### 1. General information

#### 1.1 Coordinating investigator 1

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#### Coordinating investigator 2

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Title of project:

English: Innate lymphoid cells: identification of their role in allergic asthma and exacerbations upon respiratory tract infection

Dutch: Innate lymfoide cellen: identificatie van hun rol in asthma en exacerbaties na luchtweginfecties

Project number: 3.2.13.067

Time schedule:

Start of project: 12-11-2012

Duