-/-}$, $Il1rl1^{-/-}$, $Tslpr^{-/-}$ mice) but fail to produce IL-5 and IL-13 upon allergic stimulation (Kim and Van Dyken, 2020). Intranasal IL-33 injection alone strongly stimulates lung ILC2s and induces severe type 2 inflammation *in vivo*. Interestingly, experimental asthma persists in IL-33 receptor knockout mice because of the emergence of a TSLP axis driving IL-9 and IL-13 production by ILC2s (Verma *et al.*, 2018). TSLP signaling appears to lead ILCs to become corticosteroid resistant *in vitro* and *in vivo* during airway inflammation (Kabata *et al.*, 2013). IL-25 and TSLP-deficient mice showed only partial reduction of ILC2 activation, indicating that IL-25 and TSLP act more as a costimulation. Also, TSLP promotes the viability of human ILC2s but does not induce their proliferation or cytokine production (Camelo *et al.*, 2017). Although TSLP is an essential cytokine for promoting allergic inflammation and asthma development, the combination of ovalbumin (OVA) and IL-33 induces a robust eosinophilic inflammation in $Tslpr^{-/-}$ mice with a magnitude equivalent to what observed in wild-type (WT) mice (Kabata *et al.*, 2013; Siracusa *et al.*, 2013). Recently, high mobility group 1 (HMGB1) was reported as another alarmin capable of promoting ILC2s proliferation and cytokine production in humans and mice (Wan *et al.*, 2020; Zhang *et al.*, 2020).

Others factors can activate ILC2 such as IL-2, IL-4, IL-7, IL-9, TL1A, TGF-β, LTD4 and prostaglandin D2. Murine lung ILC2s can be activated by LTD4 in mice challenged with *Alternaria* species and produce IL-4, which is not induced by IL-33 (Doherty *et al.*, 2013). Moreover, ILC2s cytokines production can be orchestrated by neuronal regulation through the expression of neurotransmitters and neuropeptides neuromedin-U (NMU), α-calcitonin gene-related peptide (α-CGRP), and vasoactive intestinale peptide (VIP) (Klose *et al.*, 2017; Wallrapp *et al.*, 2017; Nagashima *et al.*, 2019). ILC2s also self-maintain through inducible T-cell costimulator (ICOS):ICOS-L signaling, thus promoting their function and homeostasis (Maazi *et al.*, 2015). Lastly, myeloid cells are potent

drivers of ILCs function through alarmins, cytokines and lipid mediators release (Mortha and Burrows, 2018).

3.5.2 Inhibition

Counter regulation of ILCs is an essential way to limit excessive inflammation. Type I and type II IFNs and IL-27 are well-described to counter-regulate ILC2s activation and therefore to restrict type 2 immunopathologies (Molofsky *et al.*, 2015; Moro *et al.*, 2016). Thus, IFN-γ has been described to regulate the innate immune response by diminishing ILC2s activity to limit excessive inflammation following helminth infection or asthma exacerbation by fungal exposure (Califano *et al.*, 2018). It is interesting to note that IFN-γ does not suppress ILC2s function through the inhibition of IL-33 signaling (Califano *et al.*, 2018). *Ex vivo*, treatment of lung ILC2s with rIFN-γ reduces IL-5, IL-13, IL-17 RB, ST2 and GATA 3 expression, and RORα inhibitor blocks the expansion of IL-25/IL-33-driven expansion of ILC2s as well as IL-13 release (Han, Hong, *et al.*, 2017).

In addition, prostagladin E2 and I2 are inhibitory lipids that suppress IL-5 and IL-13 production by ILC2s in humans and mice (Zhou *et al.*, 2016; Maric *et al.*, 2018). Similarly, lipoxin A4, short-chain fatty acids butyrate and corticosteroids also function as negative regulators of ILC2s (Duerr and Fritz, 2016; Thio *et al.*, 2018). Soluble mediators are not the only actors regulating ILC2s as intercellular contacts may also be involved. Tregs inhibit the production of ILC2-derived IL-5 and IL-13 *ex vivo* and *in vivo*, through ICOS:ICOS-L contact, alongside with the suppressive cytokines TGF-β and IL-10 (Rigas *et al.*, 2017).

3.6 Interactions

In the lung, ILC2s can affect the function of their neighboring cells through the cytokines that they produce. Mast cells, basophils, eosinophils, macrophages, and DCs are potential target cells because of the expression of receptors responsive to cytokines derived from ILC2s (Spits and Di Santo, 2011; Mortha and Burrows, 2018). Lung ILC2s can also promote, probably through soluble factors, B cell production of early antibodies to a respiratory antigen even in the absence of T cells (Drake *et al.*, 2016).

In addition to their interactions via cytokines production, ILC2s also act through direct cell-cell interactions. As such, a fraction of ILC2s express or acquire MHC-II molecules on their surface, playing a role as nonprofessional APCs. This promotes TH2 cells differentiation and maintenance, although this observation for ILC2s has only been described in the gut (See: Box 6: ILC2s, are they antigen-presenting cells?) (Hepworth *et al.*, 2013; Hepworth and Sonnenberg, 2014; Oliphant *et al.*, 2014; Schuijs, Hammad and Lambrecht, 2019). ILC2s also express ICOS-L that may offer an important costimulation to shape

the T cell response (Maazi *et al.*, 2015). In addition to the expression of their ligand, they also express ICOS and may play a major role in activating B cells and DCs (Maazi *et al.*, 2015). OX40L and ICOS-L expression by ILC2s is also required to sustain expansion of Tregs (Molofsky *et al.*, 2015; Halim *et al.*, 2018). ILC2s dynamically express the ligand PD-L1 during immune responses in the lung. Its ligation with PD-1 on TH2 cells acts as innate regulatory checkpoint for the adaptive response leading to GATA 3 and IL-13 upregulation by T cells (Schwartz *et al.*, 2017). In fact, robust expressions of FLT3L, ICOS-L, and PD-L1 allow ILC2s to regulate B and T cells functions, making ILC2s a central bridge between innate and adaptive immune responses.

Box 6: ILC2s, are they antigen-presenting cells?

Expression of MHC-II by ILC2s has been described as well as expression of costimulatory molecules CD80 and to a lesser extent CD86, suggesting antigen-presenting capacities. Following *T.spiralis* parasite infection, activated ILC2s upregulate surface MHC-II expression and enhance capacity of effector TH cells (Angkasekwinai *et al.*, 2017). Interestingly, the expression of MHC-II by ILC2s activates T cells and promotes IL-13 dependent-expulsion of *N.brasiliensis* (Oliphant *et al.*, 2014). On the other hand, *ex vivo* T cells enhance ILC2 proliferation through the production of IL-2, showing a beneficial crosstalk (Oliphant *et al.*, 2014).

The MHC-II expression is lower than in antigen-presenting cells and the cell-surface expression by ILC2s is at least partially due to trogocytosis, likely acquired from other professional APC (Oliphant *et al.*, 2014). It is important to note that those observations were made in the gut and lymph nodes and that MHC-II expression in the lung is minimal. No data on antigen-presenting capacities in ILC2s have been reported in the respiratory tract and further investigations are needed.

3.7 Tolerance and memory

Immunological memory has been defined as the ability of the immune system to recall previous exposure to pathogens and to strongly react to a new encounter with the same pathogen. Some ILC2s persist long after the resolution of the allergen-induced inflammation and respond to unrelated allergens more potently than naive ILC2s until 3 to 4 months after the initial allergen exposure (Martinez-Gonzalez *et al.*, 2016). The authors speculated that this observation may explain why asthmatics are often sensitized to multiple allergens. ILC2 could also promote memory TH2 cell responses to secondary exposure to same antigen (Bird, 2016). Transcriptome analysis of ‘stimulated-ILC2s’ at 4 months after IL-33 stimulation and in comparison with ‘unstimulated-ILC2s’ revealed that IL-17RB (receptor of IL-25) mRNA expression is 2-fold higher in ‘stimulated-ILC2s’ than in naive ILC2s (Martinez-Gonzalez *et al.*, 2016). The fact that memory ILC2s display higher capacity to respond to IL-25 may explain why they show enhanced responses compared to naive ILC2s, since it is known that IL-25 synergizes with

IL-33 to activate ILC2s. Analysis of IL-33$^{-/-}$ mice showed that IL-33 is not required for maintenance and activation of memory ILC2s (Martinez-Gonzalez *et al.*, 2016). However, at birth, IL-33 seems to ensure functional maturation and fitness of further long-lasting resident ILC2s. Indeed, adult ILC2s coming from IL-33$^{-/-}$ mice respond less intensively to exogenous stimulation than naive ILC2s coming from WT mice (Steer *et al.*, 2020). In the end, ILC2s go through 5 phases after allergen exposure: expansion, contraction, memory generation, memory maintenance and recollection (Martinez-Gonzalez *et al.*, 2018). Even if this is not strictly immunological memory as defined above and that implies antigen specificity, the term memory is widely accepted to describe this state of innate immune cells.

Preliminary results show that long-term memory ILC2 responses are impaired in Rag1-deficient mice (lacking B and T cells), suggesting that memory ILC2s require T cells to respond to a secondary challenge (I. Martinez-Gonzalez, M. Ghaedi, F. Takei, unpublished results) (Martinez-Gonzalez *et al.*, 2018).

Box 7: Are mouse ILC2s similar to human ILC2s and therefore represent a relevant translational-experimental model?

Similarly to their mouse counterparts, human ILC2s respond to IL-25, IL-33 and TSLP by producing the TH2 cytokines IL-4, IL-5, IL-9, IL-13 and amphiregulin. They also accumulate during type 2 inflammation in the airways, skin and liver (Guia and Narni-Mancinelli, 2020). Different in the expression of surface markers, humans ILC2s can be identified by the expression of prostanglandin D2 receptor 2, CRTH2 and CD161 which are not observed in mouse ILC2s (Mjösberg *et al.*, 2011). CRTH2, receptor for PGD2 contributes to the maintenance of ILC2s activity in an autocrine manner. The second major difference concerns the frequency of ILC2s in bloodstream: ILC2s is the most prevalent population of ILCs in human blood, whereas circulating ILCs in mice mostly consist of very few ILC1s (Dutton *et al.*, 2019).

The use of murine models has allowed to deeply dissect the mechanisms by which ILC2s contribute to type 2 responses in the lung. However, these mouse models are mainly based on allergic eosinophilic asthma models. While those results can be transposed to the early-onset childhood, it may be difficult to translate them to some asthmatic patients who may present non-allergic form. For this latter type, the study of ILC2s in murine model may be interesting to address the issue of corticosteroid resistance of some severe eosinophilic asthmatics (De Grove *et al.*, 2016). For example, the persistence of type 2 airway inflammation in subgroups of patients with asthma, despite corticosteroid treatment, could reflect the fact that cytokines production by ILC2s is resistant to steroid treatment, particularly in ILC2s stimulated by IL-7 and TSLP (Liu *et al.*, 2018). In some mouse models, however, and in some disease states, ILC2s are steroid sensitive and undergo apoptosis (Walford *et al.*, 2014). In summary, the study of ILC2s in murine model remains a very powerful research model due to its accuracy and editability. However, it must always be taken carefully and put into perspective when translated to human health.

3.8 Other roles and dysregulation

In addition to their TH2 properties, pulmonary ILC2s also exert homeostatic roles particularly against sepsis during which lung is one of the most vulnerable organs (Xu *et al.*, 2018; Akama *et al.*, 2020). They are not just limited to the respiratory tract area, but are dispersed throughout the body where they can have various functions. They are present in lymphoid and non-lymphoid tissues orchestrating innate and adaptive immune responses in the setting of tissue damage, helminth infection and allergen exposure. Also present in adipose tissue, they maintain tissue homeostasis and promote adipose tissue beiging, avoiding obesity and metabolic dysfunction (M. W. Lee *et al.*, 2015). They also drive allergic skin inflammation (Leyva-Castillo *et al.*, 2020). In tumor context, they can play a protective or deleterious role, although in most cases, ST2 expression and high levels of IL-33 are associated with a worse prognosis (Bruchard and Ghiringhelli, 2019).

ILC2s maintain the balance between inflammation and homeostasis and when dysregulated, they can promote inflammation-driven pathology (Buonocore *et al.*, 2010) (**Figure 17**). For this reason, some blocking treatments may lead to adverse side effects such as psoriasis, colitis, loss of metabolic homeostasis or fibrosis due to ILC2 dysregulation. As examples, inhibiting PD-L1 during early life bacterial infection of the respiratory system prevents more severe allergic airway inflammation later in life (Starkey *et al.*, 2016). However, the same treatment during the first two weeks postpartum, in the absence of pathogenic infection, maintains exaggerated responsiveness to HDM challenge of mice in early life (Gollwitzer *et al.*, 2014). Those different effects observed following a same treatment reveal the delicate balance of ILC2 regulation.

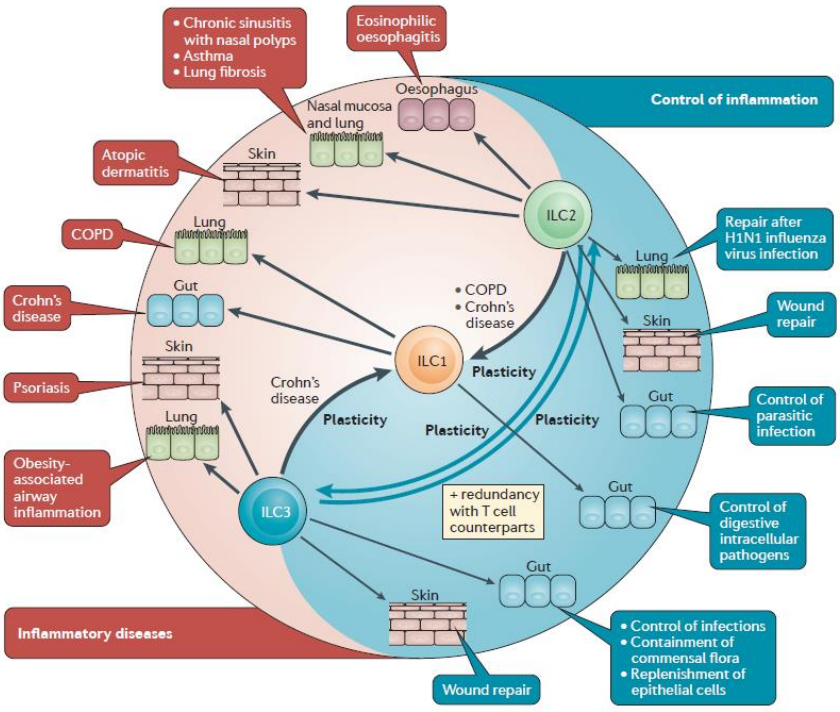


Figure 17. The Yin – Yang of ILC.

Tissue sentinels, ILCs display both pro and anti-inflammatory roles throughout the body (Ebbo et al., 2017).

3.9 Type 2 innate lymphoid cells and viral infections

NK cells are the most extensively described ILCs acting during viral infection. Indeed, they have the ability to recognize and kill virally infected cells. Despite being non-cytotoxic, ILC1s contribute to early host protection through rapid production of IFN-γ at initial sites of infection and maintain an antiviral vigilance in the mucosal epithelium (Weizman *et al.*, 2017; Shannon *et al.*, 2021). They are the first producers of IFN-γ following murine cytomegalovirus infection (Weizman *et al.*, 2019). Although NK and ILC1s both produce IFN-γ, they remain different by their distinct developmental pathways and their migration properties. Indeed, ILC1s are mostly tissue residents while NK cells recirculate throughout the body (Kim and Kim, 2016). The role of ILC3s in lung diseases is more obscure, albeit they may promote lung homeostasis through the IL-17/IL-22 axis (Ardain *et al.*, 2019). Finally, given that ILC2s constitute the main population of ILCs in the lung and central actors in asthma pathology, it is therefore not surprising that viruses, known to trigger or exacerbate asthmatic symptoms, do so via activation of ILC2s (Kim, Umetsu and Dekruyff, 2016). Here, we review the current understanding of the role of ILC2s in viral lung diseases.

3.9.1 Inflammatory role of ILC2s

ILC2s have been shown to be activated following early-life viral infections that may lead to long-term alterations of the lung environment, such as RSV and RV (Fonseca *et al.*, 2021). Respiratory viral infections alter AEC, resulting sometimes in necroptosis (Miura, 2019). Not all infection cause worsening of respiratory symptoms in every individual. This is usually observed in patients with chronic airway diseases and in many non-allergic forms of asthma, which are triggered by environmental factors, including air pollutants such as cigarette smoke and obesity. In fact, inflamed airways produce more IL-33 after RV infection than healthy ones (Jurak *et al.*, 2018). Similarly, AEC from asthmatic children are more reactive and produce significantly higher levels of TSLP after RSV infection than cells from healthy children (Lee *et al.*, 2012). Nonetheless, similar levels of IL-33 can sometimes be observed in both healthy and asthmatic patients (Jackson *et al.*, 2014). Chronic disabilities result in a greater amount of cells that may be more reactive to inflammatory cytokines and able to induce a faster and stronger deleterious immune response. *In vitro,* Influenza A Virus, RSV and RV are all potent inducers of IL-33 in mice and humans (Harker and Lloyd, 2021). The damage of epithelium by viral infection leads to the release of pro-inflammatory cytokines that can activate ILC2s. In 2011, influenza was the first viral infection described to induce AHR through the newly discovered pathway of IL-13-producing natural helper cells (later renamed innate lymphoid cells) (Chang *et al.*, 2011). Early-life RV infection activates ILC2s through an interplay between IL-25, IL-33 and TSLP (Han, Rajput, *et al.*, 2017) in mice, whereas in humans, IL-33 appears to remain the major activator. *In vitro*, bronchial epithelium produces IL-33 in response to RV infection, and then directly drives human ILC2s to produce a large amount of type 2 cytokines (Jackson *et al.*, 2014). Nasopharyngeal samples from children infected with RV and RSV

bronchiolitis have elevated levels of TSLP and IL-33, which attenuate during recovery; those with severe influenza infection show only elevated IL-33 levels (Perez *et al.*, 2014; Saravia *et al.*, 2015). Finally, TSLP activates IL-13-producing ILC2s after RSV infection in both mice and humans (Stier *et al.*, 2016).

Production of IL-13 and IL-5 by ILC2s can be stimulated by immune cells themselves such as NKT, DC and AMs (Chang *et al.*, 2011; Gorski, Hahn and Braciale, 2013; Maazi *et al.*, 2017). For example, the main source of IL-33 after influenza was more related to alveolar macrophages than epithelial cells (Chang *et al.*, 2011). As explained before in the introduction others potential players may also activate ILC2s. For example, recently, Loh et *al.* have described HMGB1 as inducer of ILC2s activation in airway remodeling with reversible effects via the use of an anti-HMGB1 antibody (Loh *et al.*, 2020). RSV infection promotes necroptosis with HMGB1 release by epithelial cells (Simpson *et al.*, 2020). Subsequent activation of ILC2s could therefore be suggested. Neuronal activation following pathogen exposure can also enhance the effect of epithelial-derived cytokines on ILC2s (Klose *et al.*, 2017). Some respiratory viruses such as RSV, influenza, coronavirus and metapneumovirus can lead to neuropathologies suggesting the existence of an ILC2s-neuronal activation (Bohmwald *et al.*, 2018; Verzele *et al.*, 2021).

ILC2s are early responders and IL-13 producing ILC2s expand 4 days after RSV infection and are more potent than TH2 cells, producing more than 10 times the amount of TH2 cytokines on a per-cell basis (Stier *et al.*, 2016). During influenza infection, ILC2s accumulate more rapidly than T cells with a peak at 4 days (Li *et al.*, 2018). Chang et *al*. have shown that influenza A virus can rapidly induce AHR by activation of ILCs and this even in absence of adaptive immune system (Chang *et al.*, 2011). Neonatal RV infection leads to IL-13 dependent mucous metaplasia and persistent AHR (Hong *et al.*, 2014). In humans, pandemic influenza-related asthma symptoms are linked to type 2 cytokines produced by ILC2s (Shim *et al.*, 2015). ILC2s can also act through direct interactions. During RSV infection, ILC2s were crucial to mediate activation of $CD4^+$ T cells via OX40/OX40L communication. Indeed, adoptive transfer of lung infected-ILC2s into naive mice enhanced both the expansion and the cytokines production of $CD4^+$ T cells (Wu *et al.*, 2019). At the same time, $CD4^+$ T cells have been shown to be essential for the activation of ILC2s during RSV infection in mice (X. Han *et al.*, 2019).

In addition to the production of cytokines, activation of ILC2s may also result in their expansion. Infants hospitalized with severe respiratory virus infection have higher numbers of ILC2s in their nasal aspirates, compared to children with mild infection (Norlander and Peebles, 2020). In mice, Influenza, RSV and Pneumovirus of mice (PVM) – relative to human RSV – infections increase the number of lung ILC2s numbers, with regulation occurring most often after resolution of the infection (W.S. Li *et al.*, 2018; Vu *et al.*, 2019; Loh *et al.*, 2020).

The pathology associated with a given viral infection varies with the age of the host. While influenza-infected newborn mice show a rapid high production of IL-33, with underlying activation of ILC2s, this is not the case in infected adults (Saravia *et al.*, 2015). Similarly, unlike adult mice, RSV infection of neonatal mice results in activation of cytokines production by ILC2s in an IL-25 dependent manner (Hong *et al.*, 2014). RV-induced asthma exacerbation has also only been reported in young mice (Rajput *et al.*, 2017). This may partly explain why early severe acute viral bronchiolitis in childhood can lead to later persistent asthma in adulthood (Bergroth *et al.*, 2020). This age-dependency seems to be more related to the "naivety" of the immune system and the resulting cytokines produced by young individuals, rather than to the direct effect of the virus itself.

Limiting or depleting ILC2s provide protection against severe disease induced by RV infection, proving their deleterious effect in this pathology (Rajput *et al.*, 2020). An activation of ILC2s is widely described following respiratory viral infections. This challenges the classical virus-TH1 immune response by showing that it may also promote type 2 immune response, especially in the context of asthma exacerbation. Their activation leads to their expansion, direct or indirect interactions with neighboring cells, and the production of type 2 cytokines that can cause numerous tissue damages such as airway obstruction, AHR, chronic infiltration, hypertrophy of bronchial smooth muscle cells and mucus overproduction. Ultimately, this may or may not lead to the initiation of asthma-like airway inflammation before any antigen sensitization.

3.9.2 Homeostatic properties of ILC2s

Airway wall remodeling is one of the most common pattern found in asthmatic patients and can be elicited by various respiratory triggers (Lambrecht and Hammad, 2015). ILC2s disrupt bronchial epithelial barrier integrity by targeting tight junctions through IL-13 in asthmatic patients (Sugita *et al.*, 2018). Despite this harmful effect, ILC2s are also essential for maintaining epithelial integrity and airway remodeling to restore lung function (Kim and Kim, 2016). Single-cell RNA-sequencing (scRNA-seq) of neonatal lung ILC2s supports the presence of two distinct effector ILC2 subsets. One is defined as pro-inflammatory through the production of type 2 cytokines, and the other, as tissue-repairing subset by the production of the epidermal growth factor – amphiregulin (Areg) (Ghaedi *et al.*, 2020). In fact, this protective role is one of the first reported in ILC2s following a viral infection. Indeed, in 2012, Monticelli et *al*. observed that the depletion of ILCs resulted in loss of airway epithelial integrity, decreased lung function and impaired airway remodeling during acute influenza virus infection. Those damages were resolved after instillation of recombinant Areg (Monticelli *et al.*, 2012). Importantly, ILC2-derived Areg prevents neonatal mortality after influenza infection under δT cells orchestration (Guo *et al.*, 2018).

Interestingly, in the context of the newly respiratory pathology COVID-19, the role of ILC2 is not well described and conflicting observations have been reported. On one hand, ILCs seem to be largely depleted from the bloodstream of severely affected patients (García *et al.*, 2020). Besides, Areg, produced in human by blood ILCs (Silverstein *et al.*, 2021), also declined in COVID-19 subjects as a function of disease severity (Harb *et al.*, 2021). ILC2s frequency is inversely correlated with tissue damage indicators such as myoglobin, troponin T and LDH. In this context, a study of a cohort of approximatively 150 individuals suggested a protective effect of ILC2s against morbidity and mortality in SARS-CoV-2 (Silverstein *et al.*, 2021). On the other hand, another studies reported that blood analysis of COVID-19 patients displays a mixed TH1 (IL-12, IFN-γ) and TH2 (IL-4, IL-5, IL-10, IL-13) orientation (Choreño-Parra *et al.*, 2021) as well as increased systemic level of IL-33 in serum and plasma with increased circulating ILC2s (Gomez-Cadena *et al.*, 2021). Further studies are then needed to better understand the role of ILC2s in COVID-19 pathology.

The following are examples of other homeostatic properties of ILC2 that may play a role during viral infections. Autocrine production of IL-9 supports ILC2 responses to coordinate epithelial cell maintenance and lung homeostasis (Mohapatra *et al.*, 2016). Patients with severe allergic asthma have high numbers of circulating ILC2s expressing CCR10 that produce Areg and IFN-γ. Depletion of those cell exacerbates AHR in allergen-challenged mice, supporting their protective role in allergic inflammation (Beuraud *et al.*, 2019). Some ILC2s produce IL-10 and can then downregulate inflammation (Seehus *et al.*, 2017). Despite the possible regulatory roles of IL-10-producing ILC2s, these cells produce more IL-5 and IL-13 compared with the non-IL-10 producers, suggesting that IL-10 producing ILC2s maintain their activity under allergic inflammation (Ebihara and Taniuchi, 2019). Surprisingly, even type 2 cytokines may have beneficial impact. Indeed, IL-5 produced by ILC2s protects mice from death in a model of idiopathic pulmonary fibrosis (Hrusch *et al.*, 2018). In the end, innate immunity appears to play a more predominant role than adaptive immunity in lung regeneration; the lung mass 14 days after pneumonectomy being unaffected by the absence of T and B lymphocytes (Lechner *et al.*, 2017).

3.9.3 Plasticity of ILC2s

Dependent on their microenvironment, ILC2s are plastic meaning that they have the capacity to adopt phenotypic similarities to other ILCs subsets. This plasticity relies on the microenvironment, and more precisely, on cytokines and chemokines produced by epithelial cells or APC (Krabbendam *et al.*, 2018) (**Figure 18**). They can transdifferentiate into an ILC1 phenotype by decreasing their expression of GATA 3 and increasing their production of IFN-γ (Spits, Bernink and Lanier, 2016). After exposure to IL-12, IL-18 or IL-1β, ILC2s and ILC3s can transdifferentiate to become phenotypically ILC1s, and these ILC1s have been associated with inflammatory autoimmune diseases (Spits, Bernink and Lanier,

2016). After influenza infection, functional plasticity of ILC2s exacerbates antiviral immunity, which may have adverse consequences in respiratory diseases such as chronic obstructive pulmonary disease (COPD) (Silver *et al.*, 2016). Human and murine ILC2s can contribute to antiviral response by converting into ILC1s in response to IL-12 and IL-18, which is promoted by IL-1β (Bal *et al.*, 2016; Ohne *et al.*, 2016). ILC2-to-ILC3 plasticity has also been described in humans (Hochdörfer *et al.*, 2019). During viral infection, plastic ILC2s are close to myeloid-derived cells that express IL-12 and IL-18 in inflamed tissues (Silver *et al.*, 2016). In addition to being described in the context of viral infections, ILC2s-to-ILC1s plasticity has been described in intestinal samples from Crohn's disease patients (Lim *et al.*, 2016). The plastic properties of ILC2s show their incredible ability to adapt their functions to environmental conditions.

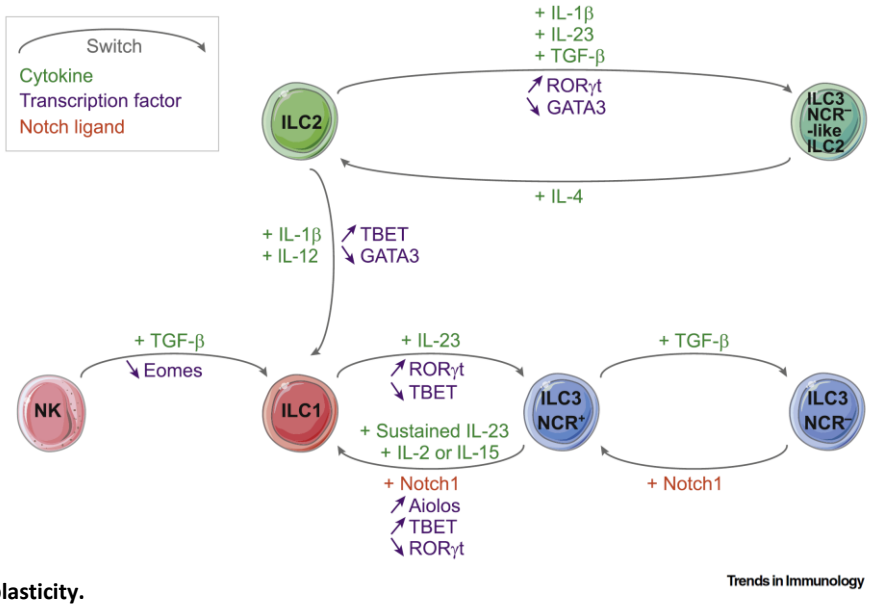


Figure 18. ILC2s plasticity.

ILC2s have the ability to transdifferentiate through another subset depending on the cytokine microenvironment (Guia and Narni-Mancinelli, 2020).

IL-1β can prone ILC2s to respond to both type 1 and type 2 cytokines, depending on the environmental stimuli, and can be released in a wide variety of infections and situations where tissue damage occurs. In a mouse model of virus-induced asthma exacerbation, IL-1β signaling induces IL-33 expression (Mahmutovic Persson *et al.*, 2018). In response to early-life RV infection in mice, IL-1β prevents ILC2 expansion, type 2 cytokine secretion and mucus metaplasia. Neonatal infection in mice lacking IL-1β signaling (NLRP3$^{-/-}$ mice or chemical inhibition of IL-1β) leads to enhanced RV-induced TH2 cytokine expression and mucus metaplasia (Han *et al.*, 2020). Nonetheless, in the presence of IL-2, IL-1β can promote proliferation of ILC2s and type 2 cytokines secretion (Ohne *et al.*, 2016), supporting an essential role of NLRP3-inflammasome activity for RV-induced airway inflammation and AHR in adult mice (M. Han *et al.*, 2019).

IL-18 – cytokine with IFN-γ-inducing properties – is mainly secreted by myeloid cells and generates strong antiviral immune responses. IL-12 and IL-18 act synergistically in anti-viral immunity (Ferreira *et al.*, 2002). However, human ILC2s stimulated with IL-18 can produce TH2 cytokine at similar levels to those seen with IL-33 stimulation, suggesting also an activating role of IL-18 (Simoni *et al.*, 2017). IL-12 drives the transdifferentiation of ILC2s to ILC1s (Lim *et al.*, 2016) although the priming with IL-1β is necessary (Ohne *et al.*, 2016). IL-$12^{-/-}$ or IL-$18^{-/-}$ mice did no shown any difference in histopathology following RSV infection wondering their role in ILC2s modulation in this pathology (Boelen *et al.*, 2002). Ratio between IL-4 and IL-12 seems to dictate the balance between activation of ILC2s and their conversion to ILC1s in the context of airway inflammation. It is therefore the synergy of all these cytokines that leads to ILC2s plasticity, as individually they can either activate or repress the activity of ILC2s during viral infections.

3.9.4 Counter regulation of ILC2s in viral infection

The well-known ILC2s inhibitor type I and type II IFNs (See: 3.5.2 Inhibition) are critical for early control of viral infection and modulation of the antiviral immune response (Lee and Ashkar, 2018). IFN-γ-receptor deficiency during primary RSV infection results in a disturbed TH1 response based on increased type 2 cytokines as well as eosinophilia (Boelen *et al.*, 2002). Intranasal administration of recombinant IFN-γ protein reduces RV-induced IL-13 production ILC2s and mucus hypersecretion (Rajput *et al.*, 2017). This subsequently blocks the development of asthma phenotype following neonatal RV infection, establishing IFN-γ deficiency as a cause of viral-induced allergic disease in immature animals (Han et al., 2017).

However, IFN-γ may also play a detrimental role by limiting ILC2s activity. Indeed, recovery of tissue and survival following influenza infection were dependent on the production of IL-5 and Areg by ILC2s (Califano *et al.*, 2018). Genetic IFN-γ deficiency or anti-IFN-γ treatment during influenza infection does not increase ILC2s, but instead enhances their release of IL-5 and Areg and improves tissue protection without affecting viral load or clearance (Califano *et al.*, 2018).

Box 8: Male and female, not the same?

It is intriguing to note that respiratory susceptibility seems to differ between males and females, leading to different outcomes. Indeed, men are more likely to develop early asthma and atopic diseases than women, who are more prone to late-onsets. In the context of respiratory viral diseases, in early life, males are more susceptible to severe disorders caused by respiratory viruses compared to females; following RSV-bronchiolitis or pneumonia, men are hospitalized twice as much as women (Chen *et al.*, 2019). One explanation may lie in a stronger TH1 immune response seen in females with higher levels of inflammatory markers than observed in males (Chen *et al.*, 2008). ILC2s may also play a role in sex bias: in neonates, the number of blood ILC2s is higher in males than in females, while remaining higher overall than in adults (Forsberg *et al.*, 2014). In adult female mice, ILC2s appear to be more reactive with greater expansion and cytokine production than in males under IL-33 stimulation, although these observations have not been demonstrated in adult humans (Wang *et al.*, 2020). Interestingly, testosterone appears to downregulate ILC2s functions and expansion during asthma, which may explain the reduction of post-puberty asthma in males (Laffont *et al.*, 2017; Wang *et al.*, 2020). Supporting these observations, Fonseca et *al*. suggest that age-associated ILC2s function, related to early-life viral responses, may be attenuated later in life by sex-associated mechanisms in males (Fonseca *et al.*, 2021).

3.9.5 ILC2 virus-memory features

As already explained above, some ILC2s persist long after the resolution of the inflammation (See: 3.7 Tolerance and memory) and they respond to unrelated allergens more potently than naïve ILC2s. Those allergen-experienced ILC2s are particularly responsive to IL-25 and probably have a potential role in IL-25-dependent virus-induced exacerbations in asthma (Martinez-Gonzalez *et al.*, 2018). In addition to the role of IL-25 signaling in memory acquisition by ILC2s, IL-33 appears to ensure early functional maturation and fitness of long-term resident ILC2s (Steer *et al.*, 2020). Expansion of ILC2s represents a peak returning to normal upon resolution of RSV inflammation. Interestingly following a second RSV infection, ILC2s proliferate more efficiently, suggesting that RSV infection may sustain the generation and responsiveness of trained ILC2s (Saravia *et al.*, 2015). IL-33 stimulated ILC2s seem responsible of a persistent inflammatory environment after early viral infection that exacerbates secondary responses later in life. Rajput et *al*. suggest that early respiratory viral infections likely enhance disease-associated "training" that may influence later pathological phenotypes (Rajput *et al.*, 2020).

Evidence of the role of epigenetic modulation as a source of long-term changes in the immunological phenotype is a well-established fact (Netea *et al.*, 2015). Although the classical adaptive immune memory is specific, antigen dependent and mediated by gene rearrangement, innate immune memory is nonspecific, antigen independent and mediated through epigenetic reprogramming (Netea and van der Meer, 2017). Cytomegalovirus infection has been described to induce MCMV-specific memory NK cells (Nabekura and Lanier, 2016) and drive adaptive epigenetic diversification of NK cells with altered signaling and effector functions (Schlums *et al.*, 2015). H3N2 influenza A virus inhibits the initiation of the host innate immune response by interfering with epigenetic control of gene expression (Marazzi and Garcia-Sastre, 2015). Coronavirus (MERS-Cov) and several influenza infections induce repressive histone modification of H3K27me3 with downregulation of IFN-stimulated genes (Menachery *et al.*, 2014). Thus, there is evidence that some viral infections have the ability to affect the immune cells through epigenetic modulation. Further studies are therefore needed to better understand the long-term changes and epigenetic reprogramming of ILC2s following respiratory infections.

3.9.6 Therapeutic potential

Targeting ILC2s may provide a therapeutic option against viral infections and specifically in TH2-associated exacerbation of asthmatic disease. Ravanetti et *al.* identified IL-33 as main player to dampen IFN-I expression and TH1-phenotype, and thus to drive influenza-induced asthma exacerbations in mice and humans (Ravanetti *et al.*, 2019). The use of IL-33 receptor knockout mice or IL-33 blocking antibodies ameliorates the RSV asthma-like inflammation (Saravia *et al.*, 2015). The treatment of viral diseases most often aims at alleviating the symptoms, and only requires the use of

antibiotics in case of bacterial superinfection. Influenza or RSV infection elicits various inflammatory profiles that differ in their sensitivity towards steroid treatment. RSV inflammation does not respond to steroid treatment, unlike influenza inflammation which is alleviated by prednisolone treatment (Mori *et al.*, 2013). However, study on a small cohort linked the use of corticosteroid in severe influenza to risk of superinfection, prolonged viral replication and increased risk of death (Lansbury *et al.*, 2019). While ILC2s seem sensitive to steroids *in vitro* and *in vivo* (Walford *et al.*, 2014), many studies designate ILC2s as the key actors to understand severe refractory asthma because of their resistance to corticosteroid therapy, in a TSLP-dependent manner (Liu *et al.*, 2018).

The use of immunotherapy to temper ILC2s is being increasingly studied. Treatment of mice with neutralizing anti-IL-2 monoclonal antibodies diminishes ILC2s activation in RSV infection (X. Han *et al.*, 2019). Anti-IL-5 (mepolizumab) decreases significantly eosinophilia in RV or SARS-CoV-2 infections but fails to prevent activation of remaining eosinophils in RV16-induced immune responses in mild asthma (Sabogal Piñeros *et al.*, 2019; Azim *et al.*, 2021). In asthma pathology, treatment using anti-TSLP antibody decreases allergen-induced bronchoconstriction before and after allergen challenge in humans (Gauvreau *et al.*, 2014). Again in humans, treatment blocking IL-13 improves lung functions parameters in asthma and reduces seasonal asthma exacerbations in a cohort of 2.148 patients (Corren *et al.*, 2011; Staton *et al.*, 2017). Finally, epigenetic modifications are explored as potential therapeutic targets in the context of viral infections (Ahmad, Assaf and Herbein, 2021). The epigenetic inhibitors of cytokine secretion (Bromodomain and iBET151) inhibit ILC2 activation and have shown efficacy in experimental allergic lung inflammation (Kerscher *et al.*, 2019).

To conclude, surprisingly, cohort study of ILCs-deficient humans appear to show equal susceptibility to viral infection as immunocompetent ones (Vély *et al.*, 2016), supporting that the precise role of ILC2s in the context of viral infection has not yet been fully understood and that further studies are needed. Viral infections can lead to chronic post-viral lung diseases or others respiratory disabilities with lifelong discomfort. This has been impressively demonstrated by the ongoing COVID-19 pandemic (Perrin *et al.*, 2020). Understanding the precise immunological mechanisms activated in the context of viral infections is therefore of prime importance to develop effective therapeutic targets against many major public health concerns.

Objectives

Objectives

The immune system is continuously shaped by environmental antigens and microorganisms which imprint subsequent immune responses. In the lung, different infectious organisms may have varying influences on the development of allergic reactions such as asthma. On the one hand, some respiratory viral infections promote the development of asthma or worsen its symptoms. On the other hand, a reduced exposure to microbes during childhood could explain the dramatic increase in allergic diseases observed in Western countries. Although widely studied, asthma remains a global socioeconomic burden whose mechanisms require better understanding.

ILC2s are widely implicated in viral pathology by driving airway hyperresponsiveness and aggravating inflammation with exacerbation of type 2 pattern such as asthma. However, they play a key role in tissue repair and maintenance of pulmonary homeostasis, especially at early post-infection stages. It is therefore necessary to maintain a delicate balance between activation and repression of ILC2s. This is particularly true for persistent viruses, such as γHVs, which are likely to induce repair/regulatory immune functions in order to promote tissue homeostasis and their long-term maintenance.

This thesis consists mainly of one study. From an immunological point of view, we aimed at investigating the **impact of a latent viral infection on pulmonary ILC2s, and at understanding the mechanisms underlying the protection conferred against airway allergy.** To help us in that purpose, we have performed experiments on mice (wild-type or genetically modified) infected with MuHV-4 - robust model of human EBV - and submitted or not to an allergic asthma protocol by instillations of mite extracts.

The global objective is obviously to better understand the **modulation of the immune system by persistent viral infections going in line with the hygiene hypothesis**.

Experimental section

Experimental section

A persistent gammaherpesvirus infection reprograms the alveolar macrophage niche for the long-term by dampening type 2 properties of group 2 innate lymphoid cells

Pauline Loos, Jérôme Baiwir, Céline Maquet, Justine Javaux, Rémy Sandor, François Lallemand, Thomas Marichal, Bénédicte Machiels*, Laurent Gillet*

* co-senior authors

Abstract

Immunological dysregulation in asthma is associated with changes in microorganism exposure early in life. Gammaherpesviruses (γHVs) are highly prevalent human viruses that establish lifelong infection and deeply shape host immunity. Using Murid herpesvirus 4 (MuHV-4), a mouse γHV, we showed that, after infection, lung resident and bone marrow-derived group 2 innate lymphoid cells (ILC2s) display lasting reduced capacity to expand and to produce type 2 cytokines in response to house dust mites, contributing to asthma protection. Importantly, we uncovered that ILC2s represent essential niche cells imprinting the tissue-specific identity of monocyte-derived alveolar macrophages upon infection. In particular, MuHV-4 infection disrupts the physiological ILC2-epithelial cells circuit that programs monocytes-derived alveolar macrophages for type 2 functions without affecting their differentiation. These results reveal that persistent γHV infection shapes the alveolar landscape much beyond the initial acute infection through long-term effect on ILC2 niche cells.

One Sentence Summary

Gammaherpesvirus infection induces lasting inhibition of ILC2 type 2 response and subverts the ILC2-Mo-derived AM crosstalk that ultimately affects the polarization of Mo-derived AMs in allergic asthma.

Introduction

Asthma is a chronic inflammatory disease of the airways that affects more than 250 million people worldwide and causes around 500,000 deaths per year[1]. Asthma is characterized by episodes of wheezing, coughing, chest tightness, and shortness of breath. These symptoms are driven by aberrant airway inflammation and subsequent processes such as mucus hypersecretion, remodeling of the airway wall and bronchial hyperresponsiveness[2]. The development and exacerbation of asthma are influenced by many environmental factors including infectious agents. On the one hand, some respiratory viral infections can trigger severe adverse outcomes in patients at risk of asthma or with existing asthma[3]. In particular, respiratory syncytial virus (RSV) and rhinovirus (RV) are the main drivers of asthma exacerbation in children and adults, respectively[4]. On the other hand, the "hygiene hypothesis" proposed that one reason for the dramatic increase in the occurrence of allergic diseases in western lifestyle countries could be linked to reduced exposure to microbes or microbial products during childhood[5–7]. In that context, we recently showed that Murid herpesvirus 4 (MuHV-4), a model for the highly prevalent human Epstein-Barr virus (EBV), inhibits the development of House Dust Mites (HDM)-induced airway allergy for the long-term[8]. Specifically, MuHV-4 infection induces the replacement of resident alveolar macrophages (AMs) by monocyte-derived AMs that block the ability of DCs to trigger a TH2 response against HDM[8]. However, the initial immunological mechanism underlying this observation is still completely unknown.

Innate lymphoid cells (ILCs) are a heterogeneous family of cells that are particularly abundant at barrier surfaces where they act as first line innate immune sensors[9–11]. Group 2 innate lymphoid cells (ILC2s) are the predominant ILC population in the lung at steady state[12]. While ILC2s are essential to promote type 2 inflammation against helminths[13–15], they also control eosinophil homeostasis[16] and play a major role in chronic type 2 inflammatory diseases such as asthma[17–21]. Thus, ILC2s are essential for the initiation and persistence of type 2 inflammation in protease and HDM-induced models of airway allergy[19,21–23]. ILC2s expand in response to various signals including epithelial cytokines (IL-25, IL-33 and thymic stromal lymphopoietin (TSLP)), lipid mediators (leukotrienes and prostaglandins) and neurotransmitters (neuromedin U, vasoactive intestinal peptide)[24–29]. Based on the integration of these signals, they can affect the function of their neighbouring cells through the cytokines they produce or *via* direct cell-cell interactions. Respiratory viruses associated with asthma exacerbation, such as RSV, RV or influenza virus, have been shown to increase the number of ILC2s and to promote their activation and production of type 2 cytokines[30]. In contrast, the long-term impact of persistent viruses, such as MuHV-4, on ILC2s function is still unknown.

In this study, we showed that MuHV-4 infection inhibits ILC2s expansion and HDM-induced type 2 response in an IFN-γ dependent manner. We identified pulmonary ILC2s as key actors that

imprinted monocyte-derived AMs identity and functional specification after virus-induced niche depletion. Our findings reveal important insights into ILC2 dependent-AM plasticity following γHVs infection with long-term consequences for host allergic susceptibility.

Results

MuHV-4 infection reduces the number of lung ILC2s and modifies their functional properties after HDM treatment

BALB/c mice were infected or not with MuHV-4 and submitted to HDM-induced airway allergy (Fig. 1A). Lung innate immune response was analyzed by flow cytometry one day after the first HDM instillation (sensitization) and three days after the last instillation (challenge). Lung ILC2s were described as $Lin^-CD45^+CD90.2^+ST2^+CD25^+$ living cells with lineage composed of B220, CD11c, CD3, CD4, CD49b, CD5, CD8α, F4/80, FcεR1, Gr1 and Siglec-F markers (Fig. 1B) and ILCs $NKp46^+$, regrouping all lung ILC1s and some ILC3s, as $CD45^+Lin^-NKp46^+$ living cells[31]. As previously shown[8], MuHV-4 infection conferred protection against HDM-induced allergic asthma as revealed by the reduced eosinophilia observed in MuHV-4 infected mice (Fig. 1C). Interestingly, in contrast with mock infected mice, we did not observe any increase of lung ILC2 number in MuHV-4 infected mice after HDM challenge (Fig. 1D). This observation was not associated with differences in ILC1 or ILC3 populations (Fig. 1D). Moreover, within MuHV-4 infected mice, ILC2s displayed decreased expression of GATA 3 and reduced production of IL-13 and IL-5 upon *ex vivo* restimulation both at sensitization and challenge phases (Fig. 1, E-G) revealing that mouse infection by MuHV-4 profoundly imprints the function of lung ILC2s after HDM treatment.

In order to directly assess IL-5 production *in vivo*, we repeated those experiments using IL-5 reporter mice (IL5-tdtomato-cre, also called Red5)[32] submitted to HDM-induced airway allergy (Fig. S1A) and analyzed the number and function of lung ILC2s (Fig. S1B). As expected, MuHV-4 infection conferred protection against HDM-induced airway allergy revealed by a reduction of lung eosinophilia upon HDM challenge (Fig. S1C). Similarly, to what was observed in BALB/c mice, MuHV-4 infection blocked the increase of lung ILC2 number observed upon HDM challenge in mock infected mice (Fig. S1D). ILC2s from MuHV-4 infected mice also displayed a lower GATA 3 expression and a lower IL-5 production compared to mock infected mice both at HDM sensitization and challenge (Fig. S1, E-G). Furthermore, this also revealed a reduced expansion of IL-5 producing T cells in MuHV-4 infected mice (Fig. S1H). Finally, while ILC2s from uninfected mice were uniformly distributed within the lung parenchyma, ILC2s from MuHV-4 infected mice were clustered in lower numbers in the bronchovascular regions (Fig. S1, I and J).

MuHV-4 infection impairs both the expansion and recruitment of pulmonary ILC2s

The reduced number of ILC2s after HDM challenge observed in MuHV-4 infected mice could reflect increased cell mortality, reduced expansion or reduced recruitment. While we did not observe any differences of ILC2s apoptosis or necrosis between groups (Fig. 2, A-C), we observed a major difference in ILC2 ability to proliferate upon HDM challenge between groups as revealed by the reduction of Ki67 positive ILC2s (Fig. 2, D and E) and by the lower mean fluorescent intensity of anti-5-Ethynyl-2'-deoxyuridine (EdU) staining following repetitive intranasal instillation of EdU (Fig. 2, F-H).

ILC2s were firstly described as resident cells and a recruitment has only been observed in some specific cases[33]. We therefore assessed if HDM treatment could induce ILC2s recruitment and if MuHV-4 infection could affect it. Briefly, CD45.2$^+$ BALB/c mice were lethally irradiated except the thoracic cavity in order to spare the lung resident cells. These recipient mice were transplanted with bone marrow (BM) from CD45.1$^+$ BALB/c congenic donors. Eight weeks after irradiation and BM transfer, mice were infected or not with MuHV-4 and then submitted to HDM sensitization and challenge (Fig. 2I). Interestingly, while no recruitment was observed upon sensitization, we showed that HDM challenge induced noticeable ILC2s recruitment (Fig. 2, J and K). Surprisingly, recruited ILC2s were already observed in the lung of MuHV-4 infected mice at steady state. However, upon HDM challenge, the recruitment of ILC2s was markedly reduced in this group in comparison with mock infected mice (Fig. 2, J-L). Analysis of both CD45.1 and CD45.2 cells revealed that cells of both origins were similarly affected by MuHV-4 infection for their capacity to produce IL-5 (Fig. 2, M and N). Altogether, these analyses revealed that MuHV-4 infection impairs both the proliferation and the recruitment of lung ILC2s following HDM challenge and affect ILC2s uniformly.

MuHV-4 infection modifies the identity of lung ILC2s

In order to characterize ILC2s after MuHV-4 infection and/or HDM instillations at the single cell level, we performed single-cell RNA sequencing on sorted ILC2s (Fig. 3A and Fig. S2, A-F). As ILC2s can acquire ILC1-like properties driven by the cytokine microenvironment, we assessed a potential plasticity of these cells by monitoring the expression of specific transcription factors following MuHV-4 infection and HDM treatments. In contrast with previous studies on ILC2s after some viral infections[34], we did not observe any increased expression of *Tbx21*[35] (T-bet) or *Rorc* (RORγt) in lung ILC2s from any group (Fig. 3B). In addition, no increase in T-bet and IFN-γ intracellular protein levels was observed after MuHV-4 infection as compared to mock-infected mice (Fig. 3C). Expression of *Gata3 a*nd *Rorα*[36] was significantly higher after HDM challenge in lung ILC2s from mock infected mice as compared to the MuHV-4 counterparts (Fig. 3B), supporting the negative regulation of ILC2s by the infection.

UMAP analysis revealed difference between ILC2s from mock and infected mice mainly after HDM challenge (Fig. 3D), in line with our previous analyses of ILC2s recruitment and proliferation (Fig. 2). Differential gene expressions highlighted higher expression of genes involved in ILC2s pro-TH2 functions (such *as Il-13, Arg1, Gata3, Rorα, Areg*) in HDM challenged mock infected mice in comparison with their MuHV-4 infected counterparts (Fig. 3, E-G). In addition, differences in expression of activation markers were observed and confirmed by flow cytometry analysis (Fig. 3H). Interestingly, at sensitization, PD-1 and KLRG1 were both overexpressed in lung ILC2s of mock mice in comparison with MuHV-4 infected mice (Fig. 3H). This suggests differences in ILC2s at the initiation of the TH2 response as PD-1 expression has been described to be upregulated on activated ILCs[37] and to be an important regulator of the maturation marker KLRG1 in ILC2s[38,39]. At the same time, Sca1 expression was higher in ILC2s from MuHV-4 infected mice (Fig. 3, F and H), however, the significance of Sca1 expression is still matter to debate[40]. Interestingly, some differences were already observed before any HDM stimulation with higher expressions of genes involved in tissue repair (*Areg*) or response to IFN-γ (*Ifrd1, Stat1*) (Fig. 3E). In particular, ST2 expression appeared to be reduced in ILC2s from MuHV-4 infected mice prior to any HDM treatment (Fig. 3, I and J). This difference was even increased upon sensitisation to HDM while IL-33 production was similar between infected and uninfected mice (Fig 3, K and L) providing a potential mechanistic explanation of the lower responsiveness of ILC2s in MuHV-4 infected mice.

Gene-set enrichment analysis of transcriptomic data, revealed that HDM challenge induced the upregulation of genes involved in TH2 immunity and lymphocyte migration in ILC2s from mock infected mice in comparison with ILC2s from MuHV-4 infected mice (Fig. 3G). In contrast, an increased expression of genes playing a role in negative regulation of innate immune response was observed in ILC2s from infected mice upon HDM challenge (Fig. 3G). Especially, it highlighted that infection with MuHV-4 induced substantially higher expression of genes involved in response to IFN-γ which is a well-known inhibitor of ILC2s[41] (Fig. 3G). Interestingly, ILC2s from MuHV-4 infected mice appeared to display a reduced orientation of their metabolism towards aerobic respiration in comparison with ILC2s from mock infected mice, suggestive of a possible involvement of metabolism in regulation of their function.

IFN-γ directs the reversible functional impairment of pulmonary ILC2s after MuHV-4 infection

To assess if MuHV-4 imprinted lung microenvironment induces persistent intrinsic changes on ILC2s, we sorted lung ILC2s and cultured them *ex vivo* with IL-2 and IL-33 (Fig. 4A). This treatment induced similar upregulation of GATA 3, KLRG1 and PD-1 expression in lung ILC2s from mock- and MuHV-4 infected mice, highlighting the necessity of the microenvironment *in vivo* to maintain the

inhibitory identity of ILC2s (Fig. 4B). Surprisingly, IL-13 production was even higher in supernatants of cultures from ILC2s from MuHV-4 infected mice previously sensitized with HDM (Fig. 4C).

MuHV-4 latency is associated with elevated levels of IFN-γ[8,42] and BubbleGUM analysis revealed a strong IFN-γ related alteration on MuHV-4 ILC2s (Fig. 3G). To determine whether inhibition of pulmonary ILC2s activation was directly dependent on IFN-γ signaling responsiveness, we generated mixed chimeras. C57BL/6 CD45.1.2^{+} mice were exposed to a complete lethal irradiation protocol and then transplanted with a mix (1:1) of BM cells from C57BL/6 CD45.1^{+} and from C57BL/6 IFN-γR$^{-/-}$ CD45.2^{+} congenic donors. 8 weeks after BM transplantation, mice were submitted to MuHV-4 infection and subsequent HDM treatment (Fig. 4D). Mixed chimera model allowed us to observe in the same mouse the production of type 2 cytokines from WT ILC2s and from ILC2s lacking IFN-γ receptor. Interestingly, the reduced production of IL-5 and IL-13 by ILC2s following infection was only observed in the WT compartment and ILC2s lacking IFN-γ receptor were producing even more IL-5 or IL-13 than WT ILC2s, indicating that IFN-γ sensing by ILC2s blocks type 2 cytokines production by these cells (Fig. 4, E and F). Accordingly, in those mice, we did not observe any protection against lung eosinophilia or any reduction in the number of ILC2s following MuHV-4 infection (Fig. 4, G and H). Altogether, these data revealed that ILC2s inhibition following MuHV-4 infection was dependent on IFN-γ sensing.

In BALF, a peak of IFN-γ production occurs at day 8 after MuHV-4 infection (Fig. 4I) mainly from CD4 and CD8 T cells (Fig. 4J). We therefore assessed the impact of MuHV-4 infection on lung ILC2s before any HDM treatment. The phenotype of ILC2s was altered as soon as 5 days post-infection with a temporary overexpression of Sca1 and of PD-1 (Fig. 4K). Interestingly, PD-1 acts as a metabolic checkpoint in ILC2s avoiding excessive inflammation[43]. We also observed a decrease of the percentage of IL-13 positive ILC2s correlating with the peak of IFN-γ (Fig. 4L). The use of the same mixed chimera model as in figure 4 confirmed the major role of IFN-γ in the inhibition of lung ILC2s at early time points post-infection (Fig. 4, M-O).

Following AM niche depletion, monocytes differentiating into AMs are in close contact with ILC2s in the lung of MuHV-4 infected mice

Next, we wondered whether MuHV-4-imprinted ILC2s may interact with monocyte (Mo)-derived AMs, which have been shown to contribute to allergic asthma protection after MuHV-4 infection[8]. As observed previously, day 8 post-MuHV-4 infection correlates with the depletion of the AMs niche and the recruitment of monocytes (Fig. 5, A and B). Concomitantly to ILC2s functional alterations, Mo-derived AMs displayed phenotypic modifications such as overexpression of MHC-II, CD86, Nos2 and PD-L1, the latter one being also overexpressed in monocytes (Fig. 5, C-E) raising the possibility that an interaction between Mo-derived AMs and ILC2s exists and may affect Mo-derived

AMs polarization. To assess whether ILC2s and AMs are in close contact, we imaged IL-5 reporter mice at different times post-infection (Fig. 5, F-H). MuHV-4 infection induced a massive infiltration of immune cells in the bronchovascular areas at day 8 post-infection. In those clusters, we observed T cells ($CD3^+$), myeloid cells ($CD68^+$) and ILC2s ($CD3^-IL\text{-}5^+$). 28 days post-infection, inflammation was resolved with some remaining T cells observed (Fig. 5, G-H). Interestingly, close contacts between ILC2s and myeloid cells were observed in each condition meaning that ILC2s and macrophages could have a direct interaction (Fig. 5, G-H).

Transcriptional profiles of AMs and pulmonary ILC2s are modified following MuHV-4 infection

To assess the modifications induced by MuHV-4 infection on ILCs and AMs and a possible crosstalk between those cells, ILCs and AMs were sorted and profiled by droplet-based single cell RNA-sequencing at different times post-infection (Fig. 6, A and B and Fig. S2, G-M). Lung ILC2s represented more than 95% of the lung ILCs populations and this proportion was maintained throughout the infection (Fig. 6C). As soon as 5 days after infection, ILC2s transcriptional profile was modified (Fig. 6D) and activation of ILC2s was observed by the increased expression of *Klrg1, Il-5 and Il-13* (Fig. 6E). Moreover, lung ILC2s seemed also to play a role in lung homeostasis following MuHV-4 infection by the production of Areg, essential for maintaining epithelial integrity and airway remodeling to restore lung function[44]. Nevertheless, as observed by FACS, a decreased activation of lung ILC2s was noticed from day 8 post-infection revealed by an overexpression of PD-1 (*Pdcd1*), downregulation of *Klrg1* and *Arg1*, reduction of cytokine production correlating with upregulation of *Ifit1* and *Ifitm3* genes related to IFN-γ signaling (Fig. 6E). Analysis of phenotypic molecular signatures with BubbleGUM confirmed that infection with MuHV-4 induced a higher expression of genes regulating negatively immune system processes as well as genes involved in viral infection, tolerance and response to IFN-γ. Finally, genes implicated in cellular respiration were downregulated in ILC2s from MuHV-4 infected mice, which could mean a reduction of aerobic respiration (Fig. 6F).

UMAP analysis confirmed the previously described[8] modification of AMs phenotype by MuHV-4 infection for the long-term (Fig. 6G). AMs from mock infected mice expressed genes encoding products that reflect an alternative activation (M2) profile such as *Marco, CD36, Chil3 and Fabp4* (Fig. 6H). In contrast, AMs from MuHV-4 infected mice showed a shift towards classical macrophages activation (M1), overexpression of MHC-II and downregulation of SiglecF (Fig. 6H). BubbleGUM analysis confirmed these observations and highlighted regulatory properties associated with production of IL-10 at days 5 and 8 post-infection (Fig. 6I).

In allergic asthma or in early life, ILC2s coordinate the polarization of AMs to a M2 phenotype[45,46]. To assess a potential crosstalk between ILC2s and AMs during MuHV-4 infection, we

extrapolated potential interactions from transcriptomic data using NicheNET analysis[47], allowing us to characterize ligands expressed by ILC2s and AMs, their associated receptors and the target genes activated by these interactions (Fig. 6J). Interestingly, this highlighted that production of GM-CSF (*Csf2*) by ILC2s at day 5 post-infection (Fig. 6K) could educate incoming monocytes towards AMs as this cytokine is essential for AM development in early life[48]. On the other hand, long-term Il-1β production by Mo-derived AMs may contribute to control lung ILC2s as previously shown[35] (Fig. 6L).

ILC2s from MuHV-4 infected mice shape Mo-derived AMs identity but are not able to promote a M2 polarization of AMs following HDM treatment

To assess the importance of the ILC2s - AMs crosstalk in the context of *in vivo* replenishment of the alveolar niche by incoming monocytes, we used ILC2s deficient mice ($Ror\alpha^{lox/lox}$ $Il7r^{Cre/+}$)[49] infected or not with MuHV-4 and submitted to HDM sensitization (Fig. S3A). As expected, no ILC2s were observed in $Ror\alpha^{lox/lox}$ $Il7r^{Cre/+}$ mice following HDM sensitization or MuHV-4 infection (Fig. S3B). We hypothesized that ILC2s from MuHV-4 infected littermate mice would not be able to induce a M2 polarization of AMs following HDM treatment similarly to the phenotype observed in ILC2s deficient mice. In mock infected littermates, AMs expressed M2 markers such as YM1 or CHIL3 at steady state as described[50]. In contrast, a decreased expression of these markers was observed in absence of ILC2s (Fig. S3, C and D). Similarly, the M2 marker RELMα was not increased in ILC2s deficient mice following HDM instillations (Fig. 3, E and F). This confirmed that ILC2s were essential to promote a M2-AMs profile at steady state and after HDM instillations in mock infected mice. In contrast, upon MuHV-4 infection, AMs from infected littermate mice showed the same profile as the one from ILC2s deficient mice, meaning an absence of M2 polarization with or without HDM (Fig. S3, D and F), confirming the absence of pro-TH2 properties of ILC2s following MuHV-4 infection.

We confirmed the potential role of lung ILC2s on AMs maturation in *ex vivo* co-cultures (Fig. 7A and Fig. S4A). To obtain monocytes in the process of differentiation to AMs, we firstly co-cultured BM monocytes with lung epithelial cells from naïve mice during 3 days (Fig. S4, B and C). Some monocytes differentiated in AM-like cells as observed by the expression of CD11c and SiglecF (Fig. 7B). ILC2s from mock or MuHV-4 infected mice were added 3 days later (Fig. 7A). ILC2s from MuHV-4 infected mice maintained their expression of Sca1 (Fig. 7C). As already described[45], Mo-derived macrophages acquired the M2 marker Arg1 when co-cultured with ILC2s from mock mice (Fig. 7D). In contrast, macrophages in culture with ILC2s from MuHV-4 infected mice showed less expression of Arg1 and an increased expression of MHC-II (Fig. 7D). As expected, no modifications were observed for SiglecF expression (Fig. 7D).

Finally, we performed transcriptomic analysis of *ex vivo* differentiated Mo-derived macrophages (defined as $Ly6G^-$, $Ly6C^-$, autofluorescent, $CD11c^+$ living cells) in the presence of lung

epithelial cells and cultured or not with lung ILC2s from mock or MuHV-4 infected mice. Macrophage identity was confirmed by the expression of associated genes (Fig. 7E). Interestingly, principal component analysis revealed major differences depending mainly on the presence or absence of ILC2s (Fig. 7F). In particular, lung ILC2s induced expression of genes related to macrophage differentiation and activation (*Csf1, Pparg, Il4ra*) and chemotaxis (*Ccr7*) (Fig. 7g). The genes that were differentially expressed between the conditions were classified with PANTHER (Fig. 7H). This revealed highly significant enrichments for pathways such as macrophage differentiation, activation or chemotaxis. In total, we observed 112 differentially expressed genes ($P < 0.05$) between monocytes cultured with ILC2s from mock or infected mice (Fig. 7G). While ILC2s from mock infected mice promoted Mo-derived AMs differentiation (*Pparg*) and a M2 polarization phenotype (*Arg1, Chil3, Ear2, Fabp4, Il1r2*), macrophages cultured with ILC2s from MuHV-4 infected mice, in addition to not overexpressing M2-gene profiles, displayed expression of genes related to immunosuppression such as *Csf3r*, or to regulatory orientation such as *Socs3* (Fig. 7, I and J). Altogether, these data indicate that, *in vitro*, besides positively regulating macrophage differentiation, lung ILC2s from MuHV-4 infected mice are not able to prone a M2 phenotype of Mo-derived AMs.

Figures

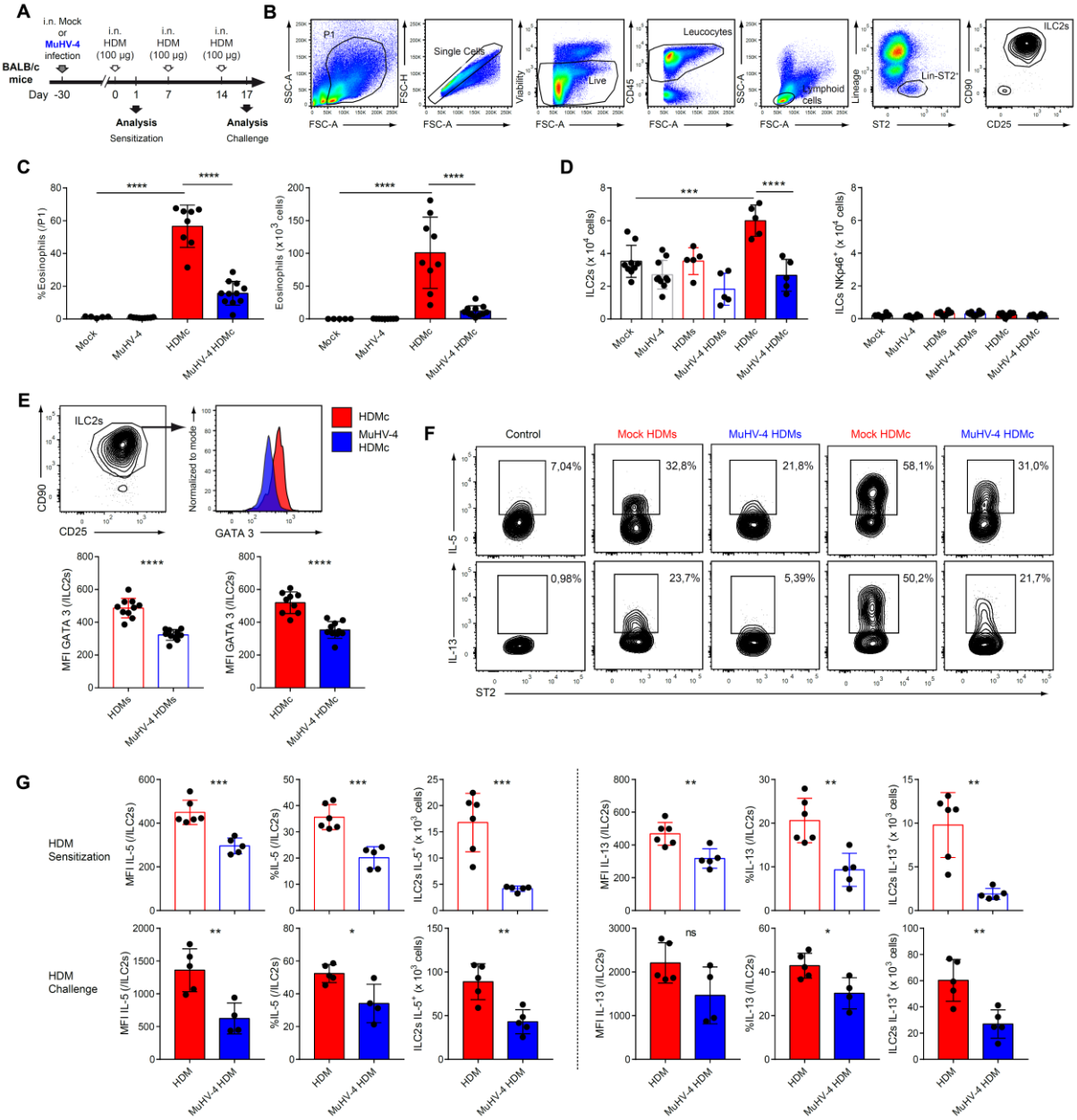


Fig. 1. MuHV-4 infection affects the number and function of pulmonary ILC2s.
(**A**) Experimental layout of MuHV-4 infection and high-dose HDM sensitization or challenge in 8-week-old-BALB/c mice (n= 5 to 10 in each group). (**B**) Strategy for the identification of lung ILC2s by flow cytometry. Lineage was defined as B220, CD11c, CD3, CD4, CD49b, CD4, CD8α, F4/80, FcεRI, Gr1 and Siglec-F. ILC2s are identified as Lin$^-$CD45$^+$ST2$^+$CD90.2$^+$CD25$^+$ living cells. (**C**) Percentage and absolute numbers of eosinophils (out AMs (autofluorescent CD11c$^+$SSC-A^{high}) Siglec-F$^+$CD11b$^+$ living cells) from BALF. (**D**) Absolute numbers of lung ILC2s and ILCs Nkp46$^+$ (Lin$^-$CD45$^+$ST2$^+$CD90.2$^+$ Nkp46$^+$ living cells). (**E**) Representative flow cytometry plots, histograms and mean fluorescence intensity (MFI) of GATA 3 staining in lung ILC2s. (**F**) Representative flow cytometry plots of intracellular staining of IL-5 and IL-13 pre-gated on lung ILC2s, numbers indicate the percentage of positive cells in each quadrant. (**G**) MFI of IL-5 and IL-13 staining in lung ILC2s, percentage and absolute numbers of IL-5 and IL-13 positive ILC2s in lung. 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, **** p<0.0001. Error bars represent SEM. Data are representative of at least three independent experiments.

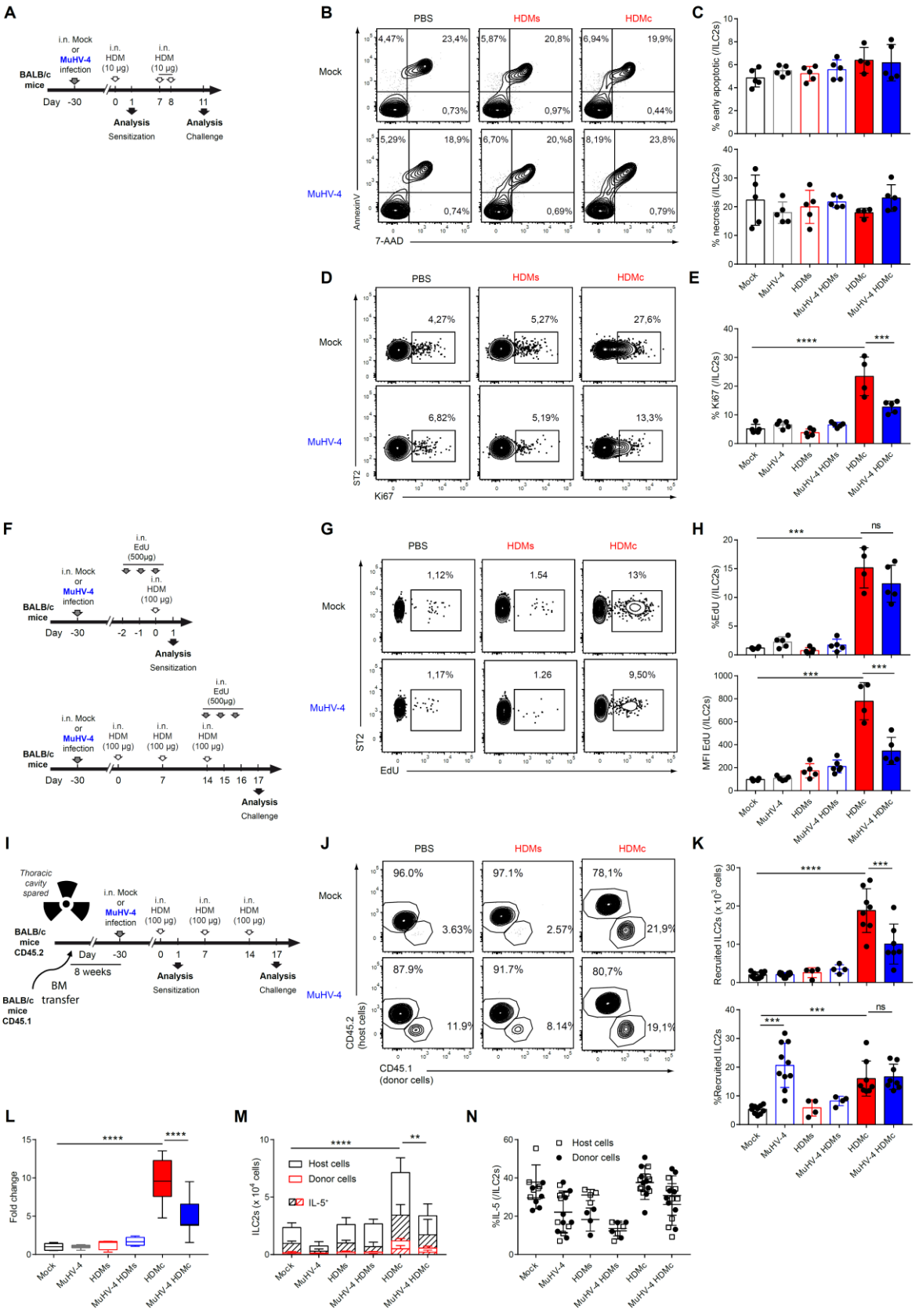

A
i.n. Mock or MuHV-4 infection
i.n. HDM (10 µg)
i.n. HDM (10 µg)
BALB/c mice
Day -30
0 1 7 8 11
Analysis
Sensitization
Analysis
Challenge
B
PBS
HDMs
HDMc
Mock
MuHV-4
4,47%
23,4%
0,73%
5,87%
20,8%
0,97%
6,94%
19,9%
0,44%
5,29%
18,9%
0,74%
6,70%
20,%8
0,69%
8,19%
23,8%
0,79%
AnnexinV
7-AAD
C
% early apoptotic (/ILC2s)
% necrosis (/ILC2s)
Mock
MuHV-4
HDMs
MuHV-4 HDMs
HDMc
MuHV-4 HDMc
D
PBS
HDMs
HDMc
Mock
MuHV-4
4,27%
5,27%
27,6%
6,82%
5,19%
13,3%
ST2
Ki67
E
% Ki67 (/ILC2s)
****
***
F
i.n. EdU (500µg)
i.n. Mock or MuHV-4 infection
i.n. HDM (100 µg)
BALB/c mice
Day -30
-2 -1 0 1
Analysis
Sensitization
i.n. EdU (500µg)
i.n. Mock or MuHV-4 infection
i.n. HDM (100 µg)
i.n. HDM (100 µg)
i.n. HDM (100 µg)
BALB/c mice
Day -30
0 7 14 15 16 17
Analysis
Challenge
G
PBS
HDMs
HDMc
Mock
MuHV-4
1,12%
1.54
13%
1,17%
1.26
9,50%
ST2
EdU
H
%EdU (/ILC2s)
MFI EdU (/ILC2s)
***
ns
I
Thoracic cavity spared
i.n. Mock or MuHV-4 infection
i.n. HDM (100 µg)
i.n. HDM (100 µg)
i.n. HDM (100 µg)
BALB/c mice CD45.2
Day -30
0 1 7 14 17
8 weeks
Analysis
Sensitization
Analysis
Challenge
BM transfer
BALB/c mice CD45.1
J
PBS
HDMs
HDMc
Mock
MuHV-4
96.0%
3.63%
97.1%
2.57%
78,1%
21,9%
87.9%
11.9%
91.7%
8.14%
80,7%
19,1%
CD45.2 (host cells)
CD45.1 (donor cells)
K
Recruited ILC2s (x 10³ cells)
%Recruited ILC2s
L
Fold change
M
ILC2s (x 10⁴ cells)
Host cells
Donor cells
IL-5⁺
N
%IL-5 (/ILC2s)
Host cells
Donor cells

Fig. 2. MuHV-4 infection impairs the expansion and recruitment of pulmonary ILC2s.
(**A-E**) 8-week-old-BALB/c mice (n= 5 in each group) were infected or not with MuHV-4 and submitted to low-dose HDM sensitization or challenge before analysis. (**A**) Experimental layout. (**B** and **C**) Representative flow cytometry plots of Annexin-V/7-AAD staining (**B**) and quantification of necrosis (Annexin-V and 7-AAD double positive) and early apoptosis (Annexin-V positive and 7-AAD negative) (**C**) in lung ILC2s. (**D** and **E**) Representative flow cytometry plots (**d**), percentage (**e**) of Ki67$^+$ cells among lung ILC2s. (**F-H**) 8-week-old-BALB/c mice were infected or not with MuHV-4 and submitted to high-dose HDM sensitization or challenge in association with EdU instillations (n= 5 in each group). (**F**) Experimental layout. (**G** and **H**) Representative flow cytometry plots (**G**) and MFI (**H**) of EdU staining in lung ILC2s. (**I-N**) 8 week-old-CD45.2$^+$ BALB/c mice were lethally irradiated, sparing the thoracic area, and transplanted with CD45.1$^+$ bone marrow (BM) before being infected or not with MuHV-4 and submitted to a high-dose HDM sensitization or challenge. (**I**) Experimental layout. (**J**) Representative flow cytometry plots for the evaluation of chimerism between recipient (CD45.2$^+$) and donor (CD45.1$^+$) cells in lung ILC2s. (**K**) Absolute numbers and percentage of CD45.1$^+$ cells among lung ILC2s. (**L**) Lung ILC2s recruitment expressed in fold change (HDM compared to mock and MuHV-4 HDM compared to the MuHV-4 group). (**M**) Absolute numbers of host or donor ILC2s producing or not IL-5. (**N**) Percentage of IL-5 positive host or donor ILC2s. 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. Data are from one experiment representative of at least three independent experiments or pooled (j-n) from multiple independent experiments and displayed as mean ± SEM of 5–10 individual mice per group and time point.

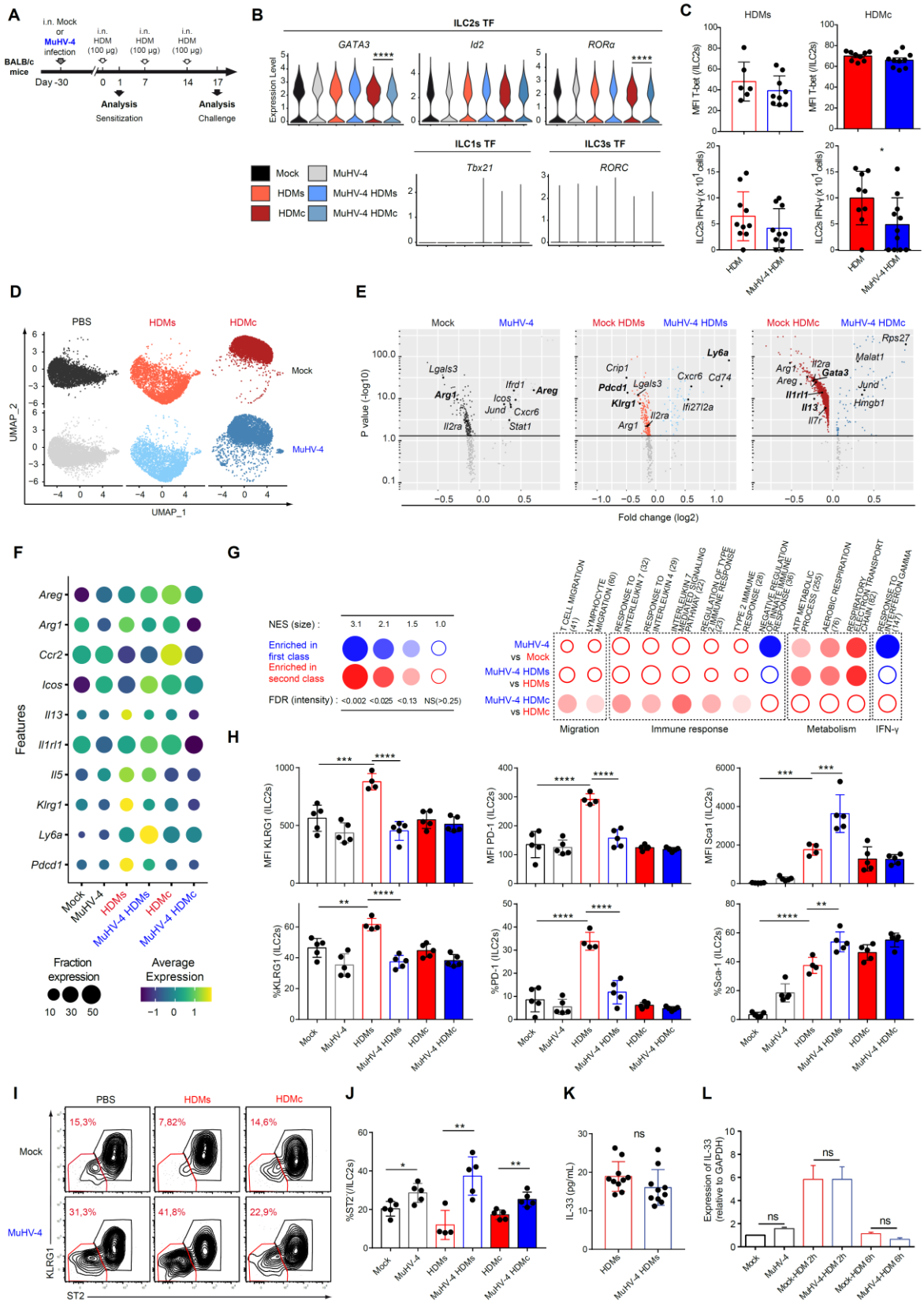

A
i.n. Mock or MuHV-4 infection
i.n. HDM (100 µg)
i.n. HDM (100 µg)
i.n. HDM (100 µg)
BALB/c mice
Day -30
0
1
7
14
17
Analysis
Sensitization
Analysis
Challenge
B
ILC2s TF
GATA3
Id2
RORα
ILC1s TF
Tbx21
ILC3s TF
RORC
Expression Level
Mock
MuHV-4
HDMs
MuHV-4 HDMs
HDMc
MuHV-4 HDMc
C
HDMs
HDMc
MFI T-bet (/ILC2s)
ILC2s IFN-γ (x 10³ cells)
HDM
MuHV-4 HDM
D
PBS
HDMs
HDMc
Mock
MuHV-4
UMAP_2
UMAP_1
E
Mock
MuHV-4
Mock HDMs
MuHV-4 HDMs
Mock HDMc
MuHV-4 HDMc
P value (-log10)
Fold change (log2)
Lgals3
Arg1
Ifrd1
Areg
Icos
Jund
Cxcr6
Il2ra
Stat1
Crip1
Pdcd1
Klrg1
Ly6a
Cd74
Ifi27l2a
Rps27
Malat1
Gata3
Areg
Il1rl1
Il13
Il7r
Hmgb1
F
Areg
Arg1
Ccr2
Icos
Il13
Il1rl1
Il5
Klrg1
Ly6a
Pdcd1
Features
Mock
MuHV-4
HDMs
MuHV-4 HDMs
HDMc
MuHV-4 HDMc
Fraction expression
Average Expression
G
NES (size) :
Enriched in first class
Enriched in second class
FDR (intensity) : <0.002 <0.025 <0.13 NS(>0.25)
T CELL MIGRATION (41)
LYMPHOCYTE MIGRATION (60)
RESPONSE TO INTERLEUKIN 7 (32)
RESPONSE TO INTERLEUKIN 4 (29)
INTERLEUKIN 7 MEDIATED SIGNALING PATHWAY (22)
REGULATION OF TYPE I IMMUNE RESPONSE (23)
TYPE 2 IMMUNE RESPONSE (28)
NEGATIVE REGULATION OF IMMUNE RESPONSE (68)
ATP METABOLIC PROCESS (255)
AEROBIC RESPIRATION (76)
RESPIRATORY ELECTRON TRANSPORT CHAIN (82)
RESPONSE TO INTERFERON GAMMA (147)
MuHV-4 vs Mock
MuHV-4 HDMs vs HDMs
MuHV-4 HDMc vs HDMc
Migration
Immune response
Metabolism
IFN-γ
H
MFI KLRG1 (ILC2s)
MFI PD-1 (ILC2s)
MFI Sca1 (ILC2s)
%KLRG1 (ILC2s)
%PD-1 (ILC2s)
%Sca-1 (ILC2s)
I
PBS
HDMs
HDMc
Mock
MuHV-4
15,3%
7,82%
14,6%
31,3%
41,8%
22,9%
KLRG1
ST2
J
%ST2 (/ILC2s)
K
IL-33 (pg/mL)
ns
L
Expression of IL-33 (relative to GAPDH)
Mock-HDM 2h
MuHV-4-HDM 2h
Mock-HDM 6h
MuHV-4-HDM 6h

Fig. 3. Single cell transcriptome of pulmonary ILC2s after MuHV-4 infection and HDM treatment.
8-week-old-BALB/c mice (n= 7 in each group) were infected or not with MuHV-4 and submitted to high-dose HDM sensitization or challenge before droplet-based single cell RNA-sequencing of ILC2s. (**A**) Experimental layout. (**B**) Violin plots of ILC1s, ILC2s and ILC3s canonical transcription factors expression in lung ILC2s. (**C**) MFI of T-bet and IFN-γ staining of lung ILC2s of mock or MuHV-4 infected mice, treated or not with HDM measured by flow cytometry. (**D**) Non-linear representation (UMAP) of the top 10 principal components (PCs) of 24.535 ILC2s split between the 6 conditions. (**E**) Volcano plot comparison of whole transcriptome gene expression of ILC2s (defined as statistically significant adjusted false-discovery rate (FDR) <0.05). (**F**) Differentially expressed genes (*y* axis) by condition (*x* axis) in lung ILC2s. Dot size represents the fraction of cells in the cluster that express the gene and colour indicates the mean expression (logTPX (see Methods)) relative to each gene. (**G**) Enrichment for transcriptomic fingerprints specific for 12 Gene Ontology sets by gene set enrichment analysis with BubbleGum software in lung ILC2s. The color indicates the cell subset showing enrichment for the gene set. The surface area of the dots is proportional to the absolute value of the normalized enrichment score (NES). The color intensity indicates the false-discovery rate (FDR). Numbers in parentheses indicate the number of genes in each gene-set. NS, not significant. (**H**) MFI and percentage of KLRG1, PD-1 and Sca1 expression in lung ILC2s from mock- or MuHV-4 infected mice submitted to high-dose HDM sensitization or challenge. (**I**) Representative flow cytometry plots of $ST2^-$ population in lung ILC2s (gated $CD45^+Lin^-CD90.2^+CD25^+$ living cells) 24h after intranasal instillation of high-dose HDM in mock- or MuHV-4 infected mice. (**J**) Percentage of IL-33R/$ST2^+$ and $ST2^-$ in lung ILC2s from mock- or MuHV-4 infected mice submitted to high-dose HDM sensitization or challenge. (**K**) ELISA measurement of IL-33 in BALF from mock- or MuHV-4 infected mice 24h after intranasal instillation of high-dose HDM. (**L**) Expression of IL-33 analyzed by RT-qPCR in lung from mock- or MuHV-4 infected-mice (n=5 mice per group) 2 or 6h after intranasal instillation of high-dose HDM. 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, **** p<0.0001. Error bars represent SEM. Analyses were performed using R.

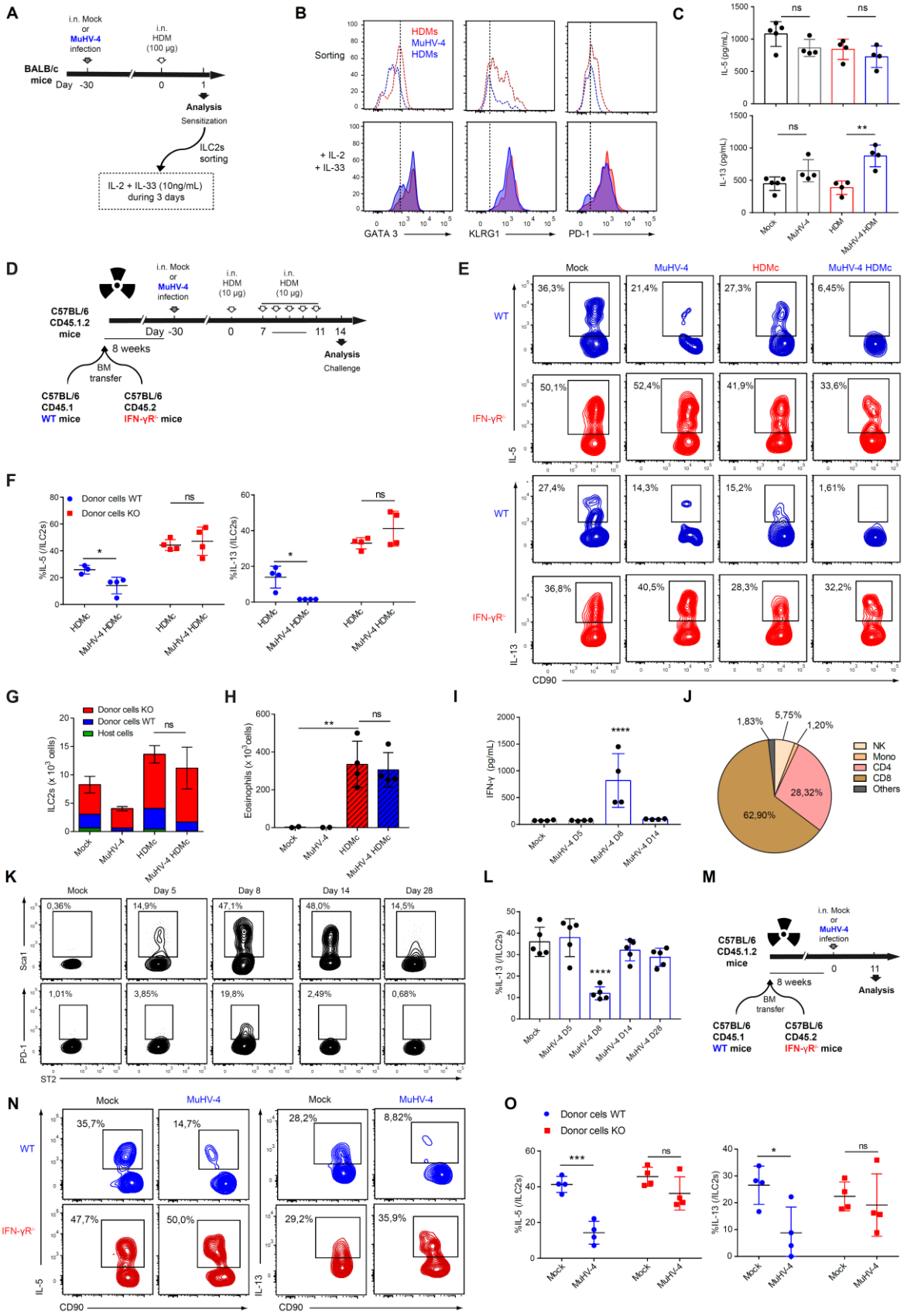

A
i.n. Mock or MuHV-4 infection
i.n. HDM (100 µg)
BALB/c mice
Day -30
0
1
Analysis
Sensitization
ILC2s sorting
IL-2 + IL-33 (10ng/mL) during 3 days
B
Sorting
HDMs
MuHV-4 HDMs
+ IL-2 + IL-33
GATA 3
KLRG1
PD-1
C
IL-5 (pg/mL)
IL-13 (pg/mL)
ns
**
Mock
MuHV-4
HDM
MuHV-4 HDM
D
C57BL/6 CD45.1.2 mice
i.n. Mock or MuHV-4 infection
i.n. HDM (10 µg)
Day -30
0
7
11
14
Analysis
Challenge
8 weeks
BM transfer
C57BL/6 CD45.1 WT mice
C57BL/6 CD45.2 IFN-γR- mice
E
Mock
MuHV-4
HDMc
MuHV-4 HDMc
WT
IFN-γR-
IL-5
IL-13
CD90
36,3%
21,4%
27,3%
6,45%
50,1%
52,4%
41,9%
33,6%
27,4%
14,3%
15,2%
1,61%
36,8%
40,5%
28,3%
32,2%
F
Donor cells WT
Donor cells KO
%IL-5 (/ILC2s)
%IL-13 (/ILC2s)
G
Donor cells KO
Donor cells WT
Host cells
ILC2s (x 10³ cells)
H
Eosinophils (x 10³cells)
I
IFN-γ (pg/mL)
****
Mock
MuHV-4 D5
MuHV-4 D8
MuHV-4 D14
J
1,83%
5,75%
1,20%
28,32%
62,90%
NK
Mono
CD4
CD8
Others
K
Mock
Day 5
Day 8
Day 14
Day 28
Sca1
PD-1
ST2
0,36%
14,9%
47,1%
48,0%
14,5%
1,01%
3,85%
19,8%
2,49%
0,68%
L
%IL-13 (/ILC2s)
MuHV-4 D28
M
C57BL/6 CD45.1.2 mice
i.n. Mock or MuHV-4 infection
0
11
Analysis
8 weeks
BM transfer
C57BL/6 CD45.1 WT mice
C57BL/6 CD45.2 IFN-γR- mice
N
Mock
MuHV-4
WT
IFN-γR-
IL-5
IL-13
CD90
35,7%
14,7%
28,2%
8,82%
47,7%
50,0%
29,2%
35,9%
O
Donor cells WT
Donor cells KO
%IL-5 (/ILC2s)
%IL-13 (/ILC2s)
***
*

Fig. 4. IFN-γ directs the reversible inhibition of pulmonary ILC2s after MuHV-4 infection.
(**A-C**) Lung ILC2s were sorted from BALB/c mice infected or not with MuHV-4 (n=6 in each group) and submitted 30 days after infection to high-dose HDM sensitization and then cultured *ex vivo* during 3 days with IL-2 (10 ng/mL) and IL-33 (10 ng/mL) before analysis. (**A**) Experimental layout. (**B**) Representative histograms of KLRG1, PD-1 and GATA 3 expression in lung ILC2s. (**C**) ELISA measurement of IL-5 and IL-13 in the supernatant of ILC2s cultured *ex vivo*. (**D-H**) CD45.1.2$^+$ C57BL/6 mice were exposed to lethal irradiation protocol (6Gy) before being transplanted with a mix (1:1) of CD45.1$^+$ WT and CD45.2$^+$ IFN-γR$^{-/-}$ congenic donors BM cells. 8 weeks after transfer, those mice were submitted to MuHV-4 infection and then to HDM low-dose sensitization and challenge (n=5 in each group). (**D**) Experimental layout. (**E**) Representative flow cytometry plots of IL-5 and IL-13 staining in lung. WT and IFN-γR$^{-/-}$ lung ILC2s from the same mouse are shown. Numbers indicate the percentage of positive cells in each quadrant. (**F**) Percentage of IL-5$^+$ and IL-13$^+$ cells among lung ILC2s. (**G** and **H**) Absolute numbers of ILC2s (**G**) in lung and eosinophils (**H**) in BALF. (**I**) ELISA measurement of IFN-γ in BALF at indicated times post-MuHV-4 infection in C57BL/6 mice. (**J**) Percentage of the different lung cell types producing IFN-γ in IFN-γ reporter mice at day 8 post-MuHV-4 infection. (**K**) Representative flow cytometry plots of Sca1 and PD-1 expression in lung ILC2s at indicated times post-MuHV-4 infection in C57BL/6 mice. (**L**) Percentage of IL-13$^+$ cells among lung ILC2s assessed by flow cytometry at different times post-MuHV-4 infection in C57BL/6 mice. (**M-O**) CD45.1.2$^+$ C57BL/6 mice were exposed to lethal irradiation protocol (6Gy) before being transplanted with a mix (1:1) of CD45.1$^+$ WT and CD45.2$^+$ IFN-γR$^{-/-}$ congenic donors BM cells. 8 weeks after transfer, those mice were submitted to MuHV-4 infection. (**M**) Experimental layout. (**N**) Representative flow cytometry plots of IL-5 and IL-13 staining in lung. WT and IFN-γR$^{-/-}$ lung ILC2s from the same mouse are shown. Numbers indicate the percentage of positive cells in each quadrant. (**O**) Percentage of IL-5$^+$ and IL-13$^+$ cells among lung ILC2s. Data were analyzed by 1way-ANOVA and Bonferroni posttests or by two-tailed Student's t-test * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Error bars represent SEM.

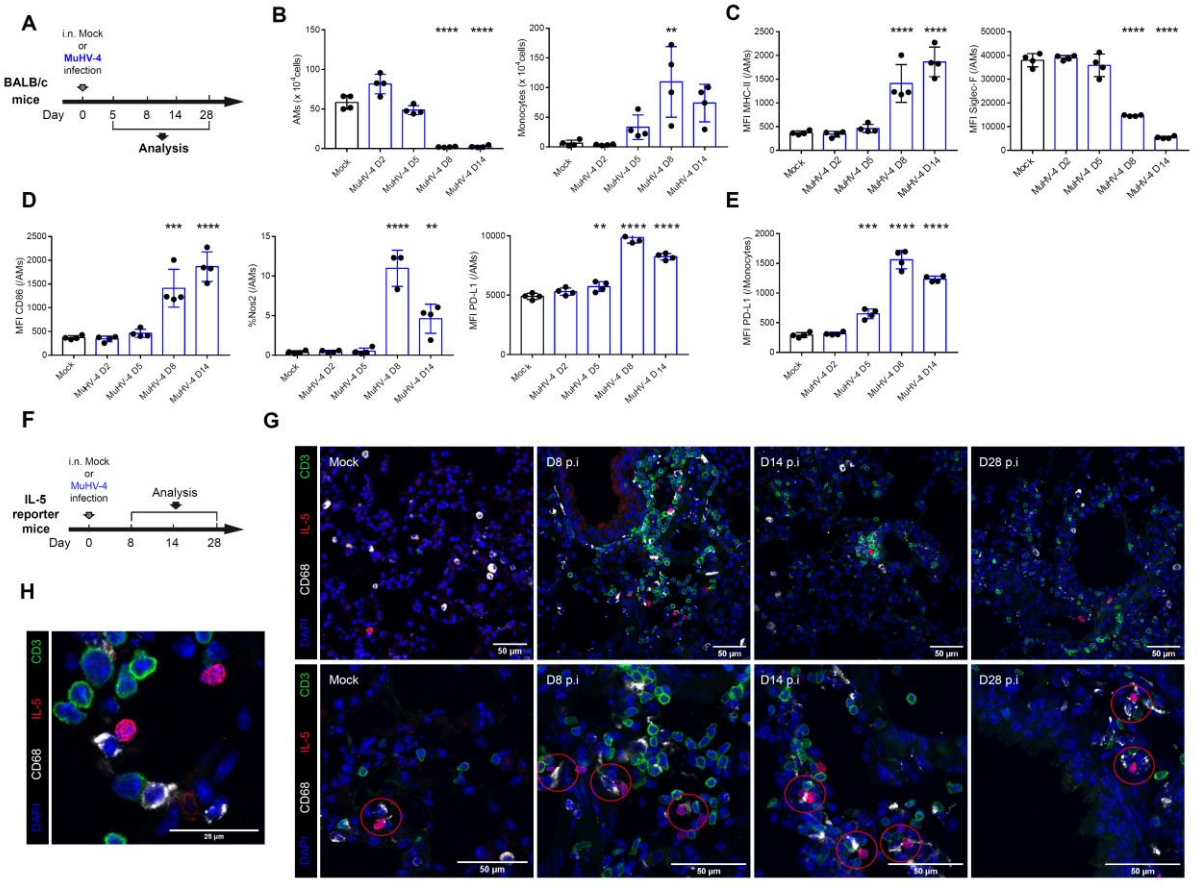


Fig. 5. Following AM niche depletion, monocytes differentiating into AMs are in close contact with ILC2s in the lung of MuHV-4 infected mice.
(**A-E**) 8-week-old BALB/c mice were infected or not with MuHV-4 (n=4 in each group). (**A**) Experimental layout. (**B**) Absolute numbers of AMs (described as autofluorescent CD11c$^+$ living cells) and monocytes (described as CD11b$^+$Ly6C$^+$ living cells) from BALF. (**C**) MFI of MHC-II and Siglec-F in BALF AMs. (**D**) MFI of indicated markers in BALF AMs. (**E**) MFI of PD-L1 in BALF monocytes. (**F-H**) 8 week-old Red5 cytokine reporter mice were infected or not with MuHV-4, submitted to immunostaining and imaged at different times post-MuHV-4 infection. (**F**) Experimental layout. (**G** and **H**) 2D thin-cut images with ILC2s (CD3$^-$ IL-5$^+$ cells), lymphocytes (CD3$^+$ cells) and myeloid cells (CD68$^+$ cells) from the indicated conditions (**G**) and 8 days post-infection (**H**). Red circles highlight close contact between ILC2s and myeloid cells. Images are representative of 3 mice. 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, **** p<0.0001. Error bars represent SEM.

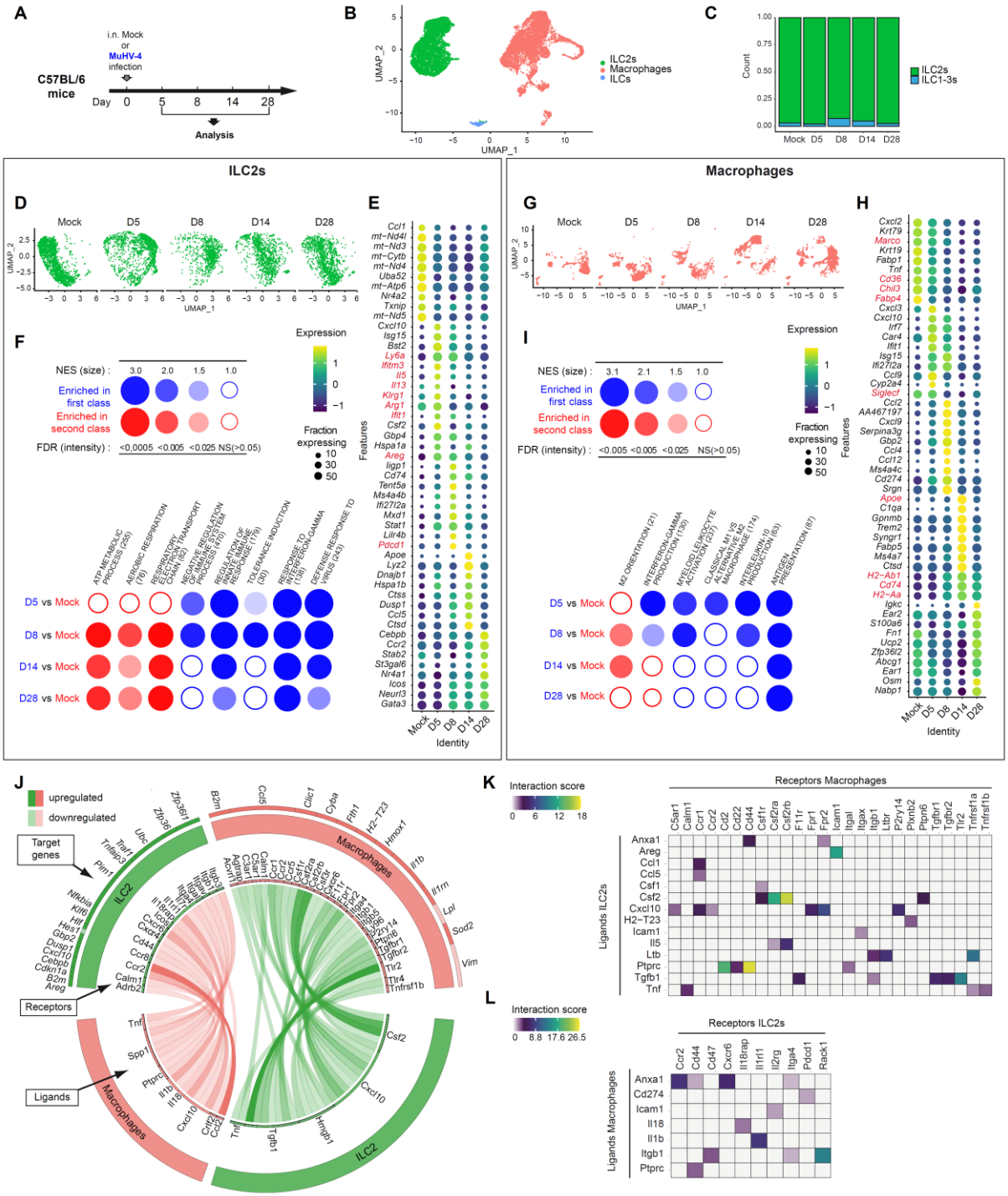


Fig. 6. Transcriptional profiles of AMs and pulmonary ILC2s are modified following MuHV-4 infection and highlight potential interactions between these cells.

Lung ILC2s (gated as CD45$^+$Lin$^-$CD90.2$^+$ living cells) and BALF AMs (gated as autofluorescent CD11c$^+$ living cells) from mock or MuHV-4 infected-8-week-old at different times post-infection. (**A**) Experimental layout. (**B**) Non linear representation (UMAP) of the top 20 principal components (PCs) of 6.291 ILC2s, 244 ILC1-3s and 9.833 AMs pooled from the different time points. (**C**) Proportions of lung ILC2s and ILC1-3s based on the transcriptomic data at different times post-infection. (**D** and **G**) UMAP of the top 20 PCs of ILC2s (**D**) and AMs (**G**) at different times post-infection. (**E** and **H**) Relative expression of the 10 most expressed genes (*y* axis) at each different times post-infection (*x* axis) in ILC2s (**E**) and AMs (**H**). 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 time points. (**F** and **I**) Enrichment for transcriptomic fingerprints specific for Gene Ontology sets by gene set enrichment analysis with BubbleGum software in ILC2s (**F**) and AMs (**I**). The color indicates the cell subset showing enrichment for the gene set. The surface area of the dots is proportional to the absolute value of the normalized enrichment score (NES) The color intensity indicates FDR. Numbers in parentheses indicate the number of genes in each gene set. NS, not significant. (**J**) Circular plot of the putative interactions between ligands (bottom), receptors (top) and the target genes activated by these interactions (up- or down-regulated) on AMs and ILC2s based on their expression at day 8 post-MuHV-4 infection. Opacity of the link correlates with the interaction score (alpha from 0,25 to 1). (**K** and **L**) Heatmap of the interaction scores between ligands and receptors expression by AMs and ILC2s at day 5 (**K**) and 28 (**L**) post-MuHV-4 infection.

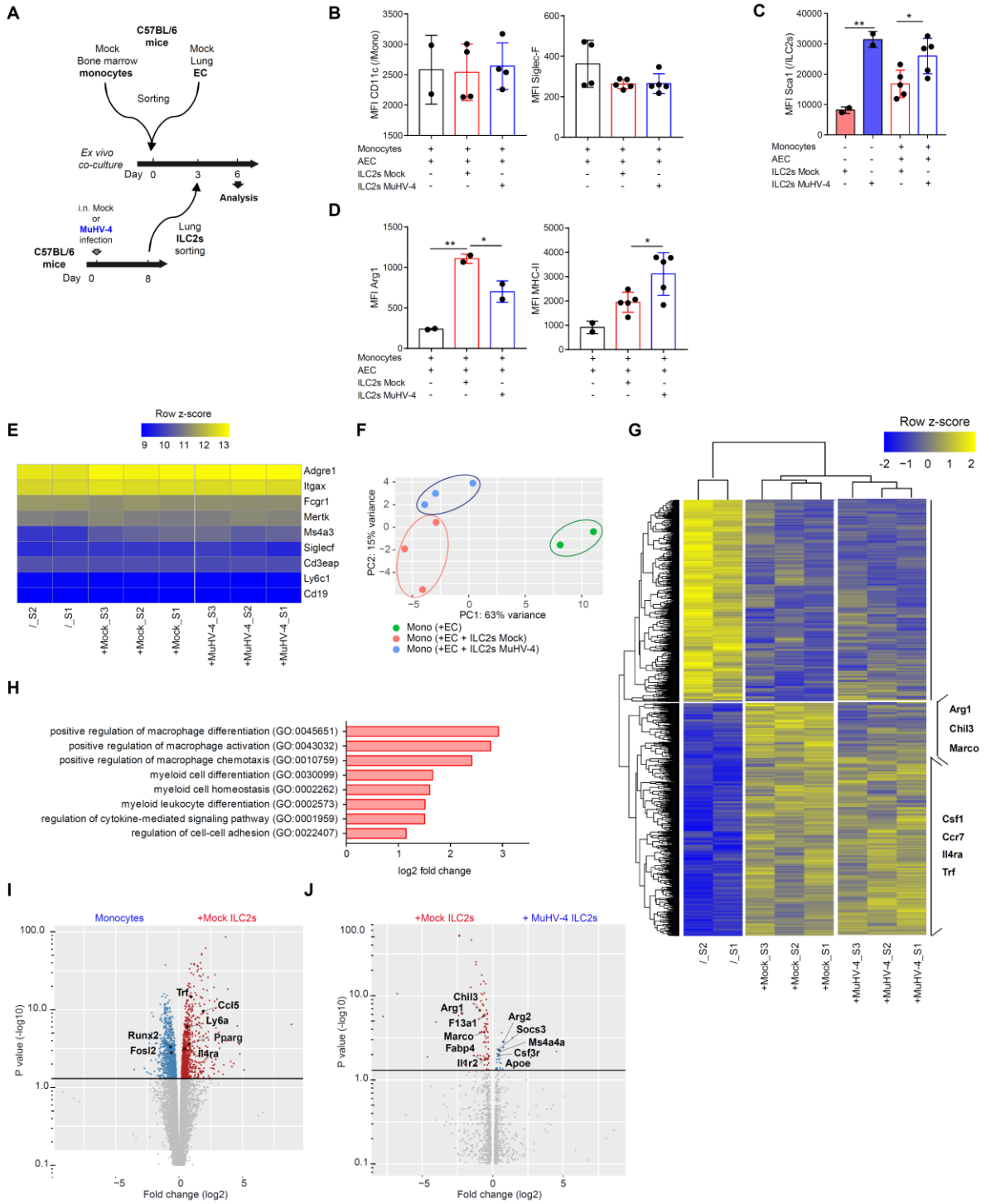


Fig. 7. MuHV-4 infection inhibits the capacity of lung ILC2s to polarize AMs towards a "M2-phenotype".
Bone marrow monocytes and lung epithelial cells (EC) from mock infected mice were cultured *ex vivo* for three days before addition or not of lung ILC2s from mice mock or MuHV-4 infected 8 days before. Flow cytometry analysis and bulk RNA sequencing of sorted Mo-derived macrophages (gated as autofluorescent CD11c$^+$ living cells) were performed 3 days after addition of ILC2s. (**A**) Experimental layout. (**B**) MFI of CD11c and Siglec-F expression in monocytes from the indicated conditions. (**C**) Sca1 expression in ILC2s from the indicated conditions. (**D**) MFI of Arg1 and MHC-II in monocytes from the indicated conditions. (**E**) Relative expression of genes associated with cell differentiation in MO-derived macrophages isolated from the different co-cultures. (**F**) Principal-component analysis (PCA) of MO-derived macrophages isolated from the different co-cultures. (**G**) Expression of all genes expressed differentially (FDR<0,05; change in expression of over twofold) in MO-derived macrophages isolated from the different co-cultures. (**H**) Biological processes identified by Panther analysis of DE upregulated genes between monocytes cultured or not with ILC2s from mock-infected mice (FDR ≤ 0,05). (**I**) Volcano plot for differentially expressed (DE) genes (FDR<0.05) by monocytes cultured or not with ILC2s from mock-infected mice. (**J**) Volcano plot for differentially expressed (DE) genes (FDR<0.05) by monocytes cultured with ILC2s of mock- or MuHV-4 infected mice. Data were analyzed by 1way-ANOVA and Bonferroni posttests or two-tailed Student's t-test * p<0.05; ** p<0.01. Error bars represent SEM.

Discussion

ILC2s are innate immune sentinels of both mucosal and non-mucosal tissues[51]. Upon activation, ILC2s secrete large amounts of cytokines such as IL-5, IL-13, IL-9 or Areg and initiate robust type 2 immune responses. ILC2s therefore play a critical role in the first line of defense against parasites or allergens. As such, ILC2s are key players in the initiation and maintenance of allergic asthma[19,23]. Viruses known to trigger or exacerbate asthmatic symptoms, such as RV or RSV, do so via expansion and activation of ILC2s[52–54]. In contrast, the effect of persistent viruses, such as herpesviruses, on ILC2s was unknown. Here, we showed for the first time that a persistent γHV infection affects the function of lung ILC2s for the long-term. In particular, ILC2s from MuHV-4 infected mice displayed decreased expression of the canonical type 2 markers and impaired capacity to expand in response to type 2 stimuli, as a consequence of both reduced recruitment and proliferation.

In asthmatic human, circulation of ILC2s into the blood has been described[55], suggesting recruitment of ILC2s to specific tissues. Although the recruitment of ILC2s has been described in mice[33], the situation is more controversial in that species, particularly in allergic asthma. Thus, some authors did not detect any recruitment of ILC2s in the lungs after administration of HDM[56], while others observed that the administration of HDM activated IL-33 responsive ILC2s in the BM suggesting potential mobilization and tissue recruitment[57]. The partial chimera model used in this article provides a definitive answer to this question. Indeed, we have demonstrated the recruitment of ILC2s into the lung not only after the HDM challenge but also a few days after viral infection. However, surprisingly, the single cell transcriptomic analysis that we conducted did not allow us to distinguish clearly different ILC2 populations. On the contrary, the evolution of gene expression within the ILC2s was rather uniform with activation in the early post-infection period and then a gradual return to the baseline situation with the maintenance of a reduced number of transcriptomic changes.

This effect of MuHV-4 infection on ILC2s appeared to be related to the microenvironment and especially to IFN-γ at the early time points following infection. IFN-γ is a known inhibitor of ILC2s[41,58]. However, while IFN-γ release induces a transient ILC2 counter regulation during influenza infection[59], we observed that MuHV-4 persistent infection sustain long-term regulation of those cells. The maintenance of this effect could rely on a dialogue of ILC2s with Mo-derived AMs as, at day 28 post-infection, Mo-derived AMs produced IL-1β, a critical regulator of ILC2 function and plasticity[60]. Similarly, this regulation could also involve potential close interaction between PD-L1 expressed by Mo-derived AMs and PD-1 immune check-point at the ILC2 surface.

Understanding how this imprinting of ILC2s is maintained following MuHV-4 infection is an important question. Besides the influence of extrinsic environmental factors, intrinsic alterations,

induced at early time points post-infection, could play a key role in sustaining ILC2 functional profile. In that context, epigenetic and metabolic modulation are now well-established as critical sources of long-term changes in the immunological phenotype of innate immune cells[61,62]. Thus, cytomegalovirus infection has been described to drive adaptive epigenetic diversification of NK cells with altered signaling and effector functions[63]. This has not yet been shown for ILC2s, even if more than 300 asthma-associated genetic polymorphisms identified in genome-wide association studies have been localized to H3K4Me2 gene regulatory elements in ILC2s[64]. Interestingly, epigenetic modifications in innate lymphoid progenitor induce glycolysis which in turn decreases ST2 expression and inhibits the development of ILC2s following IL-33 activation[54]. A similar mechanism could be involved here since a reduction of ST2 expression by ILC2s is also observed following MuHV-4 infection. It will therefore be important to investigate whether MuHV-4 infection confers a form of innate immune memory to pulmonary ILC2s through epigenetic modifications and metabolic reprogramming.

In addition to the direct influences on lung immunity, we showed important role for those cells in shaping the alveolar landscape upon infections. Tissue resident macrophages, such as AMs, display unique transcriptomic profiles[65,66]. However, how they acquire these profiles is still poorly understood. During physiological lung development, crosstalk between ILC2s, basophils and alveolar type II epithelial cells seems to modulate the development of alveolar macrophages[67,68]. This crosstalk occurs notably during first breath after birth when the alveolar expansion correlates with production of IL-33, which expand and activate ILC2s. In these conditions, ILC2s produce IL-13 that polarizes newly differentiating AMs to a M2 phenotype with homeostatic functions[46]. How circulating monocytes acquire macrophage properties and establish in tissues later in life is much largely unknown[68].

The macrophage niche model states that embryonic or adult macrophage precursors have an almost identical potential to develop into tissue-resident macrophages, while competing for a restricted number of niches per tissue[69]. However, pathways promoting macrophage replenishment and phenotype, appears to be challenge-dependent and could rely on key immune shaping by specific microbes. Here, we uncovered an essential role for lung ILC2 to elicit monocyte differentiation into AMs after virus-induced niche depletion. In addition, we demonstrated that MuHV-4 imprinted-ILC2s were still able to coordinate the differentiation of recruited monocytes into Mo-derived AMs but did not confer them a M2 polarization as it could have been expected from previous studies[46,67]. Indeed, we established that ILC2s and epithelial cells alone can reproduce in vitro an alveolar niche, which is sufficient to promote the differentiation of monocytes into AM-like cells as revealed by the expression of PPAR-γ, a transcription factor which is essential for the identity and function of AMs[70]. In vivo, we showed that MuHV-4 infection activates ILC2s, likely through the induction of IL-33 production[71]. Although these activated ILC2s produced GM-CSF which is required for the development of AMs[48], they secreted significantly less IL-13 than their counterparts in uninfected mice. Interestingly, transcriptomic analysis

after *ex vivo* co-cultures showed that Mo-derived AMs cultured with ILC2s from MuHV-4 infected mice displayed a similar expression profile to the ones of their counterparts cultured with ILC2s from mock infected mice, except for the expression of type 2 genes. This therefore demonstrates that ILC2s from MuHV-4 infected mice have only lost their type 2 properties without altering their other roles in lung physiology. Moreover, single cell transcriptomic analysis showed that MuHV-4 infection does not induce replacement or plasticity of ILC2s towards an ILC1 profile as observed for other viruses[72], but rather regulates TH2 functions of ILC2s.

This work substantially expands the understanding of γHV imprinting of lung immunity. In particular, it reveals the central importance of ILC2s to confer AM identity to MOs filling the niche following viral infection. As such, MuHV-4-imprinted ILC2s exhibit long-term alterations regulating the *in vivo* phenotypic and functional dynamics of AMs during allergic asthma. From a more general point of view, these results also suggest a central role for ILC2 in maintaining the delicate balance between γHVs and the host immune system.

Materials and Methods

Study design.

In most of the experiments, 4 to 10 mice per group per timepoint were sufficient to identify differences between groups with at least 80% power and 5% significance level. No data were excluded from the analysis and all attempts at replication were successful and gave similar readout. Allocation into experimental groups was done randomly. The specific number and genotype of mice, the experimental replicates and the statistics performed are incluced in each figure legend.

Mice.

This study was conducted in strict accordance with guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (CETS 123). Animal experiments were performed as specified in protocols approved by Committee on the Ethics of Animal Experiments of the University of Liege (permit number: 1845). All inoculations were performed under isoflurane anesthesia and every effort was made to minimize suffering. Dissections were performed after euthanasia of the animals.

Female BALB/c or C57BL/6 wild-type mice were purchased from Charles River (l'Arbresle, France). Red5 (IL5-tdtomato-cre) cytokine reporter, 'Great' (IFN-gamma reporter with endogenous polyA tail) and BALB/c CD45.1^{+} mice were from Jackson Laboratories (030926-017580-006584) (Maine, United States). C57BL/6 Rora$^{fl/sg}$ Il7r^{Cre} were provided by A. N.J. McKenzie (Cambridge, UK) and H. Rodewald (Heidelberg, Ger) and C57BL/6 IFN-γR$^{-/-}$ by E. Muraille (ULB). C57BL/6 CD45.1.2^{+} and CD45.1^{+} were bred in the GIGA animal facility (ULiege, Belgium). Except where otherwise stated, all mice used were 8 weeks of age. Animals were housed in the University of Liege according to the guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (CETS 123).

Viruses.

The wild-type MHV-68 strain of MuHV-4 was used[73]. Viral stocks were grown on BHK (baby hamster kidney) cells and were purified and titrated as described[74].

Mouse infection.

Intranasal administration of virions was performed under isoflurane anesthesia, with 1×10^4 PFU in 50 µl of PBS for MuHV-4 and specified for other routes of infection.

Administration of HDM extracts.

Isoflurane anesthetized mice received intranasal instillation of PBS or HDM in 50 µL. To induce allergic airway inflammation, two different protocols were used. In the high-dose protocol, mice were treated with 100 µg HDM extracts on day 0 and were subsequently challenged with 100 µg HDM on days 7 and 14. In an alternative protocol (HDM low-dose), mice were sensitized with 10 µg HDM on day 0 and were subsequently challenged with 10 µg HDM on days 7 to 8 or on days 7 to 11. In both models, analyses were performed on euthanized mice 3 days after the final HDM administration. To assess the early innate response to HDM, mice were sensitized with 10 or 100 µg HDM and were euthanized after one day.

Thin section Immunofluorescence microscopy.

After euthanasia, 1 mL of 2% PFA were injected intratracheally by catheterization and lungs were perfused through the right ventricle with 5 mL of 2% PFA. Tissues were harvested and fixed for 2 h in 2% PFA, washed for 4 h with PBS, cryoprotected overnight with 30% of sucrose, and embedded in OCT (Scigen) prior to freezing in blocks. Sections were processed on a Leica CM 3050S cryomicrotome (7-10 µm), dried on slides for 30 min, and kept at -80°C until staining. Tissues were blocked with 3% goat serum and 2% BSA and maintained in PBS + 0.2% triton X100 throughout antibody treatments. Primary antibodies were incubated for 4 h at 4°C and secondary antibodies for 45 min at room temperature (RT). Primary antibodies include rabbit anti-dsRed (Takara, 1:200), anti-CD3 (17A2, Biolegend, 1:100), anti-CD68 (FA-11, Invitrogen, 1:100). Secondary antibodies were used at 1:500 dilution at RT for 1 h, conjugated to AF488, AF555, and AF647 (Life Technologies, Thermo-Fisher). Slides were mounted with ProLong™ Gold Antifade Mountant mounting media (ThermoFisher). Slides were examined with a Nikon A1R confocal microscope (GIGA platform, ULiege). Analysis was performed with ImageJ (image calculator).

BAL, cytology and cytokine measurement.

After euthanasia, trachea was catheterized and BAL was performed by two consecutive flushes of the lungs with 1 mL of ice-cold PBS containing protease inhibitors (Complete, Roche). Cell density in BALF was evaluated using a hemocytometer after staining with Tuerk solution (Sigma-Aldrich). Differential cell counts were determined by flow cytometry. Cytokine production was measured by specific ELISA (Ready-SET-Go, eBioscience) according to manufacturer's instructions.

Cell suspension preparation from organs.

To harvest lung cells, mice were perfused with ice-cold PBS through the right ventricle. Then, lung lobes were collected into a C-Tube (Miltenyi) containing complete RPMI medium, 50 µg/mL liberase TM (Roche) and 100 µg/mL DNase I (Roche), before being processed with a gentleMACS dissociator (Miltenyi) and, finally incubated for 30 min at 37 °C. For epithelial cells sorting, lung were previously digested 10 min at RT in DMEM medium with 10 U/mL of Dispase (Sigma-Aldrich) before C-Tube process. BM cells were obtained from adult mice by crushing the femurs and tibiae. Blood was acquired by cardiac puncture and was immediately suspended in ice-cold PBS complemented with 5 mM EDTA. Suspensions of cells were finally washed and treated for lysis of erythrocytes (1X RBC Lysis Buffer, ThermoFisher). For all preparations, cells were finally strained through a 70-µm filter.

Antibodies and flow cytometry.

For intracellular staining, single-cell suspensions were stimulated for 4 h at 37°C in RPMI with 50 ng/mL phorbol 12-myristate 13-acetate (Sigma-Aldrich), 1 µg/mL ionomycin (Sigma-Aldrich), 8 mg/mL monensin and brefeldin (BD Biosciences) and 2 mM β-mercaptoethanol (ThermoFisher). Cells were firstly blocked with anti-FcR antibody (CD16/32, Biolegend) during 20 min. Labeling of single-cell suspensions was performed on ice in PBS containing 0.5% BSA and 0,1% Sodium azide with various panels of fluorochrome-conjugated antibodies for 30min. Antibodies to B220/CD45R (clone RA3-6B2, APC), CD11b (clone M1/70, BV605 and FITC), CD11c (clone N418, APC), , CD19 (clone 6D5, APC/Cyanine7), CD25 (clone PC61, Alexa Fluor 700), CD274 (clone 10F.9G2, BV711 and APC), CD279 (clone 29F.1A12, APC/Fire750), CD3e (clone 145-2C11, APC and BV421 and FITC), CD4 (clone RM 4-5, APC and FITC), CD45 (clone 30-F11, BV510 and PE/Cyanine7), CD45.1 (clone A20, BV421 and APC), CD45.2 (clone 104, BV510 and PE/Cyanine7), CD49b (clone DX5, APC), CD5 (clone 53-7.3, APC), CD86 (clone GL-1, APC/Cyanine7), CD8α (clone 53-6.7, APC and PerCP/Cyanine5.5), CD90.2 (53-2.1, BV421), F4/80 (clone BM8, APC), FCεRIα (clone MAR-1, APC), Gr-1 (clone RB6-8C5, APC), I-A/I-E (clone M5/114.15.2, FITC and PE/Cyanine7), IFN-γ (clone XMG1.2, BV711), IL-5 (clone TRFK5, BV421 and PE), Ki-67 (clone 16A8, Alexa Fluor 488 and PE), KLRG1 (clone 2F1, BV711), Ly6A/E (clone D7, FITC), Ly6C (clone HK1.4, BV785), SiglecF (clone E50-2440, APC) and ST2 (clone DIH9, BV421 and PE) all from Biolegend; antibodies to CD11b (clone M1/70, BV711), CD3e (clone 145-2C11, APC-Cy7), CD90.2 (clone 53-2.1, BV711), KLRG1 (clone 2F1, BV786), Ly6G (clone 1A8, APC-Cy7), SiglecF (clone E50-2440, PE and PE-CF594), Streptavidin (APC) all from BD Biosciences; antibodies to Arginase 1 (clone AexF, PE-Cyanine7), CD11c (clone N418, Alexa Fluor 700), GATA-3 (clone TWAJ, PE), IL-13 (clone eBio13A, Alexa Fluor 488), iNOS (clone CXNFT, PE), Ly6C (clone HK1.4, PE), NK1.1 (clone PK136, PE-Cyanine7), RELM alpha (clone DS8RELM, PE), T-bet (clone eBio4B10, PE) and Streptavidin (FITC) all from ThermoFisher and antibody to YM1/Chitinase 3-like 3 (Biotinylated) from R&D Systems. The gating strategy to

identify the different cell populations included successive forward- and side-scatter gating, exclusion of multiplets and selection of living cells with the viability marker Zombie Aqua™ or Violet™ (Biolegend) or Fixable Viability Dye eFluor™ 780 (eBioscience). Annexin V FITC and 7-AAD were purchased from Biolegend and apoptosis/necrosis assays were performed by using specific annexin buffer according to manufacturer's instructions. The Foxp3 Transcription Factor Staining kit was purchased from eBioscience and used for intranuclear staining. Samples were processed on a BD LSR Fortessa X-20 equipped with 50-mW violet 405-nm, 50-mW blue 488-nm, 50-mW yellow-green 561-nm and 40-mW red 633-nm lasers and an ND1.0 filter in front of the FSC photodiode.

EdU.

Mice received 3 daily intranasal injections of 0,5 mg of 5-Ethynyl-2-deoxyuridine (EdU) in 50 µL of PBS starting on the last administration of HDM (high-dose). Single- cell suspensions were prepared from lung tissues 12 h after the last injection of EdU and cells were stained to identify living ILC2s as described above. Cells were stained with freshly prepared click staining mix containing 10 mM Alexa Fluor 488-azide, 1 mM CuSO4, and 10 mM sodium ascorbate in PBS in the presence of 1 mM THPTA, and 10 mM amino-guanidine at room temperature for 2 hours in the dark[75].

Real-time quantitative PCR (RT-qPCR).

Lung tissue were homogenized in TRIzol (ThermoFisher) and RNA extracted using RNeasy Mini Kit (Qiagen) according the manufacturer's instructions. RNA was reverse transcribed using iScript™ cDNA Synthesis Kit (Bio-Rad) and real-time qPCR was performed using SYBR Green IQ supermix (Bio-Rad) and primers detailed in STAR METHODS key resource table. The comparative ΔCt method was used to represent relative expression normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Generation of BM chimeras.

BM chimeras were constructed by exposure of BALB/c CD45.2$^+$ or C57BL/6 CD45.1.2$^+$ mice to a lethal irradiation protocol that preserves or not the thoracic cavity. These recipient mice were then given intravenous injection of 5×10^6 BM cells isolated from the femur and tibia of BALB/c CD45.1$^+$ WT or a mix (1:1) of C57BL/6 CD45.1$^+$ WT and C57BL/6 IFN-γR$^{-/-}$ CD45.2$^+$ congenic BM cells. The host mice were given broad-spectrum antibiotics (endotrim, Ecuphar) at a concentration of 1.5 mg/ml, for 4 weeks in drinking water. Experimental manipulations on chimeric mice were allowed 8 weeks after the BM transplantation.

Mouse irradiation protocol.

A dose of 6 Gy one fraction was delivered to the whole body and 7,5 Gy when the thoracic cavity was spared with a dedicated small animal radiotherapy device (SmART Irradiator from Precision X-Ray Inc). Radiation was delivered using a photon beam (maximum energy of 225 kV and 13 mA), which provided a dose rate of 3 Gy/min. The planning system SmART-plan (version 1.3.9 Precision X-ray, North Branford, CT) was used to establish and deliver the treatment. To target the whole body except the thoracic cavity, we used two opposite beams to irradiate the head and the same schedule to irradiate the abdominal cavity. The dose delivered was almost 0 Gy to the lungs, 7.5 Gy to the soft tissue and 20.5 Gy to the bones. Fluoroscopy was used to check mouse positioning before each beam to avoid thoracic irradiation. During irradiation, mice received continuous isoflurane anesthesia gas via a nose cone (0.4 l/min oxygen with 1.5% isoflurane).

Cell sorting of ILC2s, ILCs and AMs.

Lung ILC2s (defined as $Lin^-CD45^+CD127^+CD90.2^+ST2^+CD25^+$ living cells) were sorted after negative enrichment against lineage markers (B220, CD11c, CD3, CD4, CD49b, CD5, CD8α, F4/80, FcεR1, Gr1 and SiglecF) using MojoSort™ Mouse anti-APC Nanobeads (Biolegend) and magnetic separation using LD columns (Miltenyi) according to the manufacturer's protocol. Lung ILCs (defined as $Lin^-CD45^+CD90.2^+$ living cells) were sorted after negative enrichment against lineage markers (B220, CD11c, CD3, CD4, CD5, CD8α, F4/80, FcεR1, Ly6G and SiglecF) using MojoSort™ Mouse anti-APC Nanobeads (Biolegend) and magnetic separation using LD columns (Miltenyi) according to the manufacturer's protocol. AMs from BALF were defined as autofluorescent, $FSC\text{-}A^{high}$ living cells. All cells were sorted on a FACSAria Illu (BD Biosciences).

***Ex vivo* culture.**

All cells were high-speed sorted using a FACSAria Illu (BD Biosciences). 5.000 lung epithelial cells from C57BL/6 naïve mice were sorted ($CD45^-CD31^-Epcam^+$ live cells) after depletion of $CD45^+$ cells using using MojoSort™ Mouse anti-CD45 Nanobeads (Biolegend) according to the manufacturer's protocol. 50.000 BM monocytes from C57BL/6 naïve mice were sorted ($CD19^-CD3^-CD11b^+Ly6C^+$ living cells), after depletion of $Ly6G^+$ and $B220^+$ cells using MojoSort™ Mouse anti-APC Nanobeads (Biolegend) and magnetic separation using LD columns (Miltenyi) according to the manufacturer's protocol. BM monocytes and lung epithelial cells were co-cultured in 200 µL of RPMI 1640 medium containing Glutamax-I with 10% FCS, 1% MEM, 50 µM 2-mercaptoethanol, 100 U/ml penicillin, 100 mg/ml streptomycin and 10 ng/ml recombinant murine GM-CSF (Peprotech). 3 days after the start of the co-culture, 2.500 ILC2s from mock- or MuHV-4 infected C57BL/6 mice (sorted as described above)

were added with IL-2 (final concentration 10 ng/mL). Cells were analyzed 3 days after the addition of ILC2s and supernatant was used for analyzing cytokine levels.

RNA preparation for bulk RNA sequencing.

From *ex vivo* co-culture, cells were harvested using Cell Dissociation Buffer (ThermoFisher) for 15 min in 37°C and then macrophages (FSC-A^{high}, CD11c^{+} living cells) were sorted using FACS and kept in TRIzol at -80°C. RNA from macrophages was extracted using Direct-zol™ RNA Miniprep (Zymo Research) according to the manufacturer's instructions, and quality was assessed on Agilent RNA 6000 Pico. cDNA was prepared using SmartSeq HT (1ng). RNA-Seq libraries were prepared from 150ng (5µl at 30pg/µl), using the Illumina Nextera XT DNA Library Preparation Kit. Libraries were quantified and normalized by qPCR. Libraries were finally sequenced using Illumina NovaSeq6000 and bioinformatics analysis was performed. Approximately 25×10^6 75-base single-end reads were generated per sample.

Subsequent analysis used R bioconductor (v.4.0.3). The nf-core/rnaseq pipeline (v.3.0) was used to generate the QC of the raw data and the count matrix[76]. The DESeq2 package was used to process the count matrix in order to get differentially expressed genes[77]. The vsn package was used to generate a variance stabilizing transformation out of the count matrix for visualization and clustering[78].

List of DE genes between Macro_ and +Mock_+MuHV-4_ were uploaded on PANTHER and GO biological process complete was selected. The results with a FDR ≤ 0.05 were considered as significant.

Single cell RNA sequencing.

Libraries preparations for single-cell immune profiling, sequencing, and post-processing of the raw data were performed at the GIGA-Genomics Core Facility (Belgium). Sorted cells were washed with 1X PBS (calcium and magnesium free) containing 0,04% weight/volume BSA (400 µg/mL). Cell concentration was adjusted to 1.000 total cells/µL and 12.800 cells were loaded on Chromium Controller (10x Genomics). Samples were further processed for droplet-based RNA sequencing and libraries were prepared using Chromium Single Cell 3' Reagent Kits v3 according to manufacturer's protocol (10x Genomics). Amplified cDNA quality controls were performed with an Agilent bioanalyzer (Agilent) and final library profile were checked on Qiaxcel (Qiagen). Sequencing libraries were loaded an Illumina Novaseq sequencer with NovaSeq SP 100 v1 kit (Illumina, CA, USA) using the following read lengths: 28 bp for Read1 (18 bp Barcode + 10 bp Randomer), 8 bp for Sample Index and 88 bp for Read2. Library quantification was processed with KAPA Library quantification kit (KAPA Biosystems).

Analysis of scRNA-seq samples.

Cell Ranger software (v.3.0.2) (10x Genomics) was used to demultiplex Illumina BCL files to FASTQ files (cellranger mkfastq), to perform alignment to mouse GRCm38/mm10 genome, filtering, UMI counting and to produce gene–barcode matrices (cellranger count). Subsequent analysis used R bioconductor (v.4.0.3) and the R package Seurat (v.2.1.5)[79]. First, individual data sets were read into R as count matrices and converted into Seurat objects (min.cells = 3, min.features = 200 genes) cells with $\leq 200 \geq 1500$ (HDM data) or ≥ 6000 (MuHV-4 data) distinct genes and $\geq 5\%$ (HDM data) or 8% (MuHV-4 data) of mitochondrial reads were filtered out. Each matrix were integrated using FindIntegrationAnchors with anchor.features = 2000 and dims = 1:30 options. Based on an elbow plot, principal components 1:10 (HDM data) or 1:20 (MuHV-4 data) were used in the subsequent analyses.

Cell types identification.

Cells were grouped in metacells (123 for HDM data and 102 for MuHV-4 data) using the FindCluster function of Seurat with a resolution of 10. Identification was made using the SingleR package[80].

UMAP representation.

Nonlinear dimensional reduction with UMAP was used to visualize the data sets, using the top 10 or 20 PCs (HDM and MuHV-4 data respectively). Non-integrated UMAP representations were generated based on the RNA assay of the Seurat object. The data were scaled on all the genes and the FindVariableFeatures with default option was run.

BubbleGUM analysis.

Bubblegum[81] analysis was used as described previously with default settings and the gene sets (containing between 20 and 500 genes) from Gene Ontology (13/11/2020) and gene set arbitrarily determined based on literature[8]. The results with a FDR ≤ 0.25 were considered as significant.

NicheNet.

To study intercellular communication the NicheNet package for R was used[47]. The top 20 ligands were kept and ‘mouse’ was selected for option organism in the function nichenet_seuratobj_aggregate.

Potential interaction scores.

In order to link NicheNet analysis with our data, we created the potential interaction score based on: average expression of ligand x average expression of receptor/target gene x weight of the interaction provide by the NicheNet package. The average expression of a gene is based on the Seurat package default calculation: log1p (RNA count for this gene / total RNA count x 10.000). The weight of an interaction is retrieved from the corresponding databases of the NicheNet package.

Circlize.

The circular visualisation was made with the R package circlize (top 5% of the ligand-target genes scores and 50% top ligand-receptor scores[82,83]. The intensity of the colour of the link between ligands and receptors is proportional to the score of the ligand-receptor link for each sender cell type (alpha from 0.25 to 1).

Quantification and statistical analysis.

Statistical tests used to analyze experiments are described in the Figure Legends. Data were analyzed using Prism software (GraphPad10, San Diego, CA). For RNA or scRNA sequencing data, all statistical analyses were performed in R v.4.0.3 and various Bioconductor packages. No statistical methods were used to predetermine sample size prior to experiments.

Acknowledgments

The authors thank A. N.J. McKenzie (Cambridge University, UK) and H-R Rodewald (German Cancer Research Center DKFZ) for the Rorα$^{lox/lox}$ Il7r$^{Cre/+}$ mice; B.G. Dewals for critical discussions and the technician and administrative team of the Immunology-Vaccinology lab (especially L. Dams and C. Espert) for very helpful assistance.

Funding:

This work was supported by

University of Liège (VIR-IMPRINT ARC)

Fonds de la Recherche Scientifique - Fonds National Belge de la Recherche Scientifique" F.R.S./FNRS ("credit de recherche" J007515F; "projet de recherche" T.0195.16; research associate support for B.M. and research fellow for P.L. and C.M.)

Institut MERIEUX (starting grant to L.G.)

EOS joint programme of F.R.S./FNRS

Fonds wetenschapellijk onderzoek – Vlaanderen - FWO (EOS ID:30981113)

B.M. is supported by

ERC Starting Grant (ERC-StG-2020 VIROME, ID:853608)

Author contributions: P.L., T.M., L.G. and B.M. designed the experiments. P.L., C.M., J.J., R.S., F.L. and B.M., and C.M. did most of the experiments. P.L. compiled the data. J.B. performed the transcriptomic analyses. P.L., L.G. and B.M. analyzed the data; P.L. and J.B. prepared the figures; P.L., B.M. and L.G. wrote the manuscript.

Competing interests: The authors declare no competing interests.

Data and materials availability: All data and codes used in this study are available from the corresponding authors upon request.

References

1. Abbafati, C. et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 396, 1204–1222 (2020).
2. Hammad, H. & Lambrecht, B. N. The basic immunology of asthma. Cell 184, 2521–2522 (2021).
3. Busse, W. W., Lemanske Jr., R. F. & Gern, J. E. Role of viral respiratory infections in asthma and asthma exacerbations. Lancet 376, 826–834 (2010).
4. Jartti, T. & Gern, J. E. Role of viral infections in the development and exacerbation of asthma in children. Journal of Allergy and Clinical Immunology vol. 140 895–906 (2017).
5. Schuijs, M. J. et al. Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. Science (80-.). 349, 1106–1110 (2015).
6. Lambrecht, B. N. & Hammad, H. The immunology of the allergy epidemic and the hygiene hypothesis. Nat Immunol in press, 1076–1083 (2017).
7. Haspeslagh, E., Heyndrickx, I., Hammad, H. & Lambrecht, B. N. The hygiene hypothesis: immunological mechanisms of airway tolerance. Curr. Opin. Immunol. 54, 102–108 (2018).
8. Machiels, B. et al. A gammaherpesvirus provides protection against allergic asthma by inducing the replacement of resident alveolar macrophages with regulatory monocytes. Nat. Immunol. 18, 1310–1320 (2017).
9. Artis, D. & Spits, H. The biology of innate lymphoid cells. Nature 517, 293–301 (2015).
10. Colonna, M. Innate Lymphoid Cells: Diversity , Plasticity , and Unique Functions in Immunity. Immunity 48, 1104–1117 (2018).
11. Kotas, M. E. & Locksley, R. M. Why Innate Lymphoid Cells? Immunity 48, 1081–1090 (2018).
12. Mindt, B. C., Fritz, J. H. & Duerr, C. U. Group 2 innate lymphoid cells in pulmonary immunity and tissue homeostasis. Front. Immunol. 9, 1–17 (2018).
13. Neill, D. R. et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature 464, 1367–1370 (2010).
14. Pulendran, B. & Artis, D. New paradigms in type 2 immunity. Science (80-.). 337, 431–435 (2012).
15. Dahlgren, M. W. & Molofsky, A. B. All along the watchtower: group 2 innate lymphoid cells in allergic responses. Curr. Opin. Immunol. 54, 13–19 (2018).
16. Nussbaum, J. C. et al. Type 2 innate lymphoid cells control eosinophil homeostasis. Nature 502, 245–248 (2013).
17. Lambrecht, B. N. & Hammad, H. The immunology of asthma. Nat Immunol 16, 45–56 (2015).
18. Hammad, H. & Lambrecht, B. N. Barrier Epithelial Cells and the Control of Type 2 Immunity. Immunity 43, 29–40 (2015).
19. Halim, T. Y. et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. Immunity 40, 425–435 (2014).

20. Licona-Limon, P., Kim, L. K., Palm, N. W. & Flavell, R. A. TH2, allergy and group 2 innate lymphoid cells. Nat Immunol 14, 536–542 (2013).
21. Christianson, C. A. et al. Persistence of asthma requires multiple feedback circuits involving type 2 innate lymphoid cells and IL-33. J Allergy Clin Immunol 136, 59-68 e14 (2015).
22. Gold, M. J. et al. Group 2 innate lymphoid cells facilitate sensitization to local, but not systemic, TH2-inducing allergen exposures. J. Allergy Clin. Immunol. 133, 1142-1148.e5 (2014).
23. Halim, T. Y. et al. Group 2 innate lymphoid cells license dendritic cells to potentiate memory TH2 cell responses. Nat Immunol 17, 57–64 (2016).
24. Fort, M. M. et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity 15, 985–95 (2001).
25. Halim, T. Y. F., Krauss, R. H., Sun, A. C. & Takei, F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. Immunity 36, 451–63 (2012).
26. Klose, C. S. N. et al. The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. Nature 549, 282–286 (2017).
27. Cardoso, V. et al. Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. Nature 549, 277–281 (2017).
28. von Moltke, J. et al. Leukotrienes provide an NFAT-dependent signal that synergizes with IL-33 to activate ILC2s. J. Exp. Med. 214, 27–37 (2017).
29. Sui, P. et al. Pulmonary neuroendocrine cells amplify allergic asthma responses. Science (80-.). 360, eaan8546 (2018).
30. Fonseca, W., Lukacs, N. W., Elesela, S. & Malinczak, C. A. Role of ILC2 in Viral-Induced Lung Pathogenesis. Frontiers in Immunology vol. 12 (2021).
31. Killig, M., Glatzer, T. & Romagnani, C. Recognition strategies of group 3 innate lymphoid cells. Front. Immunol. 5, 1–8 (2014).
32. Molofsky, A. B. et al. Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. J. Exp. Med. 210, 535–549 (2013).
33. Huang, Y. et al. S1P-dependent interorgan trafficking of group 2 innate lymphoid cells supports host defense. Science (80-.). 359, 114–119 (2018).
34. Silver, J. S. et al. Inflammatory triggers associated with exacerbations of COPD orchestrate plasticity of group 2 innate lymphoid cells in the lungs. Nat. Immunol. 17, 626–635 (2016).
35. Bal, S. M. et al. IL-1β, IL-4 and IL-12 control the fate of group 2 innate lymphoid cells in human airway inflammation in the lungs. Nat. Immunol. 17, 636–645 (2016).
36. Rajput, C. et al. RORα-dependent type 2 innate lymphoid cells are required and sufficient for mucous metaplasia in immature mice. Am. J. Physiol. - Lung Cell. Mol. Physiol. 312, L983–L993 (2017).

37. Yu, Y. et al. Single-cell RNA-seq identifies a PD-1hiILC progenitor and defines its development pathway. Nature 539, 102–106 (2016).
38. Taylor, S., Huang, Y., Mallett, G. & Amarnath, S. PD-1 regulates KLRG1+ group 2 innate lymphoid cells. J. Exp. Med 214, 1663–1678 (2017).
39. Hoyler, T. et al. The Transcription Factor GATA-3 Controls Cell Fate and Maintenance of Type 2 Innate Lymphoid Cells. Immunity 37, 634–648 (2012).
40. Entwistle, L. J. et al. Pulmonary Group 2 Innate Lymphoid Cell Phenotype Is Context Specific: Determining the Effect of Strain, Location, and Stimuli. Front. Immunol. 10, (2019).
41. Moro, K. et al. Interferon and IL-27 antagonize the function of group 2 innate lymphoid cells and type 2 innate immune responses. Nat Immunol 17, 76–86 (2016).
42. Barton, E. S. et al. Herpesvirus latency confers symbiotic protection from bacterial infection. Nature 447, 326–9 (2007).
43. Helou, D. G. et al. PD-1 pathway regulates ILC2 metabolism and PD-1 agonist treatment ameliorates airway hyperreactivity. Nat. Commun. 11, 3998 (2020).
44. Monticelli, L. A. et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. Nat Immunol 12, 1045–1054 (2011).
45. Kim, J. et al. Innate immune crosstalk in asthmatic airways: Innate lymphoid cells coordinate polarization of lung macrophages. J. Allergy Clin. Immunol. 143, 1769-1782.e11 (2019).
46. Saluzzo, S. et al. First-Breath-Induced Type 2 Pathways Shape the Lung Immune Environment. Cell Rep. 18, 1893–1905 (2017).
47. Browaeys, R., Saelens, W. & Saeys, Y. NicheNet: modeling intercellular communication by linking ligands to target genes. Nat. Methods 17, 159–162 (2020).
48. Guilliams, M. et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. J. Exp. Med. 210, 1977–1992 (2013).
49. Oliphant, C. J. et al. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4+T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. Immunity 41, 283–295 (2014).
50. Svedberg, F. R. et al. The lung environment controls alveolar macrophage metabolism and responsiveness in type 2 inflammation. Nat. Immunol. 20, 571–580 (2019).
51. Mathä, L., Martinez-Gonzalez, I., Steer, C. A. & Takei, F. The Fate of Activated Group 2 Innate Lymphoid Cells. Frontiers in Immunology vol. 12 (2021).
52. Beale, J. et al. Rhinovirus-induced IL-25 in asthma exacerbation drives type 2 immunity and allergic pulmonary inflammation. Sci. Transl. Med. 6, 256ra134-256ra134 (2014).
53. Stier, M. T. et al. Respiratory syncytial virus infection activates IL-13–producing group 2 innate lymphoid cells through thymic stromal lymphopoietin. J. Allergy Clin. Immunol. 138, 814-824.e11 (2016).

54. Li, B. W. S. S. et al. T cells and ILC2s are major effector cells in influenza-induced exacerbation of allergic airway inflammation in mice. Eur. J. Immunol. 31, 1–32 (2018).
55. Bartemes, K. R., Kephart, G. M., Fox, S. J. & Kita, H. Enhanced innate type 2 immune response in peripheral blood from patients with asthma. J. Allergy Clin. Immunol. 134, (2014).
56. Miller, M. M. et al. BATF acts as an essential regulator of IL-25–responsive migratory ILC2 cell fate and function. Sci. Immunol. 5, eaay3994 (2020).
57. Boberg, E., Johansson, K., Malmhäll, C., Weidner, J. & Rådinger, M. House dust mite induces bone marrow il-33responsive ILC2S and TH cells. Int. J. Mol. Sci. 21, (2020).
58. Duerr, C. U. et al. Type I interferon restricts type 2 immunopathology through the regulation of group 2 innate lymphoid cells. Nat Immunol 17, 65–75 (2016).
59. Califano, D. et al. IFN-γ increases susceptibility to influenza A infection through suppression of group II innate lymphoid cells. Mucosal Immunol. 11, 209–219 (2018).
60. Ohne, Y. et al. IL-1 is a critical regulator of group 2 innate lymphoid cell function and plasticity. Nat. Immunol. 17, 646–655 (2016).
61. Netea, M. G. & Joosten, L. A. B. Trained Immunity and Local Innate Immune Memory in the Lung. Cell 175, 1463–1465 (2018).
62. Netea, M. G. et al. Trained immunity: A program of innate immune memory in health and disease. Science (80-.). 352, aaf1098 (2016).
63. Schlums, H. et al. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. Immunity 42, 443–456 (2015).
64. Stadhouders, R. et al. Epigenome analysis links gene regulatory elements in group 2 innate lymphocytes to asthma susceptibility. J. Allergy Clin. Immunol. (2018) doi:10.1016/j.jaci.2017.12.1006.
65. Lavin, Y. et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell 159, 1312–1326 (2014).
66. Gosselin, D. et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. Cell 159, 1327–1340 (2014).
67. Cohen, M. et al. Lung Single-Cell Signaling Interaction Map Reveals Basophil Role in Macrophage Imprinting. Cell 175, 1031-1044.e18 (2018).
68. Guilliams, M., Thierry, G. R., Bonnardel, J. & Bajenoff, M. Establishment and Maintenance of the Macrophage Niche. Immunity vol. 52 434–451 (2020).
69. van de Laar, L. et al. Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an Empty Niche and Develop into Functional Tissue-Resident Macrophages. Immunity 44, (2016).
70. Schneider, C. et al. Induction of the nuclear receptor PPAR-γ 3 by the cytokine GM-CSF is critical for the differentiation of fetal monocytes into alveolar macrophages. Nat. Immunol. 15, 1026–1037 (2014).
71. Bonilla, W. V et al. The Alarmin Interleukin-33 Drives. (2012) doi:10.1126/science.1215418.

72. Bal, S. M., Golebski, K. & Spits, H. Plasticity of innate lymphoid cell subsets. Nature Reviews Immunology vol. 20 552–565 (2020).
73. Adler, H., Messerle, M., Wagner, M. & Koszinowski, U. H. Cloning and Mutagenesis of the Murine Gammaherpesvirus 68 Genome as an Infectious Bacterial Artificial Chromosome. JOURNAL OF VIROLOGY vol. 74 (2000).
74. Machiels, B., Stevenson, P. G., Vanderplasschen, A., Gillet, L. & Hutt-Fletcher, L. A Gammaherpesvirus Uses Alternative Splicing to Regulate Its Tropism and Its Sensitivity to Neutralization. (2013) doi:10.1371/journal.ppat.1003753.
75. Wang, I. H. et al. Tracking viral genomes in host cells at single-molecule resolution. Cell Host Microbe 14, 468–480 (2013).
76. DI Tommaso, P. et al. Nextflow enables reproducible computational workflows. Nature Biotechnology vol. 35 316–319 (2017).
77. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550 (2014).
78. Huber, W., Von Heydebreck, A., Sültmann, H., Poustka, A. & Vingron, M. Variance stabilization applied to microarray data calibration and to the quantification of differential expression. in Bioinformatics vol. 18 96–104 (Oxford University Press, 2002).
79. Stuart, T. et al. Comprehensive Integration of Single-Cell Data. Cell 177, 1888-1902.e21 (2019).
80. Aran, D. et al. Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage. Nat. Immunol. 20, 163–172 (2019).
81. Spinelli, L., Carpentier, S., Montanana Sanchis, F., Dalod, M. & Vu Manh, T. P. BubbleGUM: automatic extraction of phenotype molecular signatures and comprehensive visualization of multiple Gene Set Enrichment Analyses. BMC Genomics 16, 814 (2015).
82. Bonnardel, J. et al. Stellate Cells, Hepatocytes, and Endothelial Cells Imprint the Kupffer Cell Identity on Monocytes Colonizing the Liver Macrophage Niche. Immunity 51, 638-654.e9 (2019).
83. Gu, Z., Gu, L., Eils, R., Schlesner, M. & Brors, B. Circlize implements and enhances circular visualization in R. Bioinformatics 30, 2811–2812 (2014).

Supplemental figures

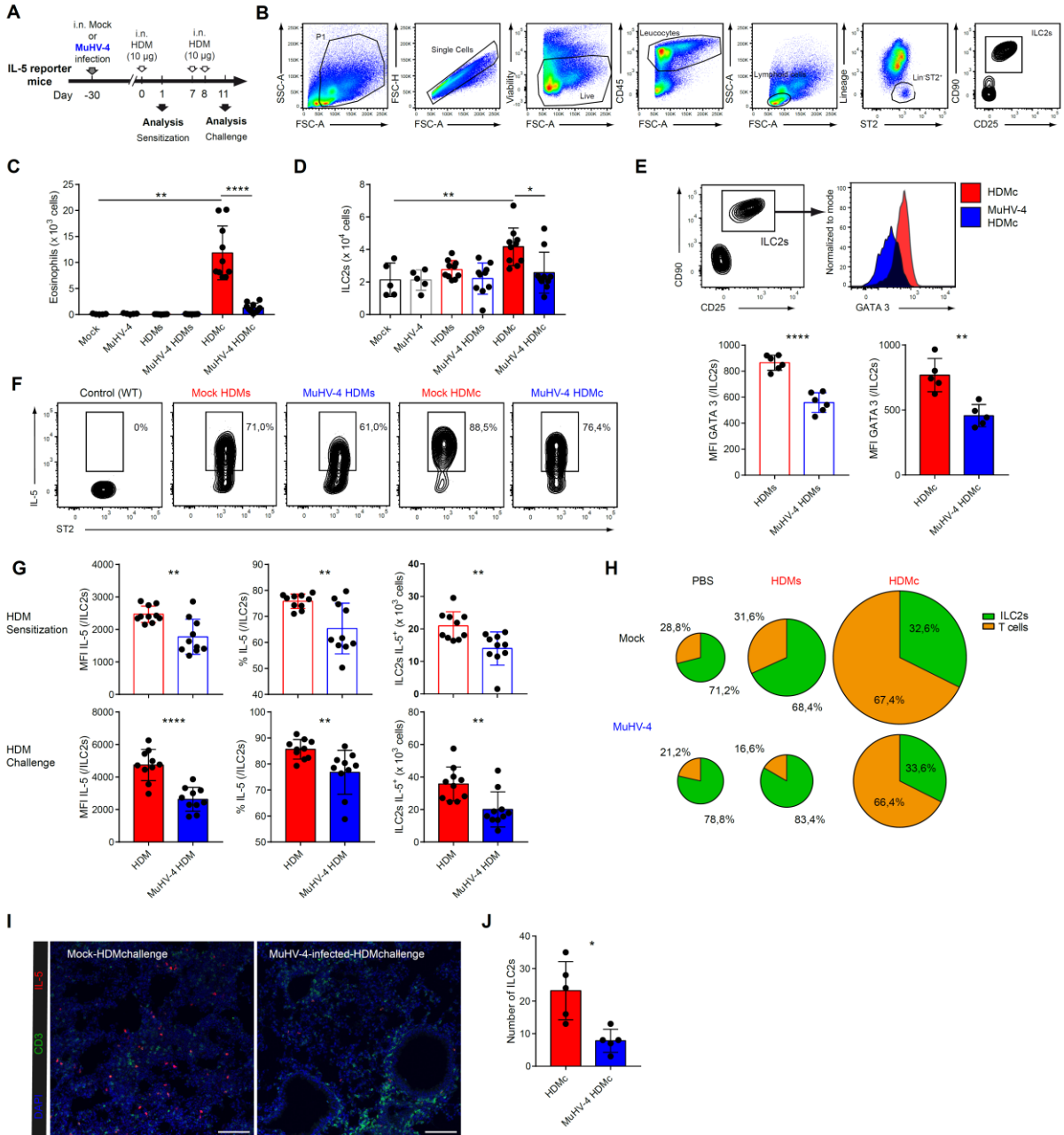
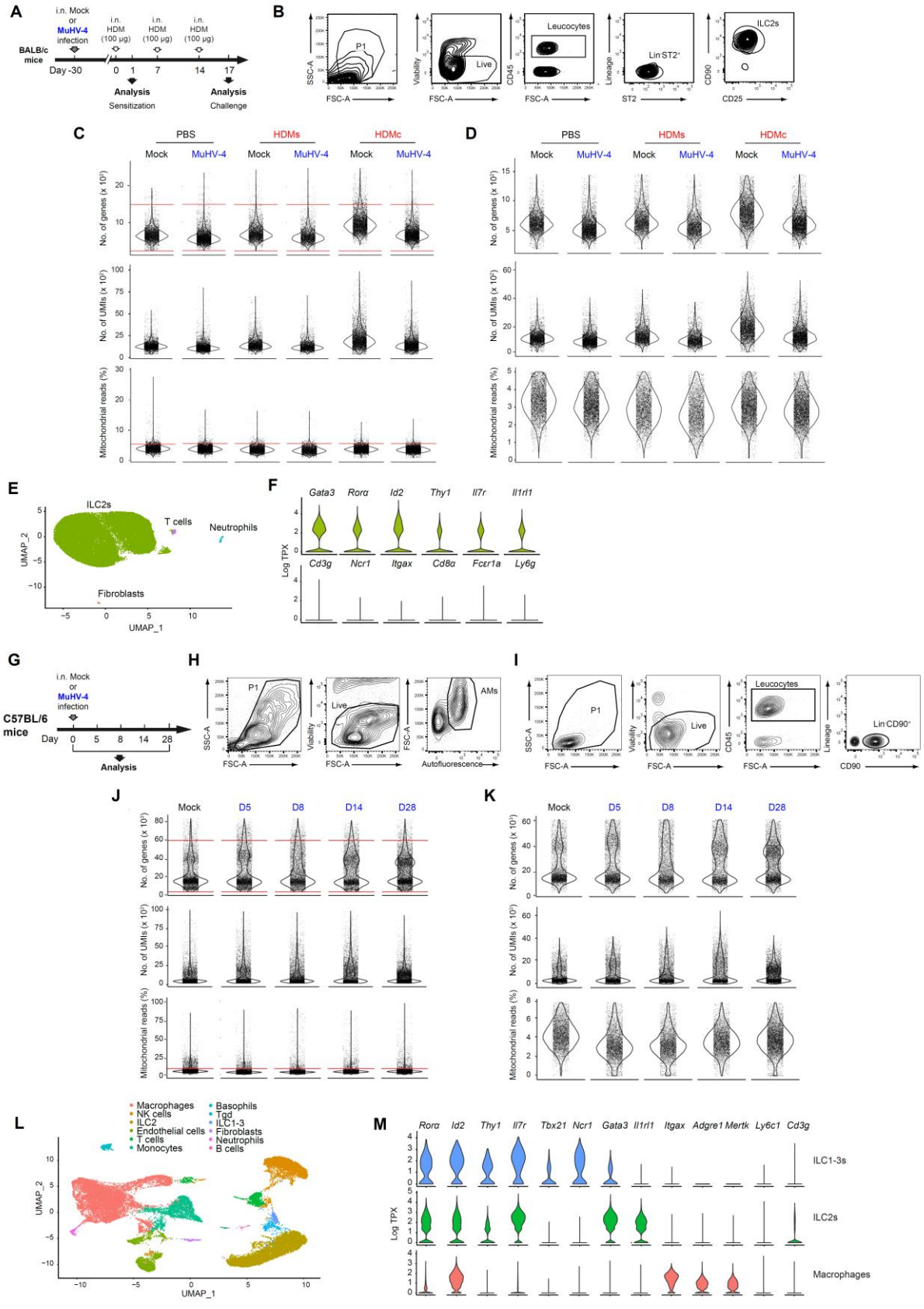


Fig. S1. MuHV-4 infection affects the number and function of pulmonary ILC2s in C57BL/6 mice.

8-week-old-Red5 (IL5-tdtomato-cre) reporter mice (n= 5 to 10 in each group) were infected or not with MuHV-4 and submitted to low-dose HDM sensitization or challenge before analysis. (**A**) Experimental layout. (**B**) Strategy for the identification of lung ILC2s by flow cytometry. Lineage was defined as B220, CD11c, CD3, CD49b, CD4, CD8α, F4/80, FcεRI, Gr1 and Siglec-F. ILC2s were described as $Lin^{-}CD45^{+}ST2^{+}CD90.2^{+}CD25^{+}$ living cells. (**C**) Absolute numbers of eosinophils (gated as gated as out AMs (autofluorescent $CD11c^{+}SSC\text{-}A^{high}$) $Siglec\text{-}F^{+}CD11b^{+}$ living cells) in BALF. (**D**) Absolute numbers of ILC2s in lung. (**E**) Representative histograms and MFI of GATA 3 staining in lung ILC2s. (**F**) Flow cytometry plots of tdTomato/IL-5 fluorescence in lung ILC2s. Numbers indicate the percentage of positive cells in each quadrant. (**G**) MFI and percentage of tdTomato/IL-5 positive cells among lung ILC2s. (**H**) Circular diagrams of $IL\text{-}5^{+}$ lung cells of the indicated conditions. Area correspond to the relative amount of $IL\text{-}5^{+}$ cells in comparison with mock-infected mice. Numbers represent the percentage of ILC2s or T cells among tdTomato/IL-5 positive cells. (**I**) Lungs of mock and MuHV-4 infected HDM challenged mice were submitted to 2D thin-cut, immunostained and imaged with ILC2s ($CD3^{-}$ $IL\text{-}5^{+}$cells) and T-lymphocytes ($CD3^{+}$ cells). Images are representative of 3 mice. (**J**) Numbers of ILC2s ($CD3^{-}$ $IL\text{-}5^{+}$ cells) observed in slides of indicated conditions. 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. Bars show mean values ± SEM. Data represent two independent experiments with similar results.


A
i.n. Mock or MuHV-4 infection
i.n. HDM (100 µg)
i.n. HDM (100 µg)
i.n. HDM (100 µg)
BALB/c mice
Day -30
0 1 7 14 17
Analysis Sensitization
Analysis Challenge
B
P1
Live
Leucocytes
Lin-ST2+
ILC2s
SSC-A
FSC-A
Viability
CD45
Lineage
ST2
CD90
CD25
C
PBS
HDMs
HDMc
Mock
MuHV-4
No. of genes (x 10³)
No. of UMIs (x 10³)
Mitochondrial reads (%)
D
E
ILC2s
T cells
Neutrophils
Fibroblasts
UMAP_1
UMAP_2
F
Gata3 Rorα Id2 Thy1 Il7r Il1rl1
Cd3g Ncr1 Itgax Cd8α Fcεr1a Ly6g
Log TPX
G
i.n. Mock or MuHV-4 infection
C57BL/6 mice
Day 0 5 8 14 28
Analysis
H
P1
Live
AMs
Autofluorescence
I
Leucocytes
Lin-CD90+
J
Mock D5 D8 D14 D28
K
L
Macrophages
NK cells
ILC2
Endothelial cells
T cells
Monocytes
Basophils
Tgd
ILC1-3
Fibroblasts
Neutrophils
B cells
M
Rorα Id2 Thy1 Il7r Tbx21 Ncr1 Gata3 Il1rl1 Itgax Adgre1 Mertk Ly6c1 Cd3g
ILC1-3s
ILC2s
Macrophages

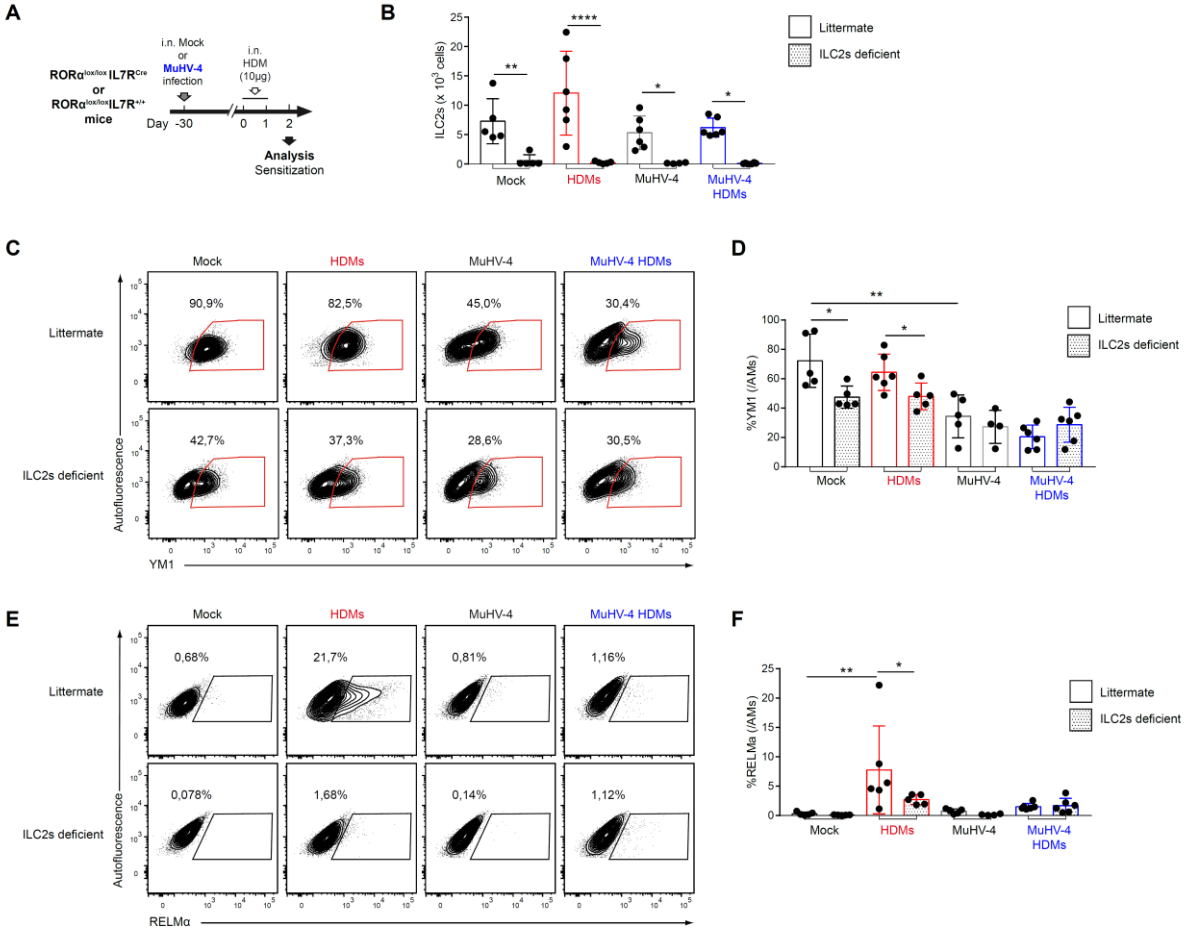
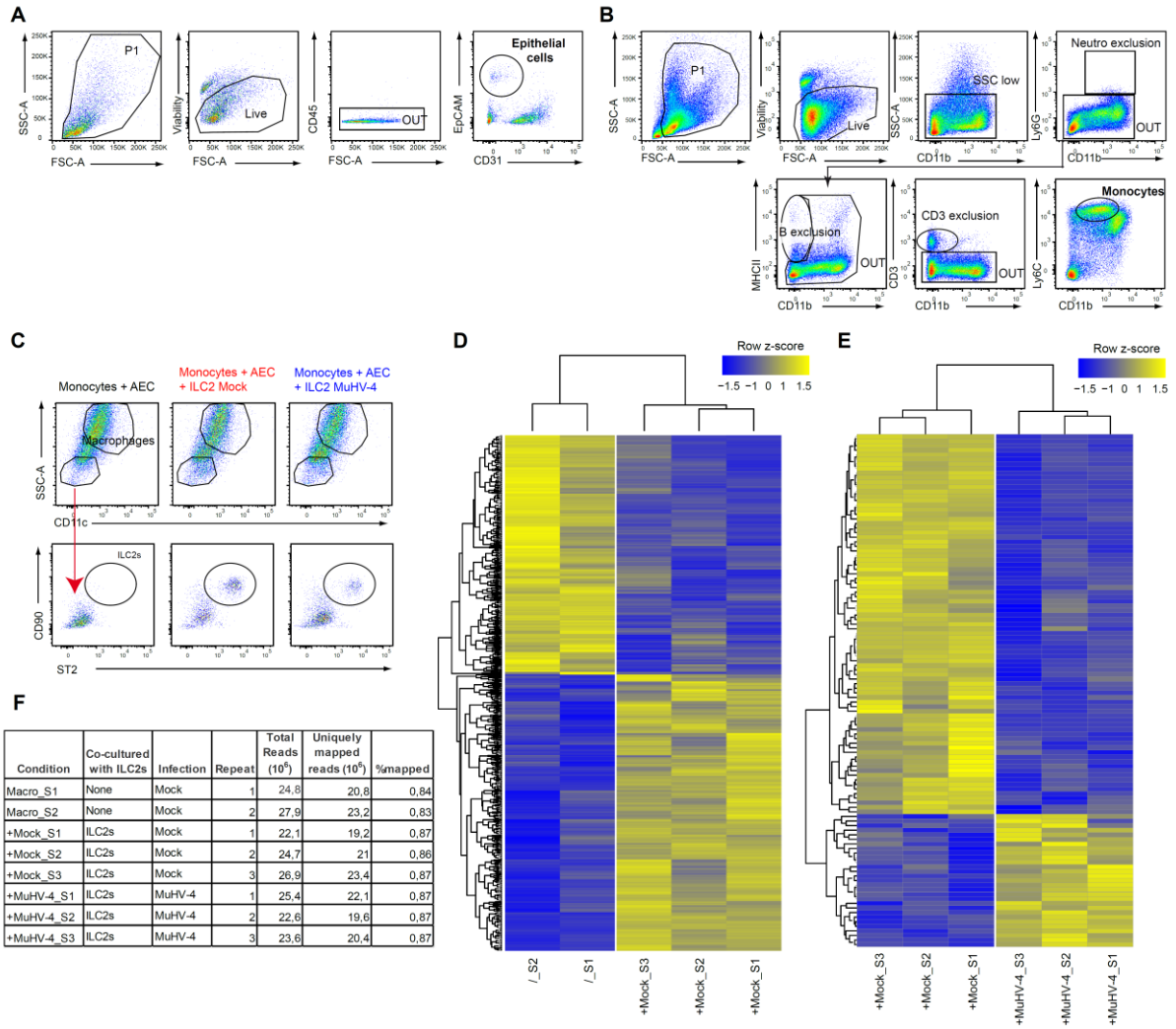


Fig. S3. MuHV-4 infection blocks the capacity of ILC2s to promote a M2 polarization of AMs.
8-week-old- ILC2s deficient mice (n= 5 to 10 in each group) were infected or not with MuHV-4 and submitted to low-dose HDM sensitization before analysis. (**A**) Experimental layout. (**B**) Quantification of lung ILC2s. (**C** and **E**) Representative flow cytometry plots of YM1 (**C**) or RELMα (**E**) expression in AMs from BALF. (**D** and **F**) Percentage of YM1 (**D**) or RELMα (**F**) positive AMs from BALF. 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; **** p<0.0001. Error bars represent SEM.



F

Condition	Co-cultured with ILC2s	Infection	Repeat	Total Reads (10^6)	Uniquely mapped reads (10^6)	%mapped
Macro_S1	None	Mock	1	24,8	20,8	0,84
Macro_S2	None	Mock	2	27,9	23,2	0,83
+Mock_S1	ILC2s	Mock	1	22,1	19,2	0,87
+Mock_S2	ILC2s	Mock	2	24,7	21	0,86
+Mock_S3	ILC2s	Mock	3	26,9	23,4	0,87
+MuHV-4_S1	ILC2s	MuHV-4	1	25,4	22,1	0,87
+MuHV-4_S2	ILC2s	MuHV-4	2	22,6	19,6	0,87
+MuHV-4_S3	ILC2s	MuHV-4	3	23,6	20,4	0,87

Fig. S4. ILC2s promote maturation of monocytes into AM like cells.
Lung monocytes, epithelial cell (EC) and ILC2s from mock- or MuHV-4 infected-mice were co-cultured as described in Figure 7. (**A**) Gating strategy for FACS sorting of lung epithelial cells, described as $CD45^-CD31^-Epcam^+$ living cells after CD45 MACS depletion. (**B**) Gating strategy for FACS sorting of BM monocytes, described as $SSC\text{-}A^{low}Ly6C^+CD11b^+$ living cells after exclusion of neutrophils ($Ly6G^+CD11b^+$), B cells ($MHCII^+CD11b^-$), T cells ($CD3^+$ $CD11b^-$) after cell enrichment through MACS negative selection (L6G and B220). (**C**) Flow cytometry plots for AMs-like and ILC2s on the indicated conditions. (**D** and **E**) Heatmap of all genes differentially expressed (FDR<0,05; change in expression of over twofold) (two to three biological replicates) in monocytes. (**F**) Sequence reads and mapping statistics for raw Illumina data.

Discussion - Perspectives

ILC2s are sentinels of the innate immunity and triggers of the type 2 immune responses through the production of cytokines, being thus the master piece of allergic asthma (Halim *et al.*, 2014, 2015). They are involved in immune responses against viruses by both mediating inflammatory responses and maintaining epithelial lung homeostasis (Fonseca *et al.*, 2021). It is therefore unsurprising that viruses known to trigger or exacerbate asthmatic symptoms do so *via* activation of ILC2s (Beale *et al.*, 2014; Stier *et al.*, 2016; W.S. Li *et al.*, 2018). In contrast, it was unknown how ILC2s react to persistent viral infections such as herpesviruses, identified as major drivers of immune imprinting. The work presented in this thesis showed, for the first time, that a γHV infection inhibits the type 2 properties of ILC2s for the long-term, in an IFN-γ-dependent manner (**Figure Discussion 1**). After HDM challenge, ILC2s expansion, recruitment and activation were affected, resulting in a decreased ability to elicit a proper type 2 immune response. The inhibition of ILC2s, occurring at early times post-infection, prevented the M2 polarization of infiltrating monocytes differentiating AMs. Inhibition of ILC2s contributed to maintain a long-lasting M1-regulatory phenotype of Mo-derived AMs, even after an allergic challenge.

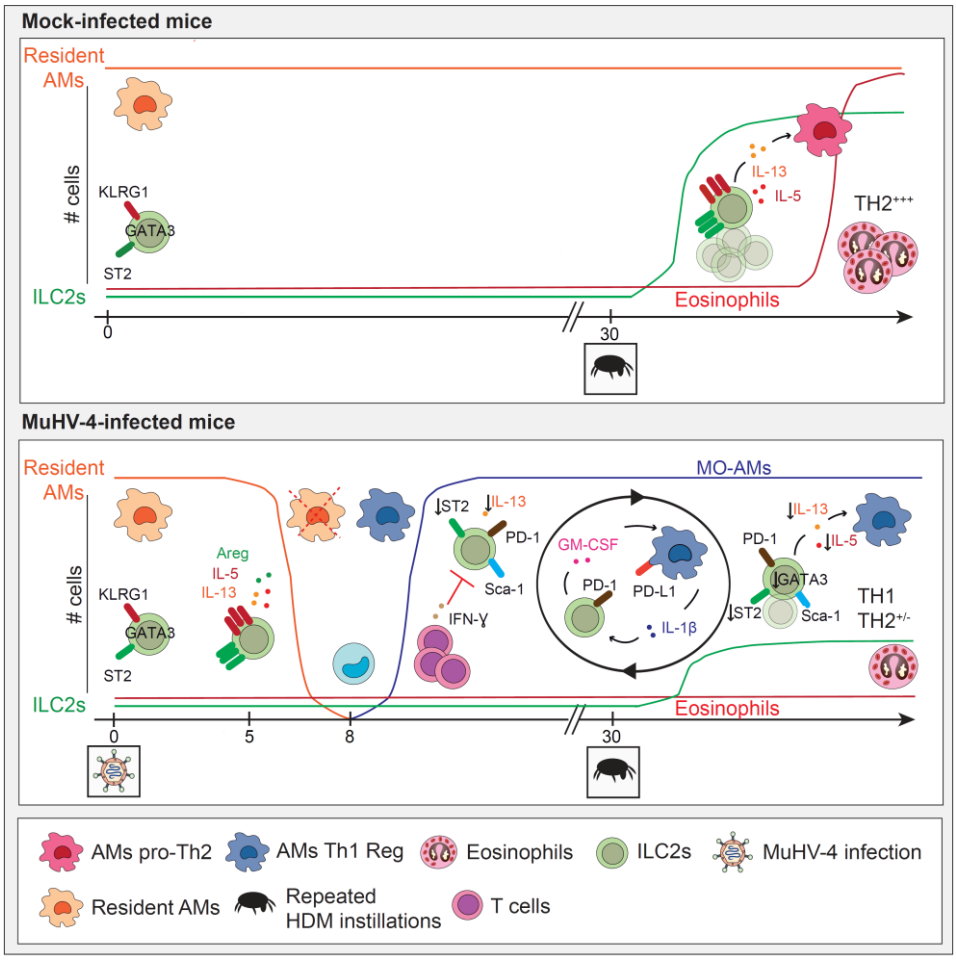


Figure Discussion 1. Graphical Summary.

MuHV-4 infection shapes ILC2s function that modulates the alveolar macrophage niche and impairs type 2 immunity to HDM.

In light of these results, we will here discuss in details the importance of pulmonary ILC2s for the development of HDM-induced allergic responses. In particular, we will emphasize the direct and indirect involvement of ILC2s in the development of the pulmonary TH2 response, both at steady-state and in the context of MuHV-4 infection. Finally, the perspectives of this work will be considered in the context of other viral infections and to a broader extent. Therefore, this discussion is structured around four major axes:

1) The functional importance of ILC2s for HDM-induced airway allergy **in a naive individual**
2) The functional importance of ILC2s modifications **consecutive to MuHV-4 infection**
3) The functional importance of ILC2s **in the context of different viral infections**
4) The functional importance of ILC2s-AMs crosstalk **throughout the body**

1) The functional importance of ILC2s for HDM-induced airway allergy in a naive individual

The contribution of both lung resident and bone-marrow-derived ILC2s to the development of HDM-induced airway allergy

As already mentioned, recruitment of ILC2s following HDM instillations had only been suggested (Stier *et al.*, 2018; Boberg *et al.*, 2020a; Boberg *et al.*, 2020b) and never demonstrated. This has been proven in the framework of this thesis by the use of partial chimeric mice which allow to differentiate resident from recruited ILC2s (CD45.1 versus CD45.2) (Fig. 2, I-N). In comparison with the parabiontic model used by Huang et *al*. in the context of parasitic infection (Huang *et al.*, 2018), we showed, in HDM-induced airway allergy, that recruited ILC2s acquire the same identity than the lung resident ILC2s and still express ST2 (Fig. 2N and data not shown). However, no mechanistic explanation for this recruitment has been proposed in the context of the manuscript submitted for publication. The expression of CCR2 was increased in ILC2s from mock infected mice following HDM challenge, compared to MuHV-4 infected mice (Fig. 3F), making CCR2 a potential candidate contributing to ILC2s recruitment following HDM challenge. Using partial mixed chimeras with BM transplantation of 50% from WT mice and 50% from $CCR2^{-/-}$ mice, we observed that ILC2s recruitment from BM cells did not depend on CCR2 expression (**Figure Discussion 2**). However, besides being a marker of BM egress, CCR2 also controls the intra-tissue trafficking of monocytes (Serbina and Pamer, 2006), suggesting that similar CCR2-dependent intra-tissue migration could exist with ILC2s. Looking at the confocal microscopy images of lung sections in Fig. S1I, we noticed a distinct distribution of ILC2s within lung parenchyma of mock or MuHV-4 infected mice, suggesting that this might be related to a different expression of CCR2. In the future, it would be interesting to repeat the same chimera model and explore, by confocal microscopy, the distribution of pulmonary ILC2s according to CCR2 expression.

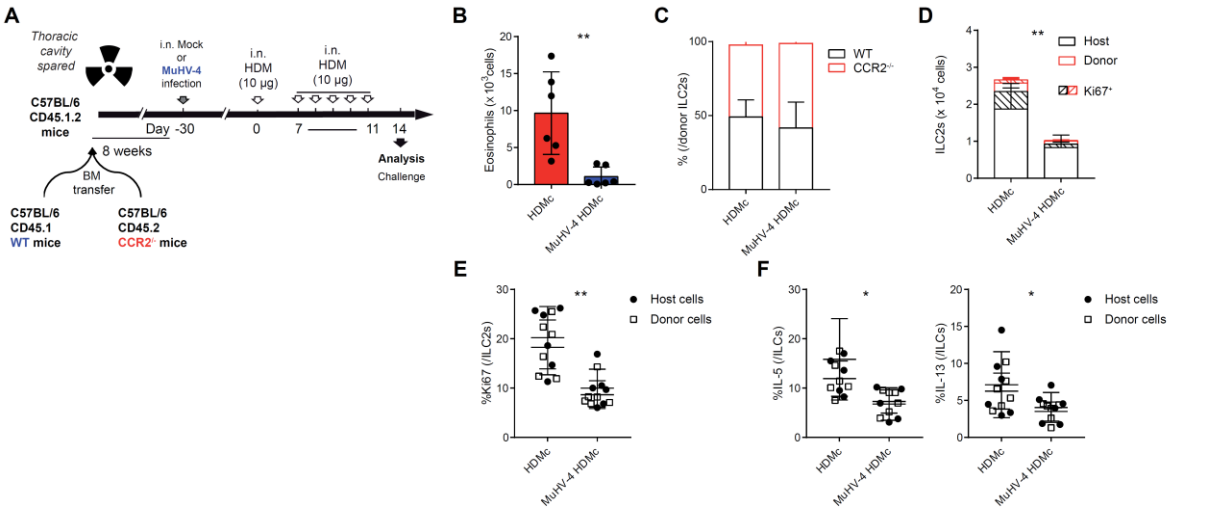


Fig. Discussion 2. ILC2s recruitment from bone marrow is not related to CCR2 expression.
(**A-C**) CD45.1.2⁺ C57BL/6 mice were lethally irradiated, sparing the thoracic area, before being transplanted with a mix (1:1) of CD45.1⁺ WT and CD45.2⁺ CCR2$^{-/-}$ congenic donors BM cells. (**A**) Experimental layout. (**B**) Quantification of BALF eosinophils. (**C**) Chimerism of donor ILC2s. (**D**) Enumeration of ILC2s from host or donor cells associated with proportion of expansion. (**E**) Percentage of Ki67 positive ILC2s. (**F**) Percentage of IL-5 and IL-13 positive ILC2s. Data were analysed by two-tailed Student's t-test * p<0,05; ** p<0,01. Errors bars represent SEM.

It has been shown that HDM challenge activates IL-33-responsive ILC2s in the BM and that IL-33 promotes egress of ST2⁺ILC2s progenitor cells from the BM (Stier *et al.*, 2018; Boberg *et al.*, 2020a; Boberg *et al.*, 2020b). Nevertheless, intranasal instillation of IL-33 in a parabiont model did not elicit any recruitment of ILC2s into the lung (Mathä *et al.*, 2021). Thus, **post-HDM recruitment appears to rely more on a network of mediators**, probably including IL-25, which is described as being produced following HDM instillations and responsible of ILC2 recruitment to the lung (Claudio *et al.*, 2019). In the future, it would then be interesting to clarify the mechanisms underlying ILC2s recruitment following HDM challenge, which are still poorly defined.

Finally, while scRNA-seq analysis has allowed us to confirm the previously described TH2 functional properties of lung ILC2 following HDM administration, these data are currently being analysed to refine our understanding of the heterogeneity of this lung ILC2s population in the context of HDM sensitization and challenge. In particular, through finer clustering, we hope to highlight the precise role of certain clusters (depending potentially on their origin) and their potential specific interactions with other cellular actors, providing potential new elements in the understanding of the development of respiratory allergies.

The direct role of ILC2s in the development of HDM-induced airway allergy

Although the direct role of ILC2s in the development of a type 2 immune response has already been extensively described, this study supports again this prominent role, here in a HDM-induced airway allergy, both at the sensitization and at the challenge steps. It has been demonstrated by the production of the type 2 cytokines, IL-5 and IL-13 (Fig. 1, F and G), as well as by the identification of activations markers (KLRG1, PD-1, Sca1, Arg1) following HDM instillations (Fig. 3, F and H). Furthermore, in

addition to this functional characterization, the administration of HDM to ILC2s deficient mice allowed us to unambiguously demonstrate the importance of these cells for the development of HDM-induced lung allergy, as ILC2s deficient mice failed to develop a proper eosinophilia following HDM challenge **(Figure Discussion 3**).

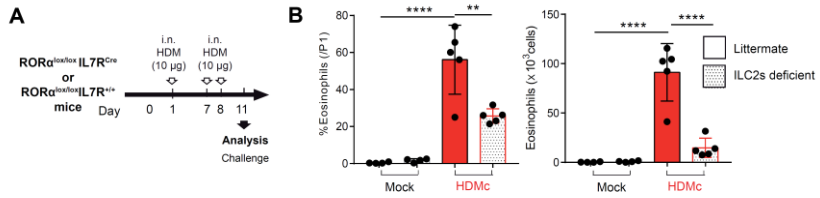


Fig. Discussion 3. ILC2s are essential for the development of a proper eosinophilia following HDM-induced airway allergy.
(**A**-**B**) 8-week-old- littermate or ILC2s deficient mice (n= 5 in each group) were submitted to low-dose HDM challenge before analysis. (**A**) Experimental layout. (**B**) Percentage and absolute numbers of eosinophils (out AMs (autofluorescent $CD11c^+SSC\text{-}A^{high}$) $Siglec\text{-}F^+CD11b^+$ living cells) from BALF. 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; **** p<0.0001. Error bars represent SEM.

The indirect role of ILC2s in the development of HDM-induced airway allergy

One important result presented in this study is **the role of ILC2s in modulating AMs polarization**. This has been firstly supported by the transcriptomic analysis (Fig. 6), observed *in vivo* (Fig. S3) and then, confirmed *ex vivo* (Fig. 7). This role of ILC2s had already been described in early life. Indeed, at birth, an IL-33/ILC2/IL-13-driven immune response maintains AMs in an M2 phenotype, at the expense of a delayed response to bacterial infections (Saluzzo *et al.*, 2017). In the context of this work, we wanted to decipher the potential functional importance of the ILC2-induced M2 polarization of AMs for the development of HDM-induced airway allergy. To address this question, we performed AMs transfer from littermate or ILC2s deficient mice and submitted the recipient mice to HDM challenge (**Figure Discussion 4**). Surprisingly, upon challenge with HDM, mice transferred with AMs displayed a higher eosinophilia compared to mice without any transferred AMs (**Figure Discussion 4C**). Very interestingly, this AM-induced TH2 exacerbation was promoted by ILC2s. Indeed, the transfer of AMs from ILC2s deficient mice did not induce any pro-TH2 function, showing that ILC2s instruct AMs toward a M2 polarization subsequently responsible for an amplification of the HDM-induced TH2 response (**Figure Discussion 4C**). It is known that the neonatal lung is immersed in a type 2 immune environment, and that "naive" resident AMs express M2-like markers such as YM1 (Saluzzo *et al.*, 2017). Moreover, Fig. S3, C and D showed that this marker is less expressed in AMs from ILC2s deficient mice, confirming that ILC2s are essential to promote a M2-AMs profile.

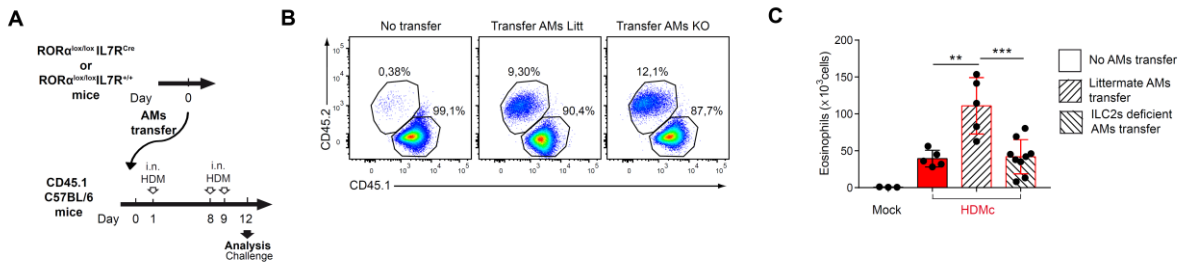


Fig. Discussion 4. ILC2s instruct AMs toward M2 polarization which amplifies a type 2 immune response.
(**A-C**) AMs were sorted by CD11c MACS enrichment from 8-week-old- littermate or ILC2s deficient or not mice (n= 15 in each group) and transferred intranasally to CD45.1 C57BL/6 mice (n= 3 to 10 in each group), mice were then submitted to low-dose HDM challenge before analysis. (**A**) Experimental layout. (**B**) Representative flow cytometry plots for the evaluation of the transfer between recipient (CD45.1$^+$) and donor (CD45.2$^+$) AMs (described as autofluorescent CD11c$^+$ living cells) in BALF (**C**) Absolute numbers of eosinophils (gated as out AMs Siglec-F$^+$CD11b$^+$ living cells) in BALF. 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; **** p<0.0001. Error bars represent SEM.

This original result questions the potential role of AMs in the mediation of the type 2 immune response. It is known that AMs regulate pro- and anti-inflammatory responses during allergic asthma (Balhara and Gounni, 2012; Y. G. Lee *et al.*, 2015), and that alternative macrophage activation (M2) is increased in asthma (Girodet *et al.*, 2016). However, one study showed that HDM-induced airway allergy is unaffected in the absence of IL-4Rα-dependent alternatively activated macrophages (Nieuwenhuizen *et al.*, 2012). Despite this last observation, the results presented here tend to suggest that AMs have a direct role in HDM-induced airway allergy, and for which it would be very interesting to study the underlying mechanism. In that context, we plan to co-culture *ex vivo* AMs from WT or ILC2s deficient mice, with OT-II CD4$^+$ T cells isolated from the spleen of naïve mice, in presence of OVA. We could then hypothesize that AMs, differentially imprinted by the presence or absence of ILC2s, will lead to a different polarization of OT-II CD4$^+$ T cells. More precisely, AMs isolated from WT mice could favor an increased TH2 profile of OT-II CD4$^+$ T cells (determinate by GATA 3 expression), in contrast to AMs isolated from ILC2 deficient mice.

2) The functional importance of ILC2s modifications consecutive to MuHV-4 infection

MuHV-4 infection induces recruitment of bone marrow ILC2s

Intriguingly, ILC2s appeared to be recruited following MuHV-4 infection, while no recruitment was observed post-HDM challenge in these mice (Fig. 2, J and K). Accordingly, scRNA-seq analysis of ILC2s highlighted changes over time suggesting the appearance of an additional population as soon as 5 days post-infection (Fig. 6D). ILC2s recruitment after a viral infection had previously never been reported. Since MuHV-4 infection depletes the alveolar macrophage niche and induces the recruitment of regulatory monocytes from the bone marrow, we might suggest that the surrounding microenvironment drives the expression of various chemokines that could also promote the recruitment of ILC2s. Transcriptomic analysis showed increased expression of *Icam1* in ILC2s (Fig. 6K) which controls ILC2s development from the bone marrow (Lei *et al.*, 2018). As a reminder, 28 days post-infection, the single-cell transcriptomic analysis performed did not clearly distinguish between different ILC2 populations likely because of a significant imprinting by antiviral microenvironment on resident

and recruited ILC2s (Fig. 3D and Fig. 6D). In the future, it would then be interesting to clarify the mechanisms underlying ILC2s recruitment following MuHV-4 infection, which has never been reported.

The direct consequence of MuHV-4 imprinting on the function of ILC2s in the context of HDM-induced airway allergy

We showed that the type 2 properties of ILC2s are inhibited by IFN-γ early after MuHV-4 infection (Fig. 4, M-O). This inhibition has a direct effect on the development of HDM-induced airway allergy, as observed by the impairment of their expansion and recruitment, as well as by the decreased production of type 2 cytokines following HDM challenge (Fig. 1 and 2). IFN-γ production, and subsequent inhibition of ILC2s were reasonably well expected. Indeed, exacerbation of asthma by RSV in infected pups mice can be blocked by IFN-γ-induced inhibition of ILC2s, establishing IFN-γ-deficiency as a cause of viral-induced allergic disease in immature animals (Han et al., 2017). Although the ILC2-inhibitory role of IFN-γ has been widely studied, we confirmed here, with the mixed chimera model, the **direct** role of IFN-γ in inhibiting, *in vivo*, the ILC2 type 2 properties (Fig. 4, D-F and M-O). Although the *in vivo* underlying mechanism are not well-elucidated, the following experiment will be done to further dissect the activation of intrinsic IFN-γ-dependent signaling in lung ILC2s following activation. Mixed chimeras (WT/IFNγR$^{-/-}$), similar to the ones generated in Fig.4 D will be repeated. Then, lung ILC2s of both genotypes will be sorted, and the transcriptomic profile of ILC2s from WT or IFNγR$^{-/-}$ compartment, within the same mouse, will be compared by bulk RNA sequencing. This could allow us to decipher further, how IFN-γ released after MuHV-4 infection, induces a direct lasting imprinting on lung ILC2s. Interestingly, herpesvirus infections are associated with chronic secretion of IFN-γ, which, among other things, maintains their latency and has been associated with a long-term classical cross protection state against various heterologous infections (Barton *et al.*, 2007). In that context, the role of MuHV-4 latency could be easily addressed by using a latency-deficient MuHV-4 mutant (MuHV-4 Del73) (Fowler *et al.*, 2003).

The indirect consequence of MuHV-4 imprinting on the function of ILC2s in the context of HDM-induced airway allergy

As described above, we have demonstrated a very important role for pulmonary ILC2s in shaping the phenotype of AMs. While uninfected mice show a population of AMs predominantly of embryonic origin, this is not the case in MuHV-4 infected mice. Indeed, our laboratory had published, and it has been confirmed again in this study, that MuHV-4 infection induces the depletion of the alveolar niche and its subsequent reconstitution by Mo-derived AMs. In this context, this would be interesting to know whether BM-derived monocytes, differentiating into AMs, are more sensitive to local imprinting by ILC2s than resident AMs.

The homeostatic functions of macrophages are regulated by so-called tissular "niche" that controls the size of the macrophage population and imprints their tissue-specific identity. Guilliams *et al.* hypothesized that a macrophage niche should fulfil four essential functions (Guilliams *et al.*, 2020):

1) the niche should provide a physical foundation or scaffold to the macrophage
2) the niche should provide trophic factors that confer macrophage self-maintenance
3) the niche should imprint the tissue-specific identity of the resident macrophage through the expression of key transcription factors
4) the macrophage should, in turn, provide a benefit for its niche

They propose to apply the concept of niche to the large family of tissue-resident immune cells, arguing that immune cells do not properly work on their own and need interactions with other cells. We hypothesized in this study that the alveolar niche is profoundly remodeled by MuHV-4 infection. In particular, we highlighted **the role of ILC2s as "niche cells" to direct the remodeling of Mo-derived AMs during adulthood**. This observation is of major importance in light of the constant evolution of the alveolar niche over time. Indeed, during the course of life of an individual exposed to environmental factors, resident embryonic AMs are progressively replaced by Mo-derived AMs (Morales-Nebreda *et al.*, 2015). The proportion of replacement is highly dependent on the degree of resident macrophage depletion, the intensity of the inflammation and the difficulty for monocytes to reach the niche. Over time, these Mo-derived AMs might persist in the lung and progressively replace the embryonic tissue-resident AMs (Morales-Nebreda *et al.*, 2015) (**Figure Discussion 5**).

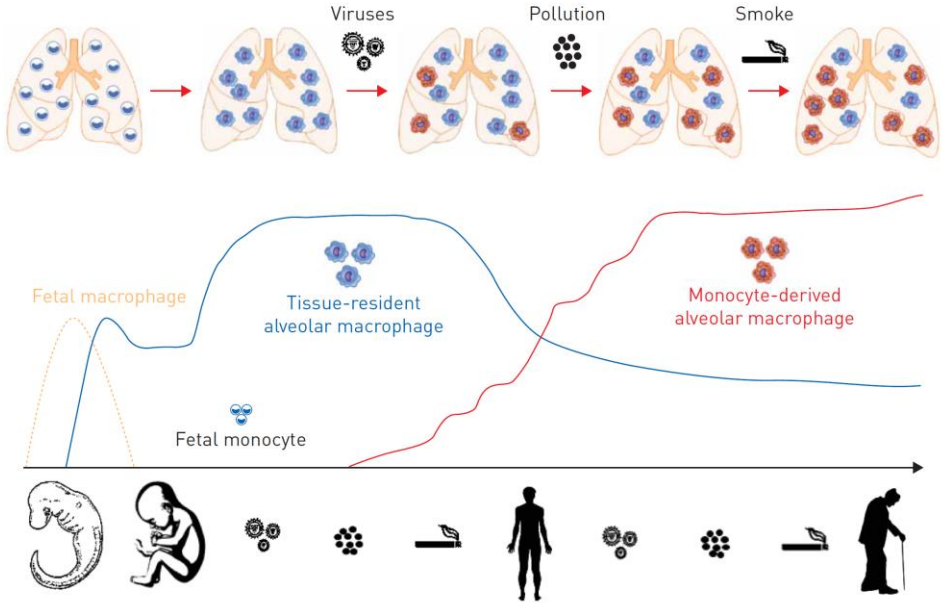


Figure Discussion 5. Lung macrophages and composition through the lifespan.

During embryogenesis, fetal monocyte cells (yellow line) populate the lung and differentiate into AMs, shortly after birth. These long-lived "tissue-resident" AMs (blue line) are capable of self-renewal in homeostatic condition. Various triggers over the lifespan may induce the recruitment of monocytes that differentiate into AMs (red line) and might persist or replace the tissue-resident AMs over time. (Morales-Nebreda et al., 2015).

The study presented in this thesis does not discriminate whether ILC2s particularly affect embryonic macrophages and/or Mo-derived AMs. However, it is a fundamental question as the AMs niche evolves over time with an increasing contribution of BM-monocytes. Therefore, we plan to investigate this question by the following three approaches. First, scRNA-seq data of macrophages at different time points post-infection (Fig. 6) will be refined in order to cluster macrophages into different subpopulations to differentiate, among others, resident AMs and Mo-derived AMs. Then, interactome extrapolation will be repeated, based on the different subpopulations to highlight potential different strengths of interaction with ILC2s. Second, we will refine imaging of lungs obtained from the same experimental layout as Fig. 5F using spectral unmixing imaging. This technique allows to segregate mixed fluorescent signals and more clearly resolve the spatial contribution of different fluorophore. It will offer the possibility to add two antibodies to the previous panel (DAPI, IL-5, CD3 and CD68) (Fig. 5, F-H), CD11c and CD11b, helping to discriminate resident AMs ($CD68^{+}CD11c^{+}$) from Mo-AMs ($CD68^{+}CD11b^{+}$) in the vicinity of pulmonary ILC2s. Third, by FACS analysis, we will carefully compare the phenotypic signature of embryonic AMs *vs* Mo-AMs in presence or absence of lung ILC2s, in the context of TH2 immunity. Preliminary results obtained from littermate or ILC2 deficient mice, submitted to HDM challenge (same experimental layout as Figure Discussion 3) already suggest that Mo-derived AMs are more sensible to the modulation of ILC2s than resident AMs (**Figure Discussion 6**). Indeed, FACS analysis allows the discrimination of embryonic from Mo-AMs based on several surface markers: resident AMs are described as autofluorescent $CD11c^{+}$ $SiglecF^{high}$ living cells and Mo-derived AMs as autofluorescent $CD11c^{+}CD11b^{+}SiglecF^{low}$ living cells (**Figure Discussion 6B**). As expected, HDM challenge induces the increased expression of the M2 markers, RELMα and Arg1, in the two populations. Interestingly, we observed that a lower percentage of Mo-AMs from ILC2s deficient mice expressed RELMα and Arg1, following HDM challenge in comparison with Mo-AMs from littermate controls (**Figure Discussion 6D**), whereas no differences was observed in resident AMs (**Figure Discussion 6C**). It is interesting to add that *ex vivo* co-culture of ILC2s, from mock infected or MuHV-4 infected mice, fails to polarize resident AMs from mock infected mice (data not shown) whereas BM-Monocytes were sensible to ILC2-induced polarization (Fig. 7). Altogether, these results strongly suggest that resident AMs are less sensitive to the ILC2-modulation than Mo-AMs. These preliminary results will have to be confirmed.

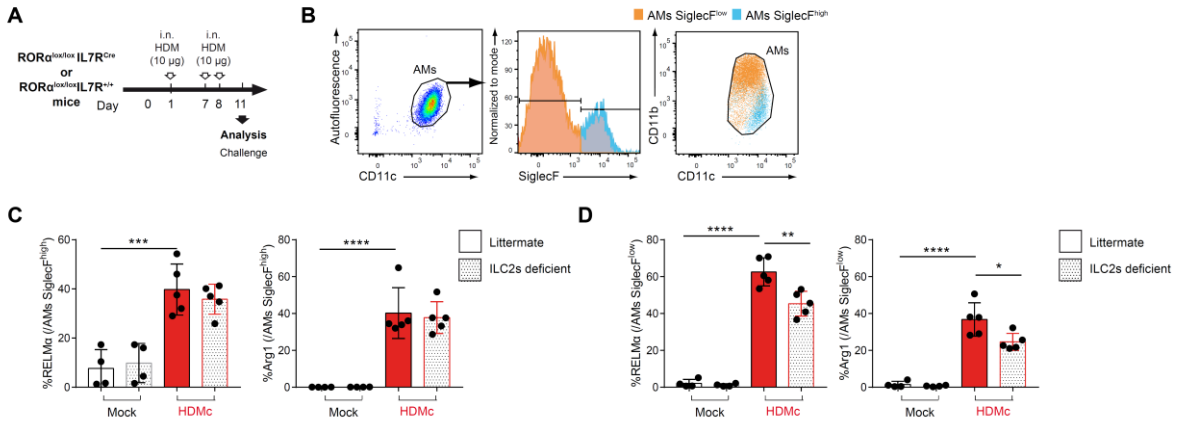


Fig. Discussion 6. ILC2s are essential for the instruction of macrophages and particularly AMs SiglecF^{low}CD11b^{+}.
(**A-D**) 8-week-old- littermate or ILC2s deficient mice (n = 5 in each group) were submitted to low-dose HDM challenge before analysis. (A) Experimental layout. (**B**) Histograms and representative flow cytometry plots of SiglecF expression on AMs. (**C and D**) Percentage of RELMα and Arg1 positive AMs SiglecF^{high} (**C**) and SiglecF^{low} (**D**). 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; **** p<0.0001. Error bars represent SEM.

The potential mechanisms underlying ILC2s-AMs crosstalk

In vitro, ILC2-mediated M2 macrophages polarization is associated with a significant amount of IL-13 (Kim *et al.*, 2019). Here, in addition to the impairment of IL-13 production by ILC2s from MuHV-4 infected mice, we highlighted potential candidate ligands and receptors for ILC2-AM interactions, using interactome extrapolation based on high-throughput data (Fig. 6, J-L). One of the main candidate is GM-CSF (*Csf2*) as its production by ILC2s calibrates macrophage defense and wound healing programs during intestinal infection and inflammation (Castro-Dopico *et al.*, 2020). We also proposed direct interactions through the expression of the receptor/ligand PD-1/PD-L1 in ILC2s and macrophages, respectively. Interestingly, Kim et *al.* had previously proposed the need for direct interaction between these cells to promote macrophage polarization, based on results obtained using PD-1 blocking antibodies and transwell assays (Kim *et al.*, 2019). Using the *ex vivo* co-culture model presented in Fig. 7, we could add anti-PD-1 or anti-GM-CSF blocking antibodies and assess the polarization of macrophages. We could also evaluate the necessity of direct contact by repeating the same model in transwell: monocytes and epithelial cells on one side and ILC2s on the other. *In vivo*, we could deplete the AMs niche of ILC2 deficient mice, transfer ILC2s from mock and MuHV-4 infected mice with BM monocytes and assess the polarization of the Mo-derived macrophages. However, besides being artificial, these approaches are unlikely to work because the maintenance of ILC2s phenotype following MuHV-4 infection requires the microenvironment as shown in Fig. 4, A-C. Therefore, transferring ILC2s from MuHV-4 infected mice into a non-infected organism may attenuate their inhibition.

In addition to IFN-γ-dependent inhibition, sustained **inhibition of ILC2s could also be supported by long-lived Mo-derived AMs.** Indeed, interactome analysis suggested a strong link between the two cell types *via* IL-1β produced by AMs (Fig. 6L). AM-derived IL-1β has already been described to inhibit type 2 inflammation and mucus metaplasia following RV infection by decreasing ILC2s functions (Han *et al.*, 2020). As previously explained, direct interactions were also proposed with

the expression of the receptor/ligand PD-1/PD-L1 in ILC2s and macrophages, respectively. Macrophage reprogramming from M2 towards M1, might increase the expression of PD-L1, which can be transcriptionally activated by STAT3 (Cai *et al.*, 2021). In addition to those two likely candidates, interactome analysis also highlighted other potential interactions (Fig. 6L): *via* IL-18, which is known as mediator of ILC2s plasticity (Silver *et al.*, 2016), *via* ICAM-1 which controls function of ILC2s (Lei *et al.*, 2018), or *via* Itgb1 whose role is ill-defined. Other factors could also be involved such as IFN-α which is produced by activated AMs and decreases the reactivity of ILC2s to IL-33 activation or fungal allergen (She *et al.*, 2021). In order to investigate the role of all of these candidates, we could use blocking antibodies *in vivo* or *ex vivo*, starting with an anti-IL-1β or anti-PD-L1 or against other potential candidates, to see if they partially revert ILC2 inhibition. However, an *in vivo* blockade by neutralizing antibodies will be difficult to assess, as they may block other pathways, and as it is difficult to estimate the most appropriate time of injection, taking into account the duration of the protocol (after infection, after HDM sensitization or after challenge). Studying ILC2s in the absence of macrophages *in vivo* would be challenging. $Csf2rb^{-/-}$ mice mainly lack AMs and $Csf1r^{-/-}$ have markedly reduced numbers of macrophages throughout the body (van de Laar *et al.*, 2016). Moreover, in the absence of niche reconstitution at young age, mice develop alveolar proteinosis characterized by abnormal accumulation of surfactant-derived lipoprotein compounds in the lung alveoli. We could artificially deplete AMs by using clodronate liposomes. However, in addition to a short duration of action, its use induces a strong local inflammation that makes it difficult to identify any specific effect of the AMs depletion. Finally, the best tool could be the use of Cre-lox mice. As a reminder, Mo-derived AMs following MuHV-4 infection express CCR2 (Machiels *et al.*, 2017). Thus, we could imagine assessing the role of PD-L1 or IL-1β expressed by Mo-derived AMs on ILC2s by using $CCR2^{Cre}$ x $PD\text{-}L1^{lox/lox}$ or $CCR2^{Cre}$ x $IL\text{-}1\beta^{lox/lox}$ mice.

Metabolic and epigenetic modifications in the context of long-term inhibition of ILC2s

PD-1 overexpression was observed 8 days post-MuHV-4 infection (Fig. 4K), concomitant to inhibitory profile. PD-1 represents an important immune checkpoint of ILC2s dampening any overactivation. Indeed, using humanized HDM mice, treatment with PD-1 agonist significantly reduced airway hyperreactivity through ILC2 regulation (Helou *et al.*, 2020). Interestingly, PD-1 deficiency in ILC2s is associated with increased glucose demand, driving a significant metabolic shift towards glycolysis and limiting ILC2s proliferation (Helou *et al.*, 2020). Accordingly, several observations in this study suggest a **shift in ILC2s metabolism following MuHV-4 infection**. This is supported by BubbleGUM analysis (Fig. 3G and Fig. 6, F and I), where genesets of cellular aerobic respiration and energy processing were enriched in ILC2s from mock infected mice, compared to MuHV-4 infected mice, following or not HDM treatment. The expression of *Arg1*, encoding the protein arginase, was also impacted following infection (Fig. 6E). *Arg1* expression is upregulated in respiratory distress and

represents an intrinsic metabolic checkpoint controlling type 2 inflammation, since its deletion in ILCs abrogated type 2 lung inflammation by decreasing ILC2s proliferation and activation (Monticelli *et al.*, 2016). Mechanistically, inhibition of Arg1 enzymatic activity disrupted several components of ILC2s metabolic programming by altering arginine catabolism, impairing polyamine biosynthesis and reducing aerobic glycolysis (Monticelli *et al.*, 2016). All these observations may therefore suggest a decrease in aerobic respiration of ILC2s following infection that may result in impairment of their functions. Several experiments could be considered to investigate this hypothesis. Inhibitors of the oxidative phosphorylation system (OXPHOS), such as oligomycin, could be injected in mock infected mice in order to mimic the altered function observed in ILC2s from MuHV-4 infected mice. Conversely, an OXPHOS activator, such as FCCP or BAM15, could be used to, at least partially, abrogate the inhibition observed in ILC2s from MuHV-4 infected mice. These experiments would allow to confirm the presence or not of a metabolic shift in ILC2s after viral infection. Moreover, *in vitro* techniques such as Seahorse™ could be considered to obtain more detailed data like OCR (oxidation consumption rate) and ECAR (extracellular acidification rate).

There is considerable evidence showing that epigenetic and metabolic alterations are closely intertwined. Profound modifications in intracellular metabolism are associated with long-term phenotypic changes linked to epigenetic reprogramming (Riksen and Netea, 2021). The inhibition of type 2 properties of ILC2s following MuHV-4 infection seems to persist at least 3 months post-infection (data not show), suggesting the acquisition of **a lasting inhibition of the type 2 properties**. In ILC2s, manipulation of glycolysis, using PKM2-pyruvate that catalyzes the conversion from phosphoenolpyruvate to pyruvate, critically modulates the expression of IL-33 receptor - ST2 - and subsequent homeostasis of ILC2s through epigenetic modification (Q. Li *et al.*, 2018). An altered expression of ST2 has been observed following MuHV-4 infection (Fig. 3, I and J, and Fig. 6E), suggesting metabolism reprogramming as well as epigenetic modification. Since epigenetic regulation is reversible, understanding the precise mechanisms driving them could help identifying potential targets for epigenetic therapies. It would therefore be interesting to analyze the modifications of ILC2s by MuHV-4 infection at the epigenetic level. Verma et *al*. have recently investigated the molecular and driving innate lymphoid cell memory and its relevance for asthma, using among others, Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) technology (Verma *et al.*, 2021). ATAC-seq allows to assess genome-wide chromatin accessibility and highlights packaging nucleosome and regulatory regions. We have already obtained preliminary ATAC-seq results on sorted lung ILC2 from mock or HDM sensitized mice. These results are encouraging as they show changes in accessibility of IL-5, KLRG1 and PD-1 peaks following HDM treatment (**Figure Discussion 7**), correlating with FACS analysis (Fig. 3, F and H). It would therefore be interesting to repeat this ATAC-seq analysis on ILC2s isolated from mock or MuHV-4 infected mice, treated or not with HDM, and to also include later

time point post-infection to identify any potential acquisition of memory properties associated with epigenetic changes.

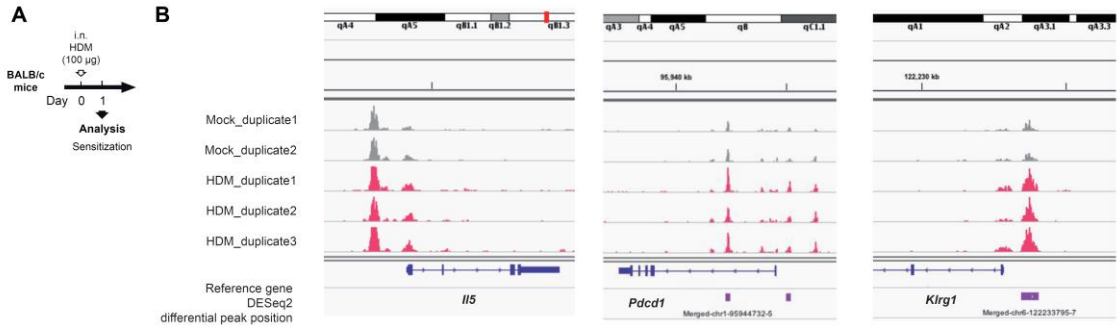


Fig. Discussion 7. ATAC-seq analysis on ILC2s from mock and HDM sensitized mice.
(**A**) 8-week-old BALB/c mice were submitted or not to high-dose HDM sensitization, before ATAC-sequencing analysis of ILC2s. (**B**) DNA accessibility tracing of three representative genes : IL-5 (*Il5*), PD-1 (*Pdcd1*) and KLRG1 (*Klrg1*).

3) The functional importance of ILC2s in the context of different viral infections

As detailed in the introduction, ILC2s **maintain lung epithelial integrity** following acute influenza infection, in a Areg-dependent manner (Monticelli *et al.*, 2012). Similarly, after MuHV-4 infection, transcriptomic analysis revealed an increase of *Areg* expression 5 days post-infection (Fig. 6E). This hypothesis is supported by the fact that ILC2 deficient mice exhibit more severe clinical signs following MuHV-4 infection revealed by a higher weight loss (data not shown). This homeostatic role of ILC2s has also been suggested in COVID-19 where patients with a severe form of the disease showed ILC2 deficiency associated with severe epithelial impairment (García *et al.*, 2020; Harb *et al.*, 2021). Understanding the protective mechanisms of ILC2s avoiding barrier dysfunction, during viral infection (latent or not) is therefore of major interest.

The study presented in this thesis does not investigate whether **ILC2 modulation is the appanage of MuHV-4 infection**. Previously, RSV infection had been reported to prevent subsequent development of OVA-induced allergic responses by inhibiting ILC2 *via* the attenuation of IL-33/ST2 axis (Wang *et al.*, 2018). It is interesting to point out that RSV is mainly described as a virus inducing a pro-TH2 immune response and remains one of the main triggers of asthma exacerbation (See: Introduction: 1.4.1. Viral infections exacerbating asthma) (Stier *et al.*, 2016). In addition to the data presented in the result section, we compared the effect of other respiratory viral infections on the lung population of ILC2s. In a pilot experiment, we infected intranasally BALB/c mice with various respiratory viruses, namely the adenovirus MAV-1, the influenza virus PR8 strain and PVM (a mouse model of RSV). 8 days post-infection, a decreased production of IL-13 associated to higher expression of PD-1 in ILC2s was only observed in mice infected with MuHV-4 (**Figure Discussion 8 A-C**). Significant protection against HDM challenge is again only associated to MuHV-4 infection, including decreased of BALF eosinophils, total ILC2 numbers and IL-5 production by these cells (**Figure**

Discussion 8 D-G). Interestingly, MAV-1 infection also decreased IL-5 production by ILC2s. However, MAV-1 infected mice did not display a significant reduction in BALF eosinophils. MAV-1 is also a double-stranded DNA virus establishing a chronic infection which can efficiently modulate innate and adaptive immunity (Goffin *et al.*, 2019; Santos, Havunen and Hemminki, 2020). Those preliminary results confirm that MuHV-4 infection induces specific changes in lung ILC2s. However, they remain incomplete and additional analyses are needed to better characterize the underlying immune response against each viral infection.

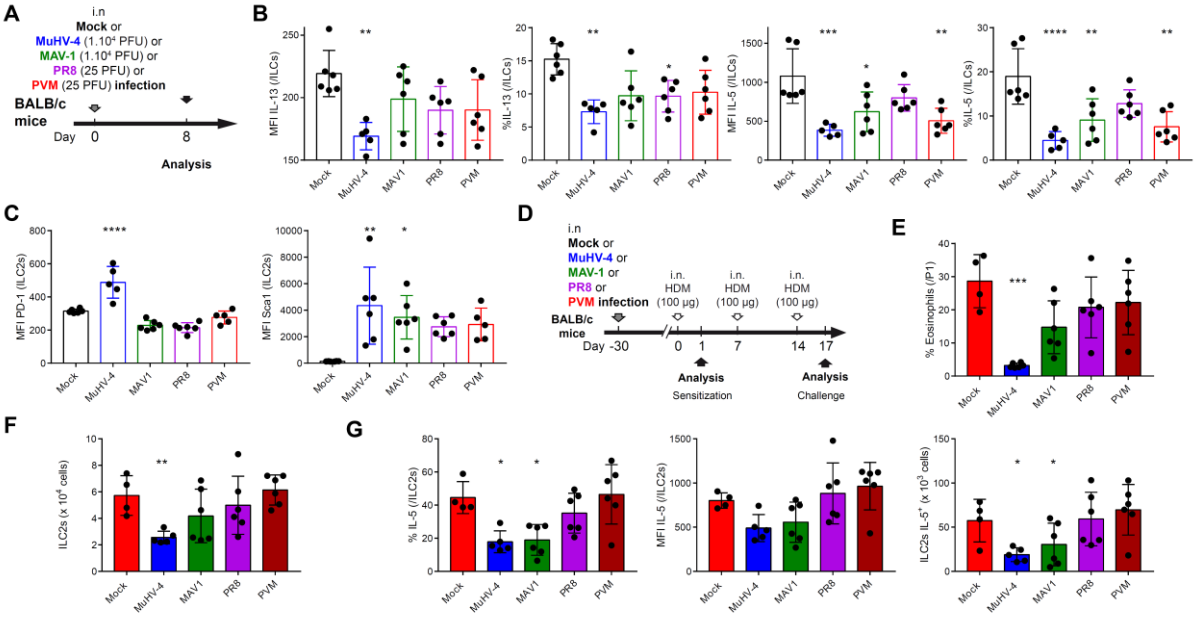


Fig. Discussion 8. Other respiratory viral infections did not induce the same phenotype of lung ILC2s as the one following MuHV-4 infection.
(**A-C**) BALB/c mice were sacrificed 8 days after infection or not with MuHV-4, MAV-1, PR8 and PVM at the indicated doses. (**A**) Experimental layout. (**B**) Quantification of cytokines production by lung ILC2s following different indicated infections. (**C**) PD-1 and Sca1 expression in lung ILC2s following the indicated infections. (**D-E**) BALB/c mice were infected with the indicated viruses and then submitted to a HDM high-dose protocol. (**D**) Experimental layout. (**E**) Quantification of eosinophils in BALF analysis. (**F**) Enumeration of lung ILC2s. (**G**) Quantification of lung IL-5 positive ILC2s. Data were analyzed by 1way-ANOVA and Bonferroni posttests * p<0,05; ** p<0,01; *** p<0,001; **** p<0,0001. Error bars represent SEM.

4) The importance of the ILC2s-AMs crosstalk throughout the body

To conclude this discussion, ILC2s and macrophages are two sentinel cells with an incredible capacity to adapt to their environment (Lavin *et al.*, 2014; Bennstein and Uhrberg, 2021) and obviously, **their crosstalk could help to maintain essential tissue homeostasis**, as already described. Indeed, after unilateral pneumectomy, ILC2s and recruited monocytes modulate the lung microenvironment to support alveolar epithelial stem cell proliferation and tissue regeneration (Lechner *et al.*, 2017). More broadly, GM-CSF, IL-4 and IL-13 produced by ILC2s can promote an alternatively activated macrophage phenotype that is essential in regulating adipocyte differentiation, lung tissue fibrosis and anti-helminth immunity (Li *et al.*, 2014; M. W. Lee *et al.*, 2015). Through IL-4 production, ILC2s polarize M2-like macrophage, contributing to expansion of regulatory T cell subset, helping to establish disease resistance in a murine model of experimental malaria (Besnard *et al.*, 2015).

However, their crosstalk might probably also lead to adverse effects and the manipulation of macrophage niche cells could offer **therapeutic opportunities**. For example, pro-tumoral properties of macrophages have been recently demonstrated. They accumulate close to tumor cells and play a critical role in early progression by promoting regulatory T cells that protect tumor cells from $CD8^+$ T cells destruction (Casanova-Acebes *et al.*, 2021)**.** In general, the role of ILC2s remains rather ambiguous regarding their antitumor activity. They are described in various tumor tissues and generally promote tumor progression by acting on microenvironment via IL-13 and IL-5 production and creating an overall pro-TH2 environment (Ercolano *et al.*, 2019). Interestingly, Shen et *al*. showed that ILC2s, in human small-cell lung carcinoma, upregulate PD-1 expression and negatively act as a positive regulator of immunosuppressive function by the polarization of M2-macrophage through IL-4 and IL-13 production (Shen *et al.*, 2021). This latest study remarkably reveals that ILC2s-dependent macrophages polarization may have deleterious effect by amplifying pro-tumor properties, supporting the need to better understand the mechanisms governing this interaction. Nevertheless, anti-tumor properties have also been described for ILC2s. They are abundantly found in colorectal cancer tissue where derived IL-9 could activate $CD8^+$T cells to inhibit tumor growth (Wan *et al.*, 2021). Importantly, antibody-mediated PD-1 blockade relieves ILC2s-intrinsic PD-1 inhibition to expand tumor-ILC2s that increase anti-tumor immunity and enhance tumor control, identifying ILC2s as positive amplifiers of anti-PD-1 immunotherapy (Moral *et al.*, 2020). The ambivalent contribution of ILC2s in tumor environment shows, once again, how important it is to decipher the regulation of those cells and their interaction with macrophages, which may offer new perspectives in immunotherapy.

Altogether, the work presented in this thesis dissects the complex role of ILC2s in the context of γHV immune imprinting. Importantly, it reveals the critical importance of ILC2s in conferring long-term AMs identity during niche replenishment following viral infection, prior to allergic asthma. Beyond the context of γHV infection, these results may help to clarify the innate cell-cell circuit that delicately maintain homeostasis throughout the body.

References

Adler, H. *et al.* (2000) ‘Cloning and mutagenesis of the Murine Gammaherpesvirus 68 Genome as an Infectious Bacterial Artificial Chromosome’, *Journal of Virology*, 74(15), pp. 6964–6974. doi: 10.1128/JVI.74.15.6964-6974.2000.

Ahmad, S. H., Assaf, M. D. and Herbein, G. (2021) ‘L’épigénétique, une nouvelle cible thérapeutique lors des infections virales’, *Virologie (Montrouge, France)*, pp. 63–92. doi: 10.1684/vir.2021.0876.

Akama, Y. *et al.* (2020) ‘The role of innate lymphoid cells in the regulation of immune homeostasis in sepsis-mediated lung inflammation’, *Diagnostics*. doi: 10.3390/diagnostics10100808.

Altman, M. C. *et al.* (2019) ‘Transcriptome networks identify mechanisms of viral and nonviral asthma exacerbations in children’, *Nature Immunology*, 20(5), pp. 637–651. doi: 10.1038/s41590-019-0347-8.

An, G. *et al.* (2020) ‘Combined blockade of IL-25, IL-33 and TSLP mediates amplified inhibition of airway inflammation and remodelling in a murine model of asthma’, *Respirology*, 25(6), pp. 603–612. doi: 10.1111/RESP.13711.

Anandan, C. *et al.* (2010) ‘Is the prevalence of asthma declining? Systematic review of epidemiological studies’, *Allergy*, 65(2), pp. 152–167. doi: 10.1111/J.1398-9995.2009.02244.X.

Angkasekwinai, P. *et al.* (2017) ‘ILC2s activated by IL-25 promote antigen-specific Th2 and Th9 functions that contribute to the control of Trichinella spiralis infection’, *PLoS ONE*, 12(9). doi: 10.1371/journal.pone.0184684.

Ardain, A. *et al.* (2019) ‘Type 3 ILCs in lung disease’, *Frontiers in Immunology*, p. 92. doi: 10.3389/fimmu.2019.00092.

Artis, D. and Spits, H. (2015) ‘The biology of innate lymphoid cells’, *Nature*, 517(7534), pp. 293–301. doi: 10.1038/nature14189.

Azim, A. *et al.* (2021) ‘Severe acute respiratory syndrome coronavirus 2 infection in those on mepolizumab therapy’, *Annals of Allergy, Asthma & Immunology*, 126(4), p. 438. doi: 10.1016/J.ANAI.2021.01.006.

Bal, S. M. *et al.* (2016) ‘IL-1β, IL-4 and IL-12 control the fate of group 2 innate lymphoid cells in human airway inflammation in the lungs’, *Nature Immunology*, 17(6), pp. 636–645. doi: 10.1038/ni.3444.

Balhara, J. and Gounni, A. S. (2012) ‘The alveolar macrophages in asthma: A double-edged sword’, *Mucosal Immunology*. Nature Publishing Group, pp. 605–609. doi: 10.1038/mi.2012.74.

Bando, J. K., Liang, H.-E. and Locksley, R. M. (2015) 'Identification and distribution of developing innate lymphoid cells in the fetal mouse intestine', *Nature Immunology*, 16(2), pp. 153–160. doi: 10.1038/ni.3057.

Barton, E., Mandal, P. and Speck, S. H. (2011) 'Pathogenesis and Host Control of Gammaherpesviruses: Lessons from the Mouse', *Annual Review of Immunology*, 29(1), pp. 351–397. doi: 10.1146/annurev-immunol-072710-081639.

Barton, E. S. *et al.* (2007) 'Herpesvirus latency confers symbiotic protection from bacterial infection', *Nature*, 447(7142), pp. 326–329. doi: 10.1038/nature05762.

Beale, J. *et al.* (2014) 'Rhinovirus-induced IL-25 in asthma exacerbation drives type 2 immunity and allergic pulmonary inflammation', *Science Translational Medicine*, 6(256), pp. 256ra134-256ra134. doi: 10.1126/SCITRANSLMED.3009124.

Bennstein, S. B. and Uhrberg, M. (2021) 'Biology and therapeutic potential of human innate lymphoid cells', *FEBS Journal*. Blackwell Publishing Ltd. doi: 10.1111/febs.15866.

Bergroth, E. *et al.* (2020) 'Rhinovirus Type in Severe Bronchiolitis and the Development of Asthma', *Journal of Allergy and Clinical Immunology*, 8(2), pp. 588-595.e4. doi: 10.1016/j.jaip.2019.08.043.

Besnard, A.-G. *et al.* (2015) 'IL-33-Mediated Protection against Experimental Cerebral Malaria Is Linked to Induction of Type 2 Innate Lymphoid Cells, M2 Macrophages and Regulatory T Cells', *PLoS Pathogens*, 11(2). doi: 10.1371/JOURNAL.PPAT.1004607.

Beuraud, C. *et al.* (2019) 'CCR10 + ILC2s with ILC1-like properties exhibit a protective function in severe allergic asthma', *Allergy*, 74(5), pp. 933–943. doi: 10.1111/all.13679.

Bigalke, J. M. and Heldwein, E. E. (2016) 'Nuclear Exodus: Herpesviruses Lead the Way', *Annual review of virology*, 3(1), p. 387. doi: 10.1146/ANNUREV-VIROLOGY-110615-042215.

Bird, L. (2016) 'Immune memory: ILC2s drive allergen recall', *Nature Reviews Immunology*, 16(2), p. 72. doi: 10.1038/nri.2016.10.

Blake, N. (2010) 'Immune evasion by gammaherpesvirus genome maintenance proteins', *Journal of General Virology*, pp. 829–846. doi: 10.1099/vir.0.018242-0.

Blaskovic, D. *et al.* (1980) 'Isolation of five strains of herpesviruses from two species of free living small rodents.', *Acta Virologica*, p. 468.

Boberg, E., Johansson, K., Malmhäll, C., Weidner, J., *et al.* (2020) 'House dust mite induces bone marrow il-33responsive ILC2S and TH cells', *International Journal of Molecular Sciences*, 21(11). doi: 10.3390/ijms21113751.

Boberg, E., Johansson, K., Malmhäll, C., Calvén, J., *et al.* (2020) 'Interplay Between the IL-33/ST2 Axis and Bone Marrow ILC2s in Protease Allergen-Induced IL-5-Dependent Eosinophilia', *Frontiers in Immunology*, 11, p. 1058. doi: 10.3389/fimmu.2020.01058.

Boelen, A. *et al.* (2002) 'Effect of lack of Interleukin-4, Interleukin-12, Interleukin-18, or the Interferon-γ receptor on virus replication, cytokine response, and lung pathology during respiratory syncytial virus infection in mice', *Journal of Medical Virology*, 66(4), pp. 552–560. doi: 10.1002/jmv.2180.

Bohmwald, K. *et al.* (2018) 'Neurologic Alterations Due to Respiratory Virus Infections', *Frontiers in Cellular Neuroscience*, 12(386). doi: 10.3389/fncel.2018.00386.

Bosteels, C. *et al.* (2020) 'Inflammatory Type 2 cDCs Acquire Features of cDC1s and Macrophages to Orchestrate Immunity to Respiratory Virus Infection', *Immunity*, 52(6), pp. 1039-1056.e9. doi: 10.1016/j.immuni.2020.04.005.

Brar, G. *et al.* (2020) 'Deletion of immune evasion genes provides an effective vaccine design for tumor-associated herpesviruses', *Vaccines*, 5(1). doi: 10.1038/s41541-020-00251-x.

Bray, F. *et al.* (2018) 'Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries', *CA: a cancer journal for clinicians*, 68(6), pp. 394–424. doi: 10.3322/CAAC.21492.

Bruchard, M. and Ghiringhelli, F. (2019) 'Deciphering the roles of innate lymphoid cells in cancer', *Frontiers in Immunology*. doi: 10.3389/fimmu.2019.00656.

Bullard, W. L. *et al.* (2019) 'Identification of murine gammaherpesvirus 68 miRNA-mRNA hybrids reveals miRNA target conservation among gammaherpesviruses including host translation and protein modification machinery', *PLOS Pathogens*, 15(8), p. e1007843. doi: 10.1371/journal.ppat.1007843.

Buonocore, S. *et al.* (2010) 'Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology', *Nature*, 464(7293), pp. 1371–1375. doi: 10.1038/nature08949.

Busse, W. W., Lemanske, R. F. and Gern, J. E. (2010) 'Role of viral respiratory infections in asthma and asthma exacerbations', *The Lancet*. Elsevier Ltd, 376(9743), pp. 826–834. doi: 10.1016/S0140-6736(10)61380-3.

Cai, H. *et al.* (2021) 'Defects in Macrophage Reprogramming in Cancer Therapy: The Negative Impact of PD-L1/PD-1', *Frontiers in Immunology*, 12, p. 690869. doi: 10.3389/fimmu.2021.690869.

Califano, D. *et al.* (2018) 'IFN-γ increases susceptibility to influenza A infection through suppression of group II innate lymphoid cells', *Mucosal Immunology*, 11(1), pp. 209–219. doi: 10.1038/mi.2017.41.

Camelo, A. *et al.* (2017) 'IL-33, IL-25, and TSLP induce a distinct phenotypic and activation profile in human type 2 innate lymphoid cells', *Blood Advances*, 1(10), pp. 577–589. doi: 10.1182/bloodadvances.2016002352.

Cañas, J. A. *et al.* (2018) 'Eosinophil-derived exosomes contribute to asthma remodelling by activating structural lung cells', *Clinical and Experimental Allergy*, 48(9), pp. 1173–1185. doi: 10.1111/cea.13122.

Casanova-Acebes, M. *et al.* (2021) 'Tissue-resident macrophages provide a pro-tumorigenic niche to early NSCLC cells', *Nature*, 595. doi: 10.1038/s41586-021-03651-8.

Castro-Dopico, T. *et al.* (2020) 'GM-CSF Calibrates Macrophage Defense and Wound Healing Programs during Intestinal Infection and Inflammation', *Cell Reports*, 32(1). doi: 10.1016/j.celrep.2020.107857.

Cayrol, C. *et al.* (2018) 'Environmental allergens induce allergic inflammation through proteolytic maturation of IL-33', *Nature Immunology*, 19(4), pp. 375–385. doi: 10.1038/s41590-018-0067-5.

Cayrol, C. and Girard, J. P. (2014) 'IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy', *Current opinion in immunology*, 31, pp. 31–37. doi: 10.1016/J.COI.2014.09.004.

Cevhertas, L. *et al.* (2020) 'Advances and recent developments in asthma in 2020', *Allergy: European Journal of Allergy and Clinical Immunology*, 75(12), pp. 3124–3146. doi: 10.1111/ALL.14607.

Chabay, P. *et al.* (2020) 'Lymphotropic Viruses EBV, KSHV and HTLV in Latin America: Epidemiology and Associated Malignancies. A Literature-Based Study by the RIAL-CYTED', *Cancers*, 12, p. 2166. doi: 10.3390/cancers12082166.

Chang, Y.-J. *et al.* (2011) 'Innate lymphoid cells mediate influenza-induced airway hyper-reactivity independently of adaptive immunity', *Nature Immunology*, 12(7), pp. 631–638. doi: 10.1038/ni.2045.

Chen, S. *et al.* (2019) 'High-mobility group box-1 protein from cc10 - club cells promotes type 2 response in the later stage of respiratory syncytial virus infection', *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 316(1), pp. L280–L290. doi: 10.1152/ajplung.00552.2017.

Chen, W. *et al.* (2008) 'Gender difference, sex hormones, and immediate type hypersensitivity reactions', *Allergy*, 63(11), pp. 1418–1427. doi: 10.1111/J.1398-9995.2008.01880.X.

Cheng, D. *et al.* (2014) 'Epithelial interleukin-25 is a key mediator in Th2-high, corticosteroid-responsive asthma', *American Journal of Respiratory and Critical Care Medicine*, 190(6), pp. 639–648. doi: 10.1164/rccm.201403-0505OC.

Cherrier, D. E., Serafini, N. and Di Santo, J. P. (2018) 'Innate Lymphoid Cell Development: A T Cell Perspective.', *Immunity*, 48(6), pp. 1091–1103. doi: 10.1016/j.immuni.2018.05.010.

Choi, Y. *et al.* (2020) 'Eosinophil extracellular traps activate type 2 innate lymphoid cells through stimulating airway epithelium in severe asthma', *Allergy: European Journal of Allergy and Clinical Immunology*, 75(1), pp. 95–103. doi: 10.1111/all.13997.

Choreño-Parra, J. A. *et al.* (2021) 'Clinical and Immunological Factors That Distinguish COVID-19 From Pandemic Influenza A(H1N1)', *Frontiers in Immunology*, 12. doi: 10.3389/fimmu.2021.593595.

Christianson, C. A. *et al.* (2015) 'Persistence of asthma requires multiple feedback circuits involving type 2 innate lymphoid cells and IL-33', *Journal of Allergy and Clinical Immunology*, 136(1), pp. 59-68.e14. doi: 10.1016/j.jaci.2014.11.037.

Claudio, E. *et al.* (2019) 'IL-25 orchestrates activation of Thelper cells via conventional dendritic cells in tissue to exacerbate chronic HDM-induced asthma pathology', *Journal of immunology*, 203(8), p. 2319. doi: 10.4049/JIMMUNOL.1900254.

Cohn, L. *et al.* (1997) 'Induction of airway mucus production by T helper 2 (Th2) cells: A critical role for interleukin 4 in cell recruitment but not mucus production', *Journal of Experimental Medicine*, 186(10), pp. 1737–1747. doi: 10.1084/jem.186.10.1737.

Corren, J. *et al.* (2011) 'Lebrikizumab Treatment in Adults with Asthma', *New England Journal of Medicine*, 365(12), pp. 1088–1098. doi: 10.1056/NEJMoa1106469.

Coverstone, A. M., Wang, L. and Sumino, K. (2019) 'Beyond Respiratory Syncytial Virus and Rhinovirus in the Pathogenesis and Exacerbation of Asthma: The Role of Metapneumovirus, Bocavirus and Influenza Virus', *Immunology and Allergy Clinics of North America*, pp. 391–401. doi: 10.1016/j.iac.2019.03.007.

Dahlgren, M. W. *et al.* (2019) 'Adventitial Stromal Cells Define Group 2 Innate Lymphoid Cell Tissue Niches', *Immunity*, 50(3), pp. 707-722.e6. doi: 10.1016/j.immuni.2019.02.002.

Davison, A. J. (2010) ‘Herpesvirus systematics’, *Veterinary microbiology*, 143(1), pp. 52–69. doi: 10.1016/J.VETMIC.2010.02.014.

Denlinger, L. C. *et al.* (2017) ‘Inflammatory and comorbid features of patients with severe asthma and frequent exacerbations’, *American Journal of Respiratory and Critical Care Medicine*, 195(3), pp. 302–313. doi: 10.1164/rccm.201602-0419OC.

Denney, L. *et al.* (2015) ‘Pulmonary Epithelial Cell-Derived Cytokine TGF-β1 Is a Critical Cofactor for Enhanced Innate Lymphoid Cell Function’, *Immunity*, 43, pp. 945–958. doi: 10.1016/j.immuni.2015.10.012.

Dierick, B. J. H. *et al.* (2020) ‘Burden and socioeconomics of asthma, allergic rhinitis, atopic dermatitis and food allergy’, *Expert review of pharmacoeconomics & outcomes research*, 20(5), pp. 437–453. doi: 10.1080/14737167.2020.1819793.

Doherty, T. A. *et al.* (2013) ‘Lung type 2 innate lymphoid cells express cysteinyl leukotriene receptor 1, which regulates TH2 cytokine production’, *Journal of Allergy and Clinical Immunology*, 132(1), pp. 205–213. doi: 10.1016/j.jaci.2013.03.048.

Döhner, K. and Sodeik, B. (2005) ‘The role of the cytoskeleton during viral infection’, *Current topics in microbiology and immunology*, 285, pp. 67–108. doi: 10.1007/3-540-26764-6_3.

Dourcy, M. *et al.* (2020) ‘A gammaherpesvirus licenses CD8 T cells to protect the host from pneumovirus-induced immunopathologies’, *Mucosal Immunology*, 13(5), pp. 799–813. doi: 10.1038/s41385-020-0293-7.

Drake, L. Y. *et al.* (2016) ‘Group 2 Innate Lymphoid Cells Promote an Early Antibody Response to a Respiratory Antigen in Mice’, *The Journal of Immunology*, 197(4), pp. 1335–1342. doi: 10.4049/jimmunol.1502669.

Duerr, C. U. and Fritz, J. H. (2016) ‘Regulation of group 2 innate lymphoid cells’, *Cytokine*, 87, pp. 1–8. doi: 10.1016/j.cyto.2016.01.018.

Dunican, E. M. *et al.* (2018) ‘Mucus plugs in patients with asthma linked to eosinophilia and airflow obstruction’, *Journal of Clinical Investigation*, 128(3), pp. 997–1009. doi: 10.1172/JCI95693.

Dutertre, C. A. *et al.* (2019) ‘Single-Cell Analysis of Human Mononuclear Phagocytes Reveals Subset-Defining Markers and Identifies Circulating Inflammatory Dendritic Cells’, *Immunity*, 51(3), pp. 573-589.e8. doi: 10.1016/J.IMMUNI.2019.08.008.

Dutton, E. E. *et al.* (2019) 'Peripheral lymph nodes contain migratory and resident innate lymphoid cell populations', *Science Immunology*, 4(35). doi: 10.1126/sciimmunol.aau8082.

Van Dyken, S. J. *et al.* (2016) 'A tissue checkpoint regulates type 2 immunity', *Nature Immunology*, 17(12), pp. 1381–1387. doi: 10.1038/ni.3582.

Ebbo, M. *et al.* (2017) 'Innate lymphoid cells: Major players in inflammatory diseases', *Nature Reviews Immunology*. Nature Publishing Group, 17(11), pp. 665–678. doi: 10.1038/nri.2017.86.

Eberl, G., Di Santo, J. P. and Vivier, E. (2015) 'The brave new world of innate lymphoid cells', *Nature Immunology*, 16(1), pp. 1–5. doi: 10.1038/ni.3059.

Ebihara, T. and Taniuchi, I. (2019) 'Exhausted-like Group 2 Innate Lymphoid Cells in Chronic Allergic Inflammation.', *Trends in immunology*, 40(12), pp. 1095–1104. doi: 10.1016/j.it.2019.10.007.

Elieh Ali Komi, D. and Bjermer, L. (2019) 'Mast Cell-Mediated Orchestration of the Immune Responses in Human Allergic Asthma: Current Insights', *Clinical Reviews in Allergy and Immunology*, pp. 234–247. doi: 10.1007/s12016-018-8720-1.

Ercolano, G. *et al.* (2019) 'ILC2s: New Actors in Tumor Immunity', *Frontiers in Immunology*. doi: 10.3389/fimmu.2019.02801.

Fahy, J. V. (2014) 'Type 2 inflammation in asthma — present in most, absent in many', *Nature Reviews Immunology*, 15(1), pp. 57–65. doi: 10.1038/nri3786.

Feldman, E. R. *et al.* (2014) 'Virus-encoded microRNAs facilitate gammaherpesvirus latency and pathogenesis in vivo', *mBio*, 5(3). doi: 10.1128/mBio.00981-14.

Ferreira, A. *et al.* (2002) 'Rhinovirus is associated with severe asthma exacerbations and raised nasal interleukin-12', *Respiration*, 69(2), pp. 136–142. doi: 10.1159/000056316.

Ferreira, D. S. *et al.* (2018) 'Airway pathology in severe asthma is related to airflow obstruction but not symptom control', *Allergy: European Journal of Allergy and Clinical Immunology*, 73(3), pp. 635–643. doi: 10.1111/all.13323.

Flaño, E. *et al.* (2000) 'Latent murine gamma-herpesvirus infection is established in activated B cells, dendritic cells, and macrophages', *Journal of immunology*, 165(2), pp. 1074–1081. doi: 10.4049/JIMMUNOL.165.2.1074.

Fonseca, W. *et al.* (2021) 'Role of ILC2 in Viral-Induced Lung Pathogenesis', *Frontiers in Immunology*.

doi: 10.3389/fimmu.2021.675169.

Forsberg, A. *et al.* (2014) 'GATA binding protein 3+ group 2 innate lymphoid cells are present in cord blood and in higher proportions in male than in female neonates', *Journal of Allergy and Clinical Immunology*, 134(1), pp. 228-230.e2. doi: 10.1016/J.JACI.2014.01.027.

Fowler, P. *et al.* (2003) 'ORF73 of murine herpesvirus-68 is critical for the establishment and maintenance of latency', *Journal of General Virology*, 84(12), pp. 3405–3416. doi: 10.1099/vir.0.19594-0.

François, S. *et al.* (2010) 'Illumination of Murine Gammaherpesvirus-68 Cycle Reveals a Sexual Transmission Route from Females to Males in Laboratory Mice', *PLoS Pathogens*, 9(4), pp. e100329–e1003292. doi: 10.1371/journal.ppat.1003292.

Friborg, J. *et al.* (1999) 'p53 Inhibition by the LANA protein of KSHV protects against cell death', *Nature*, 402(6764), pp. 889–894. doi: 10.1038/47266.

Gadani, S. P. *et al.* (2012) 'Interleukin-4: A Cytokine to Remember', *Journal of immunology*, 189(9), p. 4213. doi: 10.4049/JIMMUNOL.1202246.

García, M. *et al.* (2020) 'Innate lymphoid cell composition associates with COVID-19 disease severity', *Clinical and Translational Immunology*, 9(12). doi: 10.1002/cti2.1224.

Gaspar, M. *et al.* (2011) 'Murid Herpesvirus-4 Exploits Dendritic Cells to Infect B Cells', *PLoS Pathogens*. Edited by S. H. Speck. Public Library of Science, 7(11), p. e1002346. doi: 10.1371/journal.ppat.1002346.

Gasteiger, G. *et al.* (2015) 'Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs', *Science*, 350(6263), pp. 981–985. doi: 10.1126/science.aac9593.

Gauvreau, G. M. *et al.* (2014) 'Effects of an Anti-TSLP Antibody on Allergen-Induced Asthmatic Responses', *New England Journal of Medicine*, 370(22), pp. 2102–2110. doi: 10.1056/NEJMoa1402895.

Ghaedi, M. *et al.* (2016) 'Common-Lymphoid-Progenitor-Independent Pathways of Innate and T Lymphocyte Development', *Cell Reports*, 15(3), pp. 471–480. doi: 10.1016/J.CELREP.2016.03.039.

Ghaedi, M. *et al.* (2020) 'Single-cell analysis of RORα tracer mouse lung reveals ILC progenitors and effector ILC2 subsets.', *The Journal of experimental medicine*, 217(3). doi: 10.1084/jem.20182293.

Giffin, L. and Damania, B. (2014) 'KSHV: Pathways to Tumorigenesis and Persistent Infection Malignancies and Syndromes Linked with KSHV Infection', *Advances in Virus Research*, 88, pp. 111–159. doi: 10.1016/B978-0-12-800098-4.00002-7.

Gillet, L. *et al.* (2007) 'The murine gammaherpesvirus-68 gp150 acts as an immunogenic decoy to limit virion neutralization', *PLoS ONE*, 2(8), p. e705. doi: 10.1371/journal.pone.0000705.

Gillet, L. *et al.* (2009) 'Glycoprotein L sets the neutralization profile of murid herpesvirus 4', *Journal of General Virology*, 90(5), pp. 1202–1214. doi: 10.1099/vir.0.008755-0.

Gillet, L., Frederico, B. and Stevenson, P. G. (2015) 'Host entry by gamma-herpesviruses — lessons from animal viruses?', *Current Opinion in Virology*, 15, pp. 34–40. doi: 10.1016/J.COVIRO.2015.07.007.

Gillet, L. and Stevenson, P. G. (2007) 'Antibody evasion by the N terminus of murid herpesvirus-4 glycoprotein B', *EMBO Journal*, 26(24), pp. 5131–5142. doi: 10.1038/sj.emboj.7601925.

Girodet, P.-O. *et al.* (2016) 'Alternative Macrophage Activation Is Increased in Asthma.', *American journal of respiratory cell and molecular biology*, 55(4), pp. 467–475. doi: 10.1165/rcmb.2015-0295OC.

Godar, M. *et al.* (2018) 'A bispecific antibody strategy to target multiple type 2 cytokines in asthma', *Journal of Allergy and Clinical Immunology*, 142(4), pp. 1185-1193.e4. doi: 10.1016/j.jaci.2018.06.002.

Goffin, E. *et al.* (2019) 'Oral Vaccination with Replication-Competent Adenovirus in Mice Reveals Dissemination of the Viral Vaccine beyond the Gastrointestinal Tract', *Journal of Virology*, 93(13), pp. 1–18. doi: 10.1128/jvi.00237-19.

Gold, M. J. *et al.* (2014) 'Group 2 innate lymphoid cells facilitate sensitization to local, but not systemic, TH2-inducing allergen exposures', *Journal of Allergy and Clinical Immunology*. Elsevier Ltd, 133(4), pp. 1142-1148.e5. doi: 10.1016/j.jaci.2014.02.033.

Gollwitzer, E. S. *et al.* (2014) 'Lung microbiota promotes tolerance to allergens in neonates via PD-L1', *Nature Medicine*, 20(6), pp. 642–647. doi: 10.1038/nm.3568.

Gomez-Cadena, A. *et al.* (2021) 'Severe COVID-19 patients exhibit an ILC2 NKG2D+ population in their impaired ILC compartment', *Cellular and Molecular Immunology*, 18(2), pp. 484–486. doi: 10.1038/s41423-020-00596-2.

Gorski, S. A., Hahn, Y. S. and Braciale, T. J. (2013) 'Group 2 Innate Lymphoid Cell Production of IL-

5 Is Regulated by NKT Cells during Influenza Virus Infection', *PLoS Pathogens*, 9(9). doi: 10.1371/journal.ppat.1003615.

Gounni, A. S. *et al.* (2000) 'IL-9 expression by human eosinophils: Regulation by IL-1β and TNF-α', *Journal of Allergy and Clinical Immunology*, 106(3), pp. 460–466. doi: 10.1067/mai.2000.109172.

De Grove, K. C. *et al.* (2016) 'Characterization and quantification of innate lymphoid cell subsets in human lung', *PLoS ONE*, 11(1), pp. 1–12. doi: 10.1371/journal.pone.0145961.

Guia, S. and Narni-Mancinelli, E. (2020) 'Helper-like Innate Lymphoid Cells in Humans and Mice', *Trends in Immunology*. Elsevier Ltd, pp. 436–452. doi: 10.1016/j.it.2020.03.002.

Guilliams, M. *et al.* (2020) 'Establishment and Maintenance of the Macrophage Niche', *Immunity*, 52(3), pp. 434–451. doi: 10.1016/J.IMMUNI.2020.02.015.

Guo, X. zhi J. *et al.* (2018) 'Lung γδ T Cells Mediate Protective Responses during Neonatal Influenza Infection that Are Associated with Type 2 Immunity', *Immunity*, 49(3), pp. 531-544.e6. doi: 10.1016/j.immuni.2018.07.011.

Halim, Timotheus Y F *et al.* (2012) 'Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation.', *Immunity*, 36(3), pp. 451–63. doi: 10.1016/j.immuni.2011.12.020.

Halim, Timotheus Y.F. *et al.* (2012) 'Retinoic-Acid-Receptor-Related Orphan Nuclear Receptor Alpha Is Required for Natural Helper Cell Development and Allergic Inflammation', *Immunity*, 37(3), pp. 463–474. doi: 10.1016/j.immuni.2012.06.012.

Halim, T. Y. F. *et al.* (2014) 'Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation', *Immunity*, 40(3), pp. 425–435. doi: 10.1016/j.immuni.2014.01.011.

Halim, T. Y. F. *et al.* (2015) 'Group 2 innate lymphoid cells license dendritic cells to potenactiate memory TH2 cell responses', *Nature Immunology*, 17(1), pp. 57–64. doi: 10.1038/ni.3294.

Halim, T. Y. F. *et al.* (2018) 'Tissue-Restricted Adaptive Type 2 Immunity Is Orchestrated by Expression of the Costimulatory Molecule OX40L on Group 2 Innate Lymphoid Cells', *Immunity*, 48(6), pp. 1195-1207.e6. doi: 10.1016/j.immuni.2018.05.003.

Hammad, H. *et al.* (2009) 'House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells', *Nature Medicine*, 15(4), pp. 410–416. doi: 10.1038/nm.1946.

Hammad, H. *et al.* (2010) 'Inflammatory dendritic cells - not basophils - are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen', *Journal of Experimental Medicine*, 207(10), pp. 2097–2111. doi: 10.1084/jem.20101563.

Hammad, H. and Lambrecht, B. N. (2008) 'Dendritic cells and epithelial cells: Linking innate and adaptive immunity in asthma', *Nature Reviews Immunology*, pp. 193–204. doi: 10.1038/nri2275.

Hammad, H. and Lambrecht, B. N. (2021) 'The basic immunology of asthma', *Cell*, pp. 1469–1485. doi: 10.1016/j.cell.2021.02.016.

Han, M., Hong, J. Y., *et al.* (2017) 'IFN-γ Blocks Development of an Asthma Phenotype in Rhinovirus-Infected Baby Mice by Inhibiting Type 2 Innate Lymphoid Cells.', *American journal of respiratory cell and molecular biology*, 56(2), pp. 242–251. doi: 10.1165/rcmb.2016-0056OC.

Han, M., Rajput, C., *et al.* (2017) 'The Innate Cytokines IL-25, IL-33, and TSLP Cooperate in the Induction of Type 2 Innate Lymphoid Cell Expansion and Mucous Metaplasia in Rhinovirus-Infected Immature Mice', *The Journal of Immunology*, p. ji1700216. doi: 10.4049/jimmunol.1700216.

Han, M. *et al.* (2019) 'Inflammasome activation is required for human rhinovirus-induced airway inflammation in naive and allergen-sensitized mice', *Mucosal Immunology*, 12(4), pp. 958–968. doi: 10.1038/s41385-019-0172-2.

Han, M. *et al.* (2020) 'IL-1β prevents ILC2 expansion, type 2 cytokine secretion, and mucus metaplasia in response to early-life rhinovirus infection in mice', *Allergy: European Journal of Allergy and Clinical Immunology*, 75(8), pp. 2001–2015. doi: 10.1111/all.14241.

Han, X. *et al.* (2019) 'Essential role of CD4+ T cells for the activation of group 2 innate lymphoid cells during respiratory syncytial virus infection in mice', *Immunotherapy*, 11(15), pp. 1303–1313. doi: 10.2217/imt-2019-0084.

Harb, H. *et al.* (2021) 'Notch4 signaling limits regulatory T-cell-mediated tissue repair and promotes severe lung inflammation in viral infections', *Immunity*, 54(6), pp. 1186-1199.e7. doi: 10.1016/j.immuni.2021.04.002.

Harker, J. A. and Lloyd, C. M. (2021) 'Overlapping and distinct features of viral and allergen immunity in the human lung', *Immunity*, 54(4), pp. 617–631. doi: 10.1016/j.immuni.2021.03.010.

Haspeslagh, E. *et al.* (2017) 'Murine models of allergic asthma', in *Methods in Molecular Biology*, pp. 121–136. doi: 10.1007/978-1-4939-6786-5_10.

Heidarieh, H., Hernáez, B. and Alcamí, A. (2015) ‘Immune modulation by virus-encoded secreted chemokine binding proteins’, *Virus Research*, 209, pp. 67–75. doi: 10.1016/j.virusres.2015.02.028.

Helou, D. G. *et al.* (2020) ‘PD-1 pathway regulates ILC2 metabolism and PD-1 agonist treatment ameliorates airway hyperreactivity’, *Nature Communications*, 11(1), p. 3998. doi: 10.1038/s41467-020-17813-1.

Hepworth, M. R. *et al.* (2013) ‘Innate lymphoid cells regulate CD4 + T cell responses to intestinal commensal bacteria’, *Nature*, 498(7452), pp. 113–117. doi: 10.1038/nature12240.

Hepworth, M. R. and Sonnenberg, G. F. (2014) ‘Regulation of the adaptive immune system by innate lymphoid cells’, *Current Opinion in Immunology*, 27(1), pp. 75–82. doi: 10.1016/j.coi.2014.01.013.

Hochdörfer, T. *et al.* (2019) ‘Expression of c-Kit discriminates between two functionally distinct subsets of human type 2 innate lymphoid cells’, *European Journal of Immunology*, 49(6), pp. 884–893. doi: 10.1002/eji.201848006.

Hondowicz, B. D. *et al.* (2016) ‘Interleukin-2-Dependent Allergen-Specific Tissue-Resident Memory Cells Drive Asthma’, *Immunity*, 44(1), pp. 155–166. doi: 10.1016/j.immuni.2015.11.004.

Hong, J. Y. *et al.* (2014) ‘Neonatal rhinovirus induces mucous metaplasia and airways hyperresponsiveness through IL-25 and type 2 innate lymphoid cells’, *Journal of Allergy and Clinical Immunology*. Mosby Inc., 134(2), p. 429. doi: 10.1016/j.jaci.2014.04.020.

Houen, G. and Trier, N. H. (2021) ‘Epstein-Barr Virus and Systemic Autoimmune Diseases’, *Frontiers in Immunology*, 11. doi: 10.3389/FIMMU.2020.587380.

Hoyler, T. *et al.* (2012) ‘The Transcription Factor GATA-3 Controls Cell Fate and Maintenance of Type 2 Innate Lymphoid Cells’, *Immunity*, 37(4), pp. 634–648. doi: 10.1016/j.immuni.2012.06.020.

Hrusch, C. L. *et al.* (2018) ‘ICOS protects against mortality from acute lung injury through activation of IL-5 þ ILC2s’, *Mucosal Immunology*, 11(1). doi: 10.1038/mi.2017.42.

Huang, Y. *et al.* (2018) ‘S1P-dependent interorgan trafficking of group 2 innate lymphoid cells supports host defense’, *Science*, 359(6371), pp. 114–119. doi: 10.1126/science.aam5809.

Hughes, D. J. *et al.* (2011) ‘Chemokine binding protein M3 of murine gammaherpesvirus 68 modulates the host response to infection in a natural host’, *PLoS Pathogens*, 7(3), p. 1001321. doi: 10.1371/journal.ppat.1001321.

Jackson, D. J. *et al.* (2008) 'Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children', *American Journal of Respiratory and Critical Care Medicine*, 178(7), pp. 667–672. doi: 10.1164/rccm.200802-309OC.

Jackson, D. J. *et al.* (2014) 'IL-33-Dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo', *American Journal of Respiratory and Critical Care Medicine*, 190(12), pp. 1373–1382. doi: 10.1164/rccm.201406-1039OC.

Jartti, T. *et al.* (2004) 'Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children', *Emerging Infectious Diseases*, 10(6), pp. 1095–1101. doi: 10.3201/eid1006.030629.

Jartti, T. and Gern, J. E. (2017) 'Role of viral infections in the development and exacerbation of asthma in children', *Journal of Allergy and Clinical Immunology*, pp. 895–906. doi: 10.1016/j.jaci.2017.08.003.

Jurak, L. M. *et al.* (2018) 'Interleukin 33 selectively augments rhinovirus-induced type 2 immune responses in asthmatic but not healthy people', *Frontiers in Immunology*, 9. doi: 10.3389/fimmu.2018.01895.

Kabata, H. *et al.* (2013) 'Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells during airway inflammation', *Nature Communications*, 4, pp. 1–7. doi: 10.1038/ncomms3675.

Kanda, A. *et al.* (2020) 'Multiple biological aspects of eosinophils in host defense, eosinophil-associated diseases, immunoregulation, and homeostasis: Is their role beneficial, detrimental, regulator, or bystander?', *Biological and Pharmaceutical Bulletin*, 43(1), pp. 20–30. doi: 10.1248/bpb.b19-00892.

Kang, H.-R. *et al.* (2014) 'Murine Gammaherpesvirus 68 Encoding Open Reading Frame 11 Targets TANK Binding Kinase 1 To Negatively Regulate the Host Type I Interferon Response', *Journal of Virology*, 88(12), pp. 6832–6846. doi: 10.1128/jvi.03460-13.

Kearley, J. *et al.* (2011) 'IL-9 governs allergen-induced mast cell numbers in the lung and chronic remodeling of the airways', *American Journal of Respiratory and Critical Care Medicine*, 183(7), pp. 865–875. doi: 10.1164/rccm.200909-1462OC.

Kerscher, B. *et al.* (2019) 'BET bromodomain inhibitor IBET151 impedes human ILC2 activation and prevents experimental allergic lung inflammation', *Frontiers in Immunology*, 10(APR), p. 678. doi: 10.3389/fimmu.2019.00678.

Khan, S. H. and Grayson, M. H. (2010) 'Cross-linking IgE augments human conventional dendritic cell production of CC chemokine ligand 28', *Journal of Allergy and Clinical Immunology*, pp. 265–267. doi: 10.1016/j.jaci.2009.09.038.

Khare, A. *et al.* (2013) 'Inhaled Antigen Upregulates Retinaldehyde Dehydrogenase In Lung CD103+ But Not Plasmacytoid Dendritic Cells To Induce Foxp3 De Novo in CD4+ T cells and Promote Airway Tolerance', *Journal of Immunology*, 191(1), p. 25. doi: 10.4049/JIMMUNOL.1300193.

Kim, C. H., Hashimoto-Hill, S. and Kim, M. (2016) 'Migration and Tissue Tropism of Innate Lymphoid Cells', *Trends in Immunology*, 37(1), pp. 68–79. doi: 10.1016/j.it.2015.11.003.

Kim, D.-H. and Van Dyken, S. J. (2020) 'Opinion ILC2s in High Definition: Decoding the Logic of Tissue-Based Immunity', *Trends in Immunology*, 41(1). doi: 10.1016/j.it.2019.11.003.

Kim, H. Y., Umetsu, D. T. and Dekruyff, R. H. (2016) 'Innate lymphoid cells in asthma: Will they take your breath away?', *European Journal of Immunology*, 46(4), pp. 795–806. doi: 10.1002/eji.201444557.

Kim, J. *et al.* (2019) 'Innate immune crosstalk in asthmatic airways: Innate lymphoid cells coordinate polarization of lung macrophages Eosinophilic Asthma', *Journal of Allergy and Clinical Immunology*, 143, pp. 1769-1782.e11. doi: 10.1016/j.jaci.2018.10.040.

Kim, M. and Kim, C. H. (2016) 'Colonization and effector functions of innate lymphoid cells in mucosal tissues', *Microbes and Infection*, 18(10), pp. 604–614. doi: 10.1016/j.micinf.2016.06.005.

Kitajima, M. and Ziegler, S. F. (2013) 'Cutting edge: identification of the thymic stromal lymphopoietin-responsive dendritic cell subset critical for initiation of type 2 contact hypersensitivity', *Journal of immunology*, 191(10), pp. 4903–4907. doi: 10.4049/JIMMUNOL.1302175.

de Kleer, I. M. *et al.* (2016) 'Perinatal Activation of the Interleukin-33 Pathway Promotes Type 2 Immunity in the Developing Lung', *Immunity*, 45(6), pp. 1285–1298. doi: 10.1016/j.immuni.2016.10.031.

Klose, C. S. N. *et al.* (2017) 'The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation', *Nature*, 549(7671), pp. 282–286. doi: 10.1038/nature23676.

Kobiler, O. *et al.* (2012) 'Virus strategies for passing the nuclear envelope barrier', *Nucleus*, 3(6). doi: 10.4161/NUCL.21979.

Kotaniemi-Syrjänen, A. *et al.* (2003) 'Rhinovirus-induced wheezing in infancy - The first sign of childhood asthma?', *Journal of Allergy and Clinical Immunology*, 111(1), pp. 66–71. doi:

10.1067/mai.2003.33.

Kotas, M. E. and Locksley, R. M. (2018) 'Why Innate Lymphoid Cells?', *Immunity*, 48(6), pp. 1081–1090. doi: 10.1016/j.immuni.2018.06.002.

Krabbendam, L. *et al.* (2018) 'New insights into the function, development, and plasticity of type 2 innate lymphoid cells', *Immunological Reviews*, 286(1), pp. 74–85. doi: 10.1111/imr.12708.

Kung, T. T. *et al.* (1995) 'Involvement of IL-5 in a murine model of allergic pulmonary inflammation: prophylactic and therapeutic effect of an anti-IL-5 antibody.', *American journal of respiratory cell and molecular biology*, 13(3), pp. 360–365. doi: 10.1165/ajrcmb.13.3.7654390.

Kurth, J. *et al.* (2000) 'EBV-infected B cells in infectious mononucleosis: Viral strategies for spreading in the B cell compartment and establishing latency', *Immunity*, 13(4), pp. 485–495. doi: 10.1016/S1074-7613(00)00048-0.

Kusel, M. H. *et al.* (2007) 'Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma', *Journal of Allergy and Clinical Immunology*, 119(5), pp. 1105–1110. doi: 10.1016/j.jaci.2006.12.669.

van de Laar, L. *et al.* (2016) 'Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an Empty Niche and Develop into Functional Tissue-Resident Macrophages', *Immunity*, 44(4), pp. 755–768. doi: 10.1016/j.immuni.2016.02.017.

Laffont, S. *et al.* (2017) 'Androgen signaling negatively controls group 2 innate lymphoid cells', *The Journal of experimental medicine*, 214(6), pp. 1581–1592. doi: 10.1084/JEM.20161807.

Lambrecht, B. and Hammad, H. (2017) 'The immunology of the allergy epidemic and the hygiene hypothesis', *Nature immunology*, 18(10), pp. 1076–1083. doi: 10.1038/NI.3829.

Lambrecht, B. N. and Hammad, H. (2015) 'The immunology of asthma', *Nature Immunology*, 16(1), pp. 45–56. doi: 10.1038/ni.3049.

Lambrecht, B. N., Hammad, H. and Fahy, J. V. (2019) 'The Cytokines of Asthma', *Immunity*, pp. 975–991. doi: 10.1016/j.immuni.2019.03.018.

Lansbury, L. *et al.* (2019) 'Corticosteroids as adjunctive therapy in the treatment of influenza', *The Cochrane database of systematic reviews*, 24(2). doi: 10.1002/14651858.CD010406.PUB3.

Lavin, Y. *et al.* (2014) 'Tissue-resident macrophage enhancer landscapes are shaped by the local

microenvironment', *Cell*. Cell Press, 159(6), pp. 1312–1326. doi: 10.1016/j.cell.2014.11.018.

Lawler, C. *et al.* (2015) 'Rhadinovirus host entry by co-operative infection', *PLoS pathogens*, 11(3), pp. 1–23. doi: 10.1371/JOURNAL.PPAT.1004761.

Lawler, C., Simas, J. P. and Stevenson, P. G. (2020) 'Vaccine protection against murid herpesvirus-4 is maintained when the priming virus lacks known latency genes', *Immunology and cell biology*, 98(1), pp. 67–78. doi: 10.1111/IMCB.12299.

Lechner, A. J. *et al.* (2017) 'Recruited Monocytes and Type 2 Immunity Promote Lung Regeneration following Pneumonectomy', *Cell Stem Cell*, 21(1), pp. 120-134.e7. doi: 10.1016/j.stem.2017.03.024.

Lee, A. J. and Ashkar, A. A. (2018) 'The dual nature of type I and type II interferons', *Frontiers in Immunology*. doi: 10.3389/fimmu.2018.02061.

Lee, H. C. *et al.* (2012) 'Thymic stromal lymphopoietin is induced by respiratory syncytial virus-infected airway epithelial cells and promotes a type 2 response to infection', *Journal of Allergy and Clinical Immunology*. Mosby Inc., 130(5), p. 1187. doi: 10.1016/j.jaci.2012.07.031.

Lee, K. S. *et al.* (2009) 'Murine gammaherpesvirus 68 infection of IFNγ unresponsive mice: a small animal model for gammaherpesvirus-associated B cell lymphoproliferative disease', *Cancer research*, 69(13), p. 5481. doi: 10.1158/0008-5472.CAN-09-0291.

Lee, M. W. *et al.* (2015) 'Activated type 2 innate lymphoid cells regulate beige fat biogenesis', *Cell*, 160(1–2), pp. 74–87. doi: 10.1016/j.cell.2014.12.011.

Lee, Y. G. *et al.* (2015) 'Recruited alveolar macrophages, in response to airway epithelial-derived monocyte chemoattractant protein 1/CCL2, regulate airway inflammation and remodeling in allergic asthma', *American Journal of Respiratory Cell and Molecular Biology*, 52(6), pp. 772–784. doi: 10.1165/rcmb.2014-0255OC.

Lei, A.-H. *et al.* (2018) 'ICAM-1 controls development and function of ILC2', *The Journal of Experimental Medicine*, 215(8), pp. 2157–2174. doi: 10.1084/jem.20172359.

Leight, E. R. and Sugden, B. (2000) 'EBNA-1: A protein pivotal to latent infection by Epstein-Barr virus', *Reviews in Medical Virology*, pp. 83–100. doi: 10.1002/(SICI)1099-1654(200003/04)10:2<83::AID-RMV262>3.0.CO;2-T.

Leyva-Castillo, J. M. *et al.* (2020) 'ILC2 activation by keratinocyte-derived IL-25 drives IL-13 production at sites of allergic skin inflammation', *Journal of Allergy and Clinical Immunology*, 145(6),

pp. 1606-1614.e4. doi: 10.1016/j.jaci.2020.02.026.

Li *et al.* (2018) 'T cells and ILC2s are major effector cells in influenza-induced exacerbation of allergic airway inflammation in mice', *Eur. J. Immunol*, 49, pp. 144–156. doi: 10.1002/eji.201747421.

Li, B. *et al.* (2016) 'T cells are necessary for ILC2 activation in house dust mite-induced allergic airway inflammation in mice', *European Journal of Immunology*, 46(6), pp. 1392–1403. doi: 10.1002/eji.201546119.

Li, D. *et al.* (2014) 'IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice', *Journal of Allergy and Clinical Immunology*, 134(6), pp. 1422-1432.e11. doi: 10.1016/j.jaci.2014.05.011.

Li, Q. *et al.* (2018) 'E3 Ligase VHL Promotes Group 2 Innate Lymphoid Cell Maturation and Function via Glycolysis Inhibition and Induction of Interleukin-33 Receptor', *Immunity*, 48(2), pp. 258-270.e5. doi: 10.1016/j.immuni.2017.12.013.

Liang, H. E. *et al.* (2012) 'Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity', *Nature Immunology*, 13(1), pp. 58–66. doi: 10.1038/ni.2182.

Liang, X. *et al.* (2009) 'Gammaherpesvirus-driven plasma cell differentiation regulates virus reactivation from latently infected B lymphocytes', *PLoS Pathogens*, 5(11). doi: 10.1371/journal.ppat.1000677.

Licona-Limón, P. *et al.* (2013) 'TH2, allergy and group 2 innate lymphoid cells', *Nature Immunology*, 14(6), pp. 536–542. doi: 10.1038/ni.2617.

Lieberman, P. M. (2013) 'Keeping it quiet: chromatin control of gammaherpesvirus latency', *Nature reviews. Microbiology*, 11(12), p. 863. doi: 10.1038/NRMICRO3135.

Lim, A. I. *et al.* (2016) 'IL-12 drives functional plasticity of human group 2 innate lymphoid cells', *The Journal of Experimental Medicine*, 213(4), pp. 569–583. doi: 10.1084/jem.20151750.

Liu, S. *et al.* (2018) 'Steroid resistance of airway type 2 innate lymphoid cells from patients with severe asthma: The role of thymic stromal lymphopoietin', *The Journal of allergy and clinical immunology*, 141(1), pp. 257-268.e6. doi: 10.1016/J.JACI.2017.03.032.

Loftus, P. A. and Wise, S. K. (2016) 'Epidemiology of asthma', *Current opinion in otolaryngology & head and neck surgery*, 24(3), pp. 245–249. doi: 10.1097/MOO.0000000000000262.

Loh, Z. *et al.* (2020) 'HMGB1 amplifies ILC2-induced type-2 inflammation and airway smooth muscle remodelling', *PLoS Pathogens*, 16(7), p. e1008651. doi: 10.1371/journal.ppat.1008651.

Lopušná, K. *et al.* (2016) 'Murine gammaherpesvirus targets type I IFN receptor but not type III IFN receptor early in infection', *Cytokine*, 83, pp. 158–170. doi: 10.1016/j.cyto.2016.04.013.

Lötvall, J. *et al.* (2011) 'Asthma endotypes: A new approach to classification of disease entities within the asthma syndrome', *Journal of Allergy and Clinical Immunology*, 127(2), pp. 355–360. doi: 10.1016/j.jaci.2010.11.037.

Lukac, D., Kirshner, J. and Ganem, D. (1999) 'Transcriptional activation by the product of open reading frame 50 of Kaposi's sarcoma-associated herpesvirus is required for lytic viral reactivation in B cells', *Journal of virology*, 73(11), pp. 9348–9361. doi: 10.1128/JVI.73.11.9348-9361.1999.

Maazi, H. *et al.* (2015) 'ICOS:ICOS-Ligand Interaction Is Required for Type 2 Innate Lymphoid Cell Function, Homeostasis, and Induction of Airway Hyperreactivity', *Immunity*, 42(3), pp. 538–551. doi: 10.1016/j.immuni.2015.02.007.

Maazi, H. *et al.* (2017) 'Activated plasmacytoid dendritic cells regulate type 2 innate lymphoid cell-mediated airway hyperreactivity', *Journal of Allergy and Clinical Immunology*. doi: 10.1016/j.jaci.2017.04.043.

Machiels, B. *et al.* (2017) 'A gammaherpesvirus provides protection against allergic asthma by inducing the replacement of resident alveolar macrophages with regulatory monocytes', *Nature Immunology*. Nature Publishing Group, 18(12), pp. 1310–1320. doi: 10.1038/ni.3857.

Mahmutovic Persson, I. *et al.* (2018) 'IL-1β mediates lung neutrophilia and IL-33 expression in a mouse model of viral-induced asthma exacerbation', *Respiratory Research*, 19(1). doi: 10.1186/s12931-018-0725-z.

Mahut, B. *et al.* (2011) 'Influenza-like illness responsible for severe exacerbations in asthmatic children during H1N1 pandemic: A survey before vaccination', *Journal of Asthma*, 48(3), pp. 224–227. doi: 10.3109/02770903.2011.555032.

Marazzi, I. and Garcia-Sastre, A. (2015) 'Interference of viral effector proteins with chromatin, transcription, and the epigenome', *Current Opinion in Microbiology*, pp. 123–129. doi: 10.1016/j.mib.2015.06.009.

Maric, J. *et al.* (2018) 'Prostaglandin E2 suppresses human group 2 innate lymphoid cell function',

Journal of Allergy and Clinical Immunology, 141(5), pp. 1761-1773.e6. doi: 10.1016/j.jaci.2017.09.050.

Martinez-Gonzalez, I. *et al.* (2016) 'Allergen-Experienced Group 2 Innate Lymphoid Cells Acquire Memory-like Properties and Enhance Allergic Lung Inflammation', *Immunity*, 45(1), pp. 198–208. doi: 10.1016/j.immuni.2016.06.017.

Martinez-Gonzalez, I. *et al.* (2018) 'ILC2 memory: Recollection of previous activation', *Immunological Reviews*, 283(1), pp. 41–53. doi: 10.1111/imr.12643.

Mathä, L. *et al.* (2021) 'Migration of Lung Resident Group 2 Innate Lymphoid Cells Link Allergic Lung Inflammation and Liver Immunity', *Frontiers in Immunology*, 12(679509). doi: 10.3389/fimmu.2021.679509.

Matricardi, P. M. *et al.* (2000) 'Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: Epidemiological study', *British Medical Journal*, 320(7232), pp. 412–417. doi: 10.1136/bmj.320.7232.412.

McGeoch, D. J., Rixon, F. J. and Davison, A. J. (2006) 'Topics in herpesvirus genomics and evolution', *Virus Research*, pp. 90–104. doi: 10.1016/j.virusres.2006.01.002.

Meininger, I. *et al.* (2020) 'Tissue-Specific Features of Innate Lymphoid Cells', *Trends in Immunology*, pp. 902–917. doi: 10.1016/j.it.2020.08.009.

Menachery, V. D. *et al.* (2014) 'Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses', *mBio*, 5(3). doi: 10.1128/mBio.01174-14.

Mesri, E. A., Cesarman, E. and Boshoff, C. (2010) 'Kaposi's sarcoma and its associated herpesvirus', *Nature reviews. Cancer*, 10(10), pp. 707–719. doi: 10.1038/NRC2888.

Michi, A. N., Love, M. E. and Proud, D. (2020) 'Rhinovirus-Induced Modulation of Epithelial Phenotype: Role in Asthma', *Viruses*. doi: 10.3390/v12111328.

Milho, R. *et al.* (2009) 'In vivo imaging of murid herpesvirus-4 infection', *Journal of General Virology*, 90(1), pp. 21–32. doi: 10.1099/vir.0.006569-0.

Milho, R. *et al.* (2012) 'A heparan-dependent herpesvirus targets the olfactory neuroepithelium for host entry', *PLoS pathogens*, 8(11). doi: 10.1371/JOURNAL.PPAT.1002986.

Miller, H. E. *et al.* (2019) 'γ-herpesvirus latency attenuates Mycobacterium tuberculosis infection in

mice', *Tuberculosis*, 116, pp. 56–60. doi: 10.1016/j.tube.2019.04.022.

Miller, M. M. *et al.* (2020) 'BATF acts as an essential regulator of IL-25–responsive migratory ILC2 cell fate and function', *Science Immunology*, 5(43), p. eaay3994. doi: 10.1126/sciimmunol.aay3994.

Minhas, V. and Wood, C. (2014) 'Epidemiology and Transmission of Kaposi's Sarcoma-Associated Herpesvirus', *Viruses*, 6, pp. 4178–4194. doi: 10.3390/v6114178.

Miura, T. A. (2019) 'Respiratory epithelial cells as master communicators during viral infections', *Current clinical microbiology reports*, 6(1), pp. 10–17. doi: 10.1007/S40588-019-0111-8.

Mjösberg, J. *et al.* (2012) 'The Transcription Factor GATA3 Is Essential for the Function of Human Type 2 Innate Lymphoid Cells', *Immunity*, 37(4), pp. 649–659. doi: 10.1016/j.immuni.2012.08.015.

Mjösberg, J. M. *et al.* (2011) 'Human IL-25-and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161', *Nature Immunology*, 12(11), pp. 1055–1062. doi: 10.1038/ni.2104.

Mohapatra, A. *et al.* (2016) 'Group 2 innate lymphoid cells utilize the IRF4-IL-9 module to coordinate epithelial cell maintenance of lung homeostasis', *Mucosal Immunology*, 9(1), pp. 275–286. doi: 10.1038/mi.2015.59.

Molofsky, A. B. *et al.* (2015) 'InterleuKin-33 And Interferon-Γ Counter-Regulate Group 2 Innate Lymphoid Cell Activation During Immune Perturbation', *Immunity*, 43(1), pp. 161–174. doi: 10.1016/j.immuni.2015.05.019.

Von Moltke, J. *et al.* (2016) 'Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit', *Nature*, 529, pp. 221–225. doi: 10.1038/nature16161.

Monticelli, L. a. *et al.* (2012) 'Innate lymphoid cells promote lung tissue homeostasis following acute influenza virus infection', *Nature Immunology*, 12(11), pp. 1045–1054. doi: 10.1031/ni.2131.Innate.

Monticelli, L. A. *et al.* (2016) 'Arginase 1 is an innate lymphoid-cell-intrinsic metabolic checkpoint controlling type 2 inflammation', *Nature Immunology*, 17(6), pp. 656–665. doi: 10.1038/ni.3421.

Moon, H. G. *et al.* (2018) 'Airway Epithelial Cell-Derived Colony Stimulating Factor-1 Promotes Allergen Sensitization', *Immunity*, 49(2), pp. 275-287.e5. doi: 10.1016/j.immuni.2018.06.009.

Moral, J. A. *et al.* (2020) 'ILC2s amplify PD-1 blockade by activating tissue-specific cancer immunity', *Nature*, 579(March), pp. 1–6. doi: 10.1038/s41586-020-2015-4.

Morales-Nebreda, L. *et al.* (2015) 'The heterogeneity of lung macrophages in the susceptibility to disease', *European Respiratory Review*, 24(137), pp. 505–509. doi: 10.1183/16000617.0031-2015.

Mori, H. *et al.* (2013) 'Differences in respiratory syncytial virus and influenza infection in a house-dust-mite-induced asthma mouse model: Consequences for steroid sensitivity', *Clinical Science*, 125(12), pp. 565–574. doi: 10.1042/CS20130098.

Morita, H., Moro, K. and Koyasu, S. (2016) 'Innate lymphoid cells in allergic and nonallergic inflammation', *Journal of Allergy and Clinical Immunology*, 138(5), pp. 1253–1264. doi: 10.1016/j.jaci.2016.09.011.

Moro, K. *et al.* (2010) 'Innate production of TH2 cytokines by adipose tissue-associated c-Kit+Sca-1+lymphoid cells', *Nature*, 463(7280), pp. 540–544. doi: 10.1038/nature08636.

Moro, K. *et al.* (2016) 'Interferon and IL-27 antagonize the function of group 2 innate lymphoid cells and type 2 innate immune responses', *Nature Immunology*, 17(1), pp. 76–86. doi: 10.1038/ni.3309.

Mortha, A. and Burrows, K. (2018) 'Cytokine Networks between Innate Lymphoid Cells and Myeloid Cells', *Frontiers in Immunology*, 9, p. 191. doi: 10.3389/fimmu.2018.00191.

Motomura, Y. *et al.* (2014) 'Basophil-derived interleukin-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation', *Immunity*, 40(5), pp. 758–771. doi: 10.1016/j.immuni.2014.04.013.

Nabekura, T. and Lanier, L. L. (2016) 'Tracking the fate of antigen-specific versus cytokineactivated natural killer cells after cytomegalovirus infection', *J Exp Med*, 213(12), pp. 2745–2758. doi: 10.1084/jem.20160726.

Naessens, T. *et al.* (2020) 'Human Lung Conventional Dendritic Cells Orchestrate Lymphoid Neogenesis during Chronic Obstructive Pulmonary Disease', *American journal of respiratory and critical care medicine*, 202(4), pp. 535–548. doi: 10.1164/RCCM.201906-1123OC.

Nagashima, H. *et al.* (2019) 'Neuropeptide CGRP Limits Group 2 Innate Lymphoid Cell Responses and Constrains Type 2 Inflammation', *Immunity*, 51(4), pp. 682-695.e6. doi: 10.1016/j.immuni.2019.06.009.

Nelms, K. *et al.* (1999) 'THE IL-4 RECEPTOR: Signaling Mechanisms and Biologic Functions', *Annual Review of Immunology*, 17, pp. 701–738. doi: 10.1146/ANNUREV.IMMUNOL.17.1.701.

Netea, M. G. *et al.* (2015) 'Innate immune memory: a paradigm shift in understanding host defense',

Nature Immunology, 16(7), pp. 675–679. doi: 10.1038/ni.3178.

Netea, M. G. and van der Meer, J. W. M. (2017) 'Trained Immunity: An Ancient Way of Remembering', *Cell Host and Microbe*. Elsevier Inc., 21(3), pp. 297–300. doi: 10.1016/j.chom.2017.02.003.

Newcomb, D. C. and Peebles, R. S. (2013) 'Th17-mediated inflammation in asthma', *Current opinion in immunology*, 25(6), pp. 755–760. doi: 10.1016/J.COI.2013.08.002.

Nieuwenhuizen, N. E. *et al.* (2012) 'Allergic airway disease is unaffected by the absence of IL-4Rα-dependent alternatively activated macrophages', *Journal of Allergy and Clinical Immunology*, 130(3), pp. 743-750.e8. doi: 10.1016/j.jaci.2012.03.011.

Nikonova, A. *et al.* (2020) 'M1-like macrophages are potent producers of anti-viral interferons and M1-associated marker-positive lung macrophages are decreased during rhinovirus-induced asthma exacerbations', *EBioMedicine*, 54. doi: 10.1016/j.ebiom.2020.102734.

Nilsson, C. *et al.* (2005) 'Does early EBV infection protect against IgE sensitization?', *Journal of Allergy and Clinical Immunology*, 116(2), pp. 438–444. doi: 10.1016/j.jaci.2005.04.027.

Nordström, I. *et al.* (2010) 'Infection of infants with human herpesvirus type 6 may be associated with reduced allergic sensitization and T-helper type 2 development', *Clinical and Experimental Allergy*, 40(6), pp. 882–890. doi: 10.1111/j.1365-2222.2010.03491.x.

Norlander, A. E. and Peebles, R. S. (2020) 'Innate type 2 responses to respiratory syncytial virus infection', *Viruses*. doi: 10.3390/v12050521.

Nussbaum, J. C. *et al.* (2013) 'Type 2 innate lymphoid cells control eosinophil homeostasis', *Nature*, 502(7470), pp. 245–248. doi: 10.1038/nature12526.

O'Flaherty, B. M. *et al.* (2014) 'The Murine Gammaherpesvirus Immediate-Early Rta Synergizes with IRF4, Targeting Expression of the Viral M1 Superantigen to Plasma Cells', *PLoS Pathogens*, 10(8). doi: 10.1371/journal.ppat.1004302.

O'Sullivan, T. E. and Sun, J. C. (2018) 'Determination of the Fate and Function of Innate Lymphoid Cells Following Adoptive Transfer of Innate Lymphoid Cell Precursors', in *Type 2 Immunity: Methods and Protocols*, pp. 109–119. doi: 10.1007/978-1-4939-7896-0_10.

Ohne, Y. *et al.* (2016) 'IL-1 is a critical regulator of group 2 innate lymphoid cell function and plasticity', *Nature immunology*, 17(6). doi: 10.1038/ni.3447.

Oliphant, C. J. *et al.* (2014) 'MHCII-mediated dialog between group 2 innate lymphoid cells and CD4+T cells potentiates type 2 immunity and promotes parasitic helminth expulsion', *Immunity*. The Authors, 41(2), pp. 283–295. doi: 10.1016/j.immuni.2014.06.016.

Owens, S. M. *et al.* (2020) 'Deletion of Murine Gammaherpesvirus Gene M2 in Activation-Induced Cytidine Deaminase-Expressing B Cells Impairs Host Colonization and Viral Reactivation', *Journal of Virology*, 95(1). doi: 10.1128/JVI.01933-20.

Pakkasela, J. *et al.* (2020) 'Age-specific incidence of allergic and non-allergic asthma', *BMC Pulmonary Medicine*, 20(1). doi: 10.1186/s12890-019-1040-2.

Pavlova, I. V, Virgin Iv, H. W. and Speck, S. H. (2003) 'Disruption of Gammaherpesvirus 68 Gene 50 Demonstrates that Rta Is Essential for Virus Replication', *Journal of Virology*, 77(10), pp. 5731–5739. doi: 10.1128/JVI.77.10.5731-5739.2003.

Peng, L. *et al.* (2010) 'Three-Dimensional Visualization of Gammaherpesvirus Life Cycle in Host Cells by Electron Tomography', *Structure*, 13(18(1)), pp. 47–58. doi: 10.1016/j.str.2009.10.017.

Perez, G. F. *et al.* (2014) 'Rhinovirus infection in young children is associated with elevated airway TSLP levels', *European Respiratory Journal*, pp. 1075–1078. doi: 10.1183/09031936.00049214.

Perrin, R. *et al.* (2020) 'Into the looking glass: Post-viral syndrome post COVID-19', *Medical hypotheses*, 144. doi: 10.1016/J.MEHY.2020.110055.

Prasad, B. V. V. and Schmid, M. F. (2012) 'Principles of virus structural organization', *Advances in experimental medicine and biology*, 726, pp. 17–47. doi: 10.1007/978-1-4614-0980-9_3.

Qin, L. *et al.* (2019) 'Respiratory syncytial virus promoted the differentiation of th17 cells in airway microenvironment through activation of notch-1/ delta3', *Journal of Medical Microbiology*, 68(4), pp. 649–656. doi: 10.1099/jmm.0.000959.

Radermecker, C. *et al.* (2018) 'Role of neutrophils in allergic asthma', *Current Opinion in Immunology*, 54, pp. 28–34. doi: 10.1016/J.COI.2018.05.006.

Radtke, K. *et al.* (2010) 'Plus-and Minus-End Directed Microtubule Motors Bind Simultaneously to Herpes Simplex Virus Capsids Using Different Inner Tegument Structures', *PLoS Pathogens*, 6(7), p. e10000991. doi: 10.1371/journal.ppat.1000991.

Raffegerst, S. *et al.* (2015) 'Prevention of Tumor Formation by Latent Gammaherpesvirus Infection', *PLoS ONE*, 10(12). doi: 10.1371/JOURNAL.PONE.0145678.

Rajput, C. *et al.* (2017) 'RORα-dependent type 2 innate lymphoid cells are required and sufficient for mucous metaplasia in immature mice', *American Journal of Physiology - Lung Cellular and Molecular Physiology*. American Physiological Society, 312(6), pp. L983–L993. doi: 10.1152/ajplung.00368.2016.

Rajput, C. *et al.* (2020) 'Early-life heterologous rhinovirus infections induce an exaggerated asthma-like phenotype', *Journal of Allergy and Clinical Immunology*, 146(3), pp. 571-582.e3. doi: 10.1016/j.jaci.2020.03.039.

Raundhal, M. *et al.* (2015) 'High IFN-γ and low SLPI mark severe asthma in mice and humans', *Journal of Clinical Investigation*, 125(8), pp. 3037–3050. doi: 10.1172/JCI80911.

Ravanetti, L. *et al.* (2019) 'IL-33 drives influenza-induced asthma exacerbations by halting innate and adaptive antiviral immunity', *Journal of Allergy and Clinical Immunology*, 143, pp. 1355-1370.e16. doi: 10.1016/j.jaci.2018.08.051.

Redhu, N. S. and Gounni, A. S. (2013) 'The high affinity IgE receptor (FcεRI) expression and function in airway smooth muscle', *Pulmonary Pharmacology and Therapeutics*, 26(1), pp. 86–94. doi: 10.1016/j.pupt.2012.04.004.

Ricardo-Gonzalez, R. R. *et al.* (2018) 'Tissue signals imprint ILC2 identity with anticipatory function', *Nature Immunology*, 19(10), pp. 1093–1099. doi: 10.1038/s41590-018-0201-4.

Ricardo-Gonzalez, R. R. *et al.* (2020) 'Tissue-specific pathways extrude activated ILC2s to disseminate type 2 immunity', *Journal of Experimental Medicine*, 217(4), p. e20191172. doi: 10.1084/jem.20191172.

Rich, H. E. *et al.* (2020) 'Insights Into Type I and III Interferons in Asthma and Exacerbations', *Frontiers in Immunology*. doi: 10.3389/fimmu.2020.574027.

Rigas, D. *et al.* (2017) 'Type 2 innate lymphoid cell suppression by regulatory T cells attenuates airway hyperreactivity and requires inducible T-cell costimulator–inducible T-cell costimulator ligand interaction', *Journal of Allergy and Clinical Immunology*. Mosby, 139(5), pp. 1468-1477.e2. doi: 10.1016/J.JACI.2016.08.034.

Van Rijt, L. S. *et al.* (2005) 'In vivo depletion of lung CD11c+ dendritic cells during allergen challenge abrogates the characteristic features of asthma', *The Journal of experimental medicine*, 201(6), pp. 981–991. doi: 10.1084/JEM.20042311.

Riksen, N. P. and Netea, M. G. (2021) 'Immunometabolic control of trained immunity', *Molecular Aspects of Medicine*, 77, p. 100897. doi: 10.1016/J.MAM.2020.100897.

Robinette, M. L. *et al.* (2017) 'IL-15 sustains IL-7R-independent ILC2 and ILC3 development', *Nature Communications*, 8. doi: 10.1038/ncomms14601.

Rochford, R. *et al.* (2019) 'The Global Landscape of EBV-Associated Tumors', *Frontiers in Oncology | www.frontiersin.org*, 1, p. 713. doi: 10.3389/fonc.2019.00713.

Romanet-Manent, S. *et al.* (2002) 'Allergic vs nonallergic asthma: what makes the difference?', *Allergy*, 57(7), pp. 607–613. doi: 10.1034/J.1398-9995.2002.23504.X.

Rubner, F. J. *et al.* (2017) 'Early life rhinovirus wheezing, allergic sensitization, and asthma risk at adolescence', *Journal of Allergy and Clinical Immunology*, 139(2), pp. 501–507. doi: 10.1016/j.jaci.2016.03.049.

Sabogal Piñeros, Y. *et al.* (2019) 'Anti-IL-5 in Mild Asthma Alters Rhinovirus-induced Macrophage, B-Cell, and Neutrophil Responses.', *American journal of respiratory and critical care medicine*, 199(4), pp. 508–517. doi: 10.1164/RCCM.201803-0461OC.

Saito, F. *et al.* (2013) 'MHV68 Latency Modulates the Host Immune Response to Influenza A Virus', *Inflammation*, 36(6), pp. 1295–1303. doi: 10.1007/s10753-013-9668-1.

Saluzzo, S. *et al.* (2017) 'First-Breath-Induced Type 2 Pathways Shape the Lung Immune Environment', *Cell Reports*, 18(8), pp. 1893–1905. doi: 10.1016/j.celrep.2017.01.071.

Santos, J., Havunen, R. and Hemminki, A. (2020) 'Modulation of the tumor microenvironment with an oncolytic adenovirus for effective T-cell therapy and checkpoint inhibition', *Methods in enzymology*, 635, pp. 205–230. doi: 10.1016/BS.MIE.2019.05.043.

Saravia, J. *et al.* (2015) 'Respiratory Syncytial Virus Disease Is Mediated by Age-Variable IL-33', *PLoS Pathogens*, 11(10). doi: 10.1371/journal.ppat.1005217.

Schleich, F. N. *et al.* (2013) 'Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation', *BMC Pulmonary Medicine*, 13(11). doi: 10.1186/1471-2466-13-11.

Schlums, H. *et al.* (2015) 'Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function', *Immunity*, 42(3), pp. 443–456. doi: 10.1016/j.immuni.2015.02.008.

Schuijs, M. J., Hammad, H. and Lambrecht, B. N. (2019) 'Professional and "Amateur" Antigen-Presenting Cells In Type 2 Immunity', *Trends in Immunology*, 40(1), pp. 22–34. doi: 10.1016/j.it.2018.11.001.

Schulz, T. F. and Cesarman, E. (2015) 'Kaposi Sarcoma-associated Herpesvirus: mechanisms of oncogenesis', *Current Opinion in Virology*, 14, pp. 116–128. doi: 10.1016/J.COVIRO.2015.08.016.

Schwartz, C. *et al.* (2017) 'ILC2s regulate adaptive Th2 cell functions via PD-L1 checkpoint control', *The Journal of Experimental Medicine*, 214(9), pp. 2507–2521. doi: 10.1084/jem.20170051.

Sebina, I. and Phipps, S. (2020) 'The contribution of neutrophils to the pathogenesis of RSV bronchiolitis', *Viruses*. doi: 10.3390/v12080808.

Šebová, R. *et al.* (2019) 'Residue mutations in murine herpesvirus 68 immunomodulatory protein M3 reveal specific modulation of chemokine binding', *Frontiers in Cellular and Infection Microbiology*, 9(JUN). doi: 10.3389/fcimb.2019.00210.

Seehus, C. R. *et al.* (2017) 'Alternative activation generates IL-10 producing type 2 innate lymphoid cells', *Nature Communications*, 8(1), p. 1900. doi: 10.1038/s41467-017-02023-z.

Serbina, N. V and Pamer, E. G. (2006) 'Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2', *Nature Immunology*, 7(3), pp. 311–318. doi: 10.1038/ni1309.

Seumois, G. *et al.* (2020) 'Single-cell transcriptomic analysis of allergen-specific T cells in allergy and asthma', *Science Immunology*, 5(48). doi: 10.1126/SCIIMMUNOL.ABA6087.

Shannon, J. P. *et al.* (2021) 'Group 1 innate lymphoid-cell-derived interferon-γ maintains anti-viral vigilance in the mucosal epithelium', *Immunity*, 54(2), pp. 276-290.e5. doi: 10.1016/j.immuni.2020.12.004.

Sharma, J. *et al.* (2015) 'A knowledgebase resource for interleukin-17 family mediated signaling', *J. Cell Commun. Signal*, 9, pp. 291–296. doi: 10.1007/s12079-015-0297-3.

She, L. *et al.* (2021) 'STING activation in alveolar macrophages and group 2 innate lymphoid cells suppresses IL-33-driven type 2 immunopathology', *JCI Insight*, 6(3). doi: 10.1172/JCI.INSIGHT.143509.

Shen, C. *et al.* (2021) 'PD-1 Affects the Immunosuppressive Function of Group 2 Innate Lymphoid Cells in Human Non-Small Cell Lung Cancer', *Small Cell Lung Cancer. Front. Immunol*, 12, p. 680055.

doi: 10.3389/fimmu.2021.680055.

Shen, Y. *et al.* (2018) 'Inhibition of murine herpesvirus-68 replication by IFN-gamma in macrophages is counteracted by the induction of SOCS1 expression', *PLoS Pathogens*, 14(8). doi: 10.1371/journal.ppat.1007202.

Shih, H. Y. *et al.* (2016) 'Developmental Acquisition of Regulomes Underlies Innate Lymphoid Cell Functionality', *Cell*, 165(5), pp. 1120–1133. doi: 10.1016/j.cell.2016.04.029.

Shim, D. H. *et al.* (2015) 'Pandemic influenza virus, pH1N1, induces asthmatic symptoms via activation of innate lymphoid cells', *Pediatric Allergy and Immunology*, 26(8), pp. 780–788. doi: 10.1111/pai.12462.

Silveira, J. S. *et al.* (2019) 'Respiratory syncytial virus increases eosinophil extracellular traps in a murine model of asthma', *Asia Pacific Allergy*, 9(4). doi: 10.5415/apallergy.2019.9.e32.

Silver, J. S. *et al.* (2016) 'Inflammatory triggers associated with exacerbations of COPD orchestrate plasticity of group 2 innate lymphoid cells in the lungs', *Nature Immunology*, 17(6), pp. 626–635. doi: 10.1038/ni.3443.

Silverstein, N. J. *et al.* (2021) 'Innate lymphoid cells and disease tolerance in SARS-CoV-2 infection.', *medRxiv : the preprint server for health sciences*. doi: 10.1101/2021.01.14.21249839.

Simoni, Y. *et al.* (2017) 'Human Innate Lymphoid Cell Subsets Possess Tissue-Type Based Heterogeneity in Phenotype and Frequency', *Immunity*. Elsevier Inc., 46(1), pp. 148–161. doi: 10.1016/j.immuni.2016.11.005.

Simpson, J. *et al.* (2020) 'Respiratory Syncytial Virus Infection Promotes Necroptosis and HMGB1 Release by Airway Epithelial Cells', *American Journal of Respiratory and Critical Care Medicine*. American Thoracic Society, 201(11), pp. 1358–1371. doi: 10.1164/rccm.201906-1149OC.

Simpson, J. L. *et al.* (2006) 'Inflammatory subtypes in asthma: Assessment and identification using induced sputum', *Respirology*, 11(1), pp. 54–61. doi: 10.1111/j.1440-1843.2006.00784.x.

Siracusa, M. C. *et al.* (2013) 'Thymic Stromal Lymphopoietin-Mediated Extramedullary Hematopoiesis Promotes Allergic Inflammation', *Immunity*, 39(6), pp. 1158–1170. doi: 10.1016/j.immuni.2013.09.016.

Skepper, J. N. *et al.* (2001) 'Herpes Simplex Virus Nucleocapsids Mature to Progeny Virions by an Envelopment → Deenvelopment → Reenvelopment Pathway', *Journal of Virology*, 75(12), pp. 5697–

5702. doi: 10.1128/JVI.75.12.5697-5702.2001/ASSET/AA5260B3-4B67-4708-9B3E-9F342516057E/ASSETS/GRAPHIC/JV12122952DF.JPEG.

Skevaki, C. *et al.* (2021) 'SARS-CoV-2 infection and COVID-19 in asthmatics: a complex relationship', *Nature Reviews Immunology*, pp. 202–203. doi: 10.1038/s41577-021-00516-z.

Smits, H. H. *et al.* (2016) 'Microbes and asthma: Opportunities for intervention', *Journal of Allergy and Clinical Immunology*, 137(3), pp. 690–697. doi: 10.1016/J.JACI.2016.01.004.

Smole, U. *et al.* (2020) 'Serum amyloid A is a soluble pattern recognition receptor that drives type 2 immunity', *Nature Immunology*, 21(7), pp. 756–765. doi: 10.1038/s41590-020-0698-1.

Sorel, O. and Dewals, B. G. (2019) 'The critical role of genome maintenance proteins in immune evasion during gammaherpesvirus latency', *Frontiers in Microbiology*. doi: 10.3389/fmicb.2018.03315.

Speck, S. H. and Ganem, D. (2010) 'Viral latency and its regulation: lessons from the gamma-herpesviruses.', *Cell host & microbe*, 8(1), pp. 100–15. doi: 10.1016/j.chom.2010.06.014.

Spits, H., Bernink, J. H. and Lanier, L. (2016) 'NK cells and type 1 innate lymphoid cells: Partners in host defense', *Nature Immunology*, 17(7), pp. 758–764. doi: 10.1038/ni.3482.

Spits, H. and Di Santo, J. P. (2011) 'The expanding family of innate lymphoid cells: Regulators and effectors of immunity and tissue remodeling', *Nature Immunology*, pp. 21–27. doi: 10.1038/ni.1962.

Stadhouders, R. *et al.* (2018) 'Epigenome analysis links gene regulatory elements in group 2 innate lymphocytes to asthma susceptibility', *Journal of Allergy and Clinical Immunology*, (2018). doi: 10.1016/j.jaci.2017.12.1006.

Starkey, M. R. *et al.* (2016) 'Programmed Death Ligand 1 Promotes Early-Life Chlamydia Respiratory Infection-Induced Severe Allergic Airway Disease', *American journal of respiratory cell and molecular biology*, 54(4), pp. 493–503. doi: 10.1165/RCMB.2015-0204OC.

Staton, T. L. *et al.* (2017) 'Seasonal variability of severe asthma exacerbations and clinical benefit from lebrikizumab', *Journal of Allergy and Clinical Immunology*, 139(5), pp. 1682-1684.e3. doi: 10.1016/j.jaci.2016.12.965.

Steer, C. A. *et al.* (2020) 'Lung group 2 innate lymphoid cells are trained by endogenous IL-33 in the neonatal period', *JCI Insight*, 5(14). doi: 10.1172/JCI.INSIGHT.135961.

Stempel, M., Chan, B. and Brinkmann, M. M. (2019) 'Coevolution pays off: Herpesviruses have the

license to escape the DNA sensing pathway', *Medical Microbiology and Immunology*, pp. 495–512. doi: 10.1007/s00430-019-00582-0.

Stevenson, P. G. (2004) 'Immune evasion by gamma-herpesviruses', *Current Opinion in Immunology*, pp. 456–462. doi: 10.1016/j.coi.2004.05.002.

Stier, M. T. *et al.* (2016) 'Respiratory syncytial virus infection activates IL-13–producing group 2 innate lymphoid cells through thymic stromal lymphopoietin', *Journal of Allergy and Clinical Immunology*. Elsevier Inc., 138(3), pp. 814-824.e11. doi: 10.1016/j.jaci.2016.01.050.

Stier, M. T. *et al.* (2018) 'IL-33 promotes the egress of group 2 innate lymphoid cells from the bone marrow', *The Journal of Experimental Medicine*, 215(1), pp. 263–281. doi: 10.1084/jem.20170449.

Strachan, D. P. (1989) 'Hay fever, hygiene, and household size', *BMJ (Clinical research ed.)*, 299(6710), pp. 1259–1260. doi: 10.1136/BMJ.299.6710.1259.

Sugita, K. *et al.* (2018) 'Type 2 innate lymphoid cells disrupt bronchial epithelial barrier integrity by targeting tight junctions through IL-13 in asthmatic patients', *Journal of Allergy and Clinical Immunology*, 141(1), pp. 300-310.e11. doi: 10.1016/j.jaci.2017.02.038.

Sun, B. *et al.* (2018) 'Characterization and allergic role of IL-33-induced neutrophil polarization', *Cellular and Molecular Immunology*, 15(8), pp. 782–793. doi: 10.1038/cmi.2017.163.

Sunil-Chandra, N. P. *et al.* (1992) 'Virological and pathological features of mice infected with murine gamma-herpesvirus 68', *The Journal of general virology*, 73 (Pt 9)(9), pp. 2347–2356. doi: 10.1099/0022-1317-73-9-2347.

Sunil-Chandra, N. P. *et al.* (1994) 'Lymphoproliferative disease in mice infected with murine gammaherpesvirus 68.', *The American Journal of Pathology*, 145(4), p. 818. Available at: /pmc/articles/PMC1887324/?report=abstract (Accessed: 16 August 2021).

Šupolíková, M. *et al.* (2018) 'The Effect of Murine Herpesvirus 68 (MHV-68) Related Growth Factor 68 (MHGF-68) on the Tumor Progression in Athymic Nude Mice', *American Journal of Biomedical and Life Sciences*, 6(6), pp. 127–131. doi: 10.11648/J.AJBLS.20180606.13.

Svensson, A. *et al.* (2010) 'Exposure to Human Herpes Virus Type 6 Protects Against Allergic Asthma in Mice', *Journal of Allergy and Therapy*, 1(1). doi: 10.4172/2155-6121.1000101.

Takeuchi, K. *et al.* (2006) 'Prevalence of Epstein-Barr virus in Japan: Trends and future prediction', *Pathology International*, 56(3), pp. 112–116. doi: 10.1111/j.1440-1827.2006.01936.x.

Tarakanova, V. L. *et al.* (2010) 'MHV68 complement regulatory protein facilitates MHV68 replication in primary macrophages in a complement independent manner', *Virology*, 396(2), pp. 323–328. doi: 10.1016/j.virol.2009.10.030.

Terrell, S. and Speck, S. H. (2017) 'Murine gammaherpesvirus M2 antigen modulates splenic B cell activation and terminal differentiation in vivo', *PLoS Pathogens*, 13(8). doi: 10.1371/journal.ppat.1006543.

Thio, C. L.-P. *et al.* (2018) 'Regulation of type 2 innate lymphoid cell–dependent airway hyperreactivity by butyrate', *Journal of Allergy and Clinical Immunology*. doi: 10.1016/j.jaci.2018.02.032.

Thomsen, S. F. *et al.* (2011) 'Increase in the heritability of asthma from 1994 to 2003 among adolescent twins', *Respiratory Medicine*, 105(8), pp. 1147–1152. doi: 10.1016/j.rmed.2011.03.007.

Uddin, M. *et al.* (2019) 'NETopethic inflammation in chronic obstructive pulmonary disease and severe asthma', *Frontiers in Immunology*, 10(FEB). doi: 10.3389/fimmu.2019.00047.

Uppal, T. *et al.* (2014) 'KSHV LANA—The Master Regulator of KSHV Latency', *Viruses*, 6(12), pp. 4691–4998. doi: 10.3390/v6124961.

Veerati, P. C. *et al.* (2020) 'Airway Epithelial Cell Immunity Is Delayed During Rhinovirus Infection in Asthma and COPD', *Frontiers in Immunology*, 11. doi: 10.3389/fimmu.2020.00974.

Veldhoen, M. *et al.* (2008) 'Transforming growth factor-β "reprograms" the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset', *Nature Immunology*, 9(12), pp. 1341–1346. doi: 10.1038/ni.1659.

Vély, F. *et al.* (2016) 'Evidence of innate lymphoid cell redundancy in humans', *Nature Immunology*, 17(11), pp. 1291–1299. doi: 10.1038/ni.3553.

Verma, M. *et al.* (2018) 'Experimental asthma persists in IL-33 receptor knockout mice because of the emergence of thymic stromal lymphopoietin–driven IL-9 + and IL-13 + type 2 innate lymphoid cell subpopulations', *Journal of Allergy and Clinical Immunology*, 142(3), pp. 793-803.e8. doi: 10.1016/j.jaci.2017.10.020.

Verma, M. *et al.* (2021) 'The molecular and epigenetic mechanisms of innate lymphoid cell (ILC) memory and its relevance for asthma', *Journal of Experimental Medicine*. J Exp Med, 218(7). doi: 10.1084/jem.20201354.

Verzele, N. A. J. *et al.* (2021) 'The impact of influenza pulmonary infection and inflammation on vagal

bronchopulmonary sensory neurons', *FASEB Journal*, 35(3). doi: 10.1096/fj.202001509R.

Vos, T. *et al.* (2020) 'Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019', *The Lancet*, 396(10258), pp. 1204–1222. doi: 10.1016/S0140-6736(20)30925-9.

Vu, L. D. *et al.* (2019) 'Elevated levels of type 2 respiratory innate lymphoid cells in human infants with severe respiratory syncytial virus bronchiolitis', *American Journal of Respiratory and Critical Care Medicine*, 200(11), pp. 1414–1423. doi: 10.1164/rccm.201812-2366OC.

W.S. Li, B. *et al.* (2018) 'T cells and ILC2s are major effector cells in influenza-induced exacerbation of allergic airway inflammation in mice', *European Journal of Immunology*. doi: 10.1002/eji.201747421.

Walford, H. H. *et al.* (2014) 'Increased ILC2s in the eosinophilic nasal polyp endotype are associated with corticosteroid responsiveness', *Clinical immunology*, 155(1), p. 126. doi: 10.1016/J.CLIM.2014.09.007.

Wallrapp, A. *et al.* (2017) 'The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation', *Nature*, 549(7672), pp. 351–356. doi: 10.1038/nature24029.

Wan, H. *et al.* (1999) 'Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions', *Journal of Clinical Investigation*, 104(1), pp. 123–133. doi: 10.1172/JCI5844.

Wan, J. *et al.* (2020) 'HMGB1-induced ILC2s activate dendritic cells by producing IL-9 in asthmatic mouse model', *Cellular Immunology*. doi: 10.1016/j.cellimm.2020.104085.

Wan, J. *et al.* (2021) 'ILC2-derived IL-9 inhibits colorectal cancer progression by activating CD8+ T cells', *Cancer Letters*, 502, pp. 34–43. doi: 10.1016/j.canlet.2021.01.002.

Wang, C. *et al.* (2020) 'Sex differences in group 2 innate lymphoid cell-dominant allergic airway inflammation', *Molecular Immunology*, 128, pp. 89–97. doi: 10.1016/j.molimm.2020.09.019.

Wang, D. *et al.* (2018) 'Respiratory syncytial virus prevents the subsequent development of ovalbumin-induced allergic responses by inhibiting ILC2 via the IL-33/ST2 pathway', *Immunotherapy*, 10(12), pp. 1065–1076. doi: 10.2217/imt-2018-0059.

Weizman, O. El *et al.* (2017) 'ILC1 Confer Early Host Protection at Initial Sites of Viral Infection', *Cell*, 171(4), pp. 795-808.e12. doi: 10.1016/j.cell.2017.09.052.

Weizman, O. El *et al.* (2019) 'Mouse cytomegalovirus-experienced ILC1s acquire a memory response dependent on the viral glycoprotein m12', *Nature Immunology*. Nature Publishing Group, 20(8), pp. 1004–1011. doi: 10.1038/s41590-019-0430-1.

White, D. W., Beard, S. R. and Barton, E. S. (2012) 'Immune modulation during latent herpesvirus infection', *Immunological Reviews*, pp. 189–208. doi: 10.1111/j.1600-065X.2011.01074.x.

Willart, M. A. M. *et al.* (2012) 'Interleukin-1α controls allergic sensitization to inhaled house dust mite via the epithelial release of GM-CSF and IL-33', *The Journal of Experimental Medicine*, 209(8), pp. 1505–1517. doi: 10.1084/jem.20112691.

Winkler, C. *et al.* (2019) 'Activation of group 2 innate lymphoid cells after allergen challenge in asthmatic patients', *Journal of Allergy and Clinical Immunology*, 144(1), pp. 61-69.e7. doi: 10.1016/j.jaci.2019.01.027.

Wohlleben, G. *et al.* (2003) 'Influenza A Virus Infection Inhibits the Efficient Recruitment of Th2 Cells into the Airways and the Development of Airway Eosinophilia', *The Journal of Immunology*, 170(9), pp. 4601–4611. doi: 10.4049/jimmunol.170.9.4601.

Wolterink, R. G. J. K. *et al.* (2012) 'Pulmonary innate lymphoid cells are major producers of IL-5 and IL-13 in murine models of allergic asthma', *European Journal of Immunology*, 42(5), pp. 1106–1116. doi: 10.1002/eji.201142018.

Wong, K. J. *et al.* (2017) 'Group 2 innate lymphoid cell activation in the neonatal lung drives type 2 immunity and allergen sensitization', *Journal of Allergy and Clinical Immunology*, 153, pp. 370–378. doi: 10.1016/j.jaci.2016.12.981.

Wong, S. H. *et al.* (2012) 'Transcription factor RORα is critical for nuocyte development', *Nature Immunology*, 13(3), pp. 229–236. doi: 10.1038/ni.2208.

Wu, J. *et al.* (2019) 'Critical role of OX40/OX40L in ILC2-mediated activation of CD4+T cells during respiratory syncytial virus infection in mice', *International Immunopharmacology*, 76. doi: 10.1016/j.intimp.2019.105784.

Wu, W. *et al.* (2014) 'Unsupervised phenotyping of Severe Asthma Research Program participants using expanded lung data', *The Journal of allergy and clinical immunology*, 133(5), pp. 1280–1288. doi: 10.1016/J.JACI.2013.11.042.

Xu, H. *et al.* (2018) 'Interleukin-33 contributes to ILC2 activation and early inflammation-associated

lung injury during abdominal sepsis', *Immunology and Cell Biology*. doi: 10.1111/imcb.12159.

Yagi, R. *et al.* (2014) 'The transcription factor GATA3 is critical for the development of all IL-7Rα-expressing innate lymphoid cells', *Immunity*, 20(403), pp. 378–388. doi: 10.1016/j.immuni.2014.01.012.

Yang, Q. *et al.* (2013) 'T Cell Factor 1 Is Required for Group 2 Innate Lymphoid Cell Generation', *Immunity*, 38(4), pp. 694–704. doi: 10.1016/j.immuni.2012.12.003.

Yu, Y. *et al.* (2015) 'The transcription factor Bcl11b is specifically expressed in group 2 innate lymphoid cells and is essential for their development', *The Journal of Experimental Medicine*, 212(6), pp. 865–874. doi: 10.1084/jem.20142318.

Yudanin, N. A. *et al.* (2019) 'Spatial and Temporal Mapping of Human Innate Lymphoid Cells Reveals Elements of Tissue Specificity', *Immunity*, 50(2), pp. 505-519.e4. doi: 10.1016/J.IMMUNI.2019.01.012.

Zalani, S., Holley-Guthrie, E. and Kenney, S. (1996) 'Epstein-Barr viral latency is disrupted by the immediate-early BRLF1 protein through a cell-specific mechanism.', *Proceedings of the National Academy of Sciences of the United States of America*, 93(17), p. 9194. doi: 10.1073/PNAS.93.17.9194.

Zeippen, C. *et al.* (2017) 'The Major Envelope Glycoprotein of Murid Herpesvirus 4 Promotes Sexual Transmission', *Journal of Virology*, 91, pp. 235–252. doi: 10.1128/JVI.00235-17.

Zeis, P. *et al.* (2020) 'In Situ Maturation and Tissue Adaptation of Type 2 Innate Lymphoid Cell Progenitors', *Immunity*, 53(4), pp. 775-792.e9. doi: 10.1016/j.immuni.2020.09.002.

Zhang, K. *et al.* (2020) 'RAGE-induced ILC2 expansion in acute lung injury due to haemorrhagic shock', *Thorax*, 75(3), pp. 209–219. doi: 10.1136/thoraxjnl-2019-213613.

Zhong, C., Zheng, M. and Zhu, J. (2018) 'Lymphoid tissue inducer—A divergent member of the ILC family', *Cytokine and Growth Factor Reviews*, pp. 5–12. doi: 10.1016/j.cytogfr.2018.02.004.

Zhong, H. *et al.* (2017) 'Increased innate type 2 immune response in house dust mite-allergic patients with allergic rhinitis', *Clinical Immunology*, 183, pp. 293–299. doi: 10.1016/j.clim.2017.09.008.

Zhou, W. *et al.* (2016) 'Prostaglandin I2 signaling and inhibition of group 2 innate lymphoid cell responses', *American Journal of Respiratory and Critical Care Medicine*, 193(1), pp. 31–42. doi: 10.1164/rccm.201410-1793OC.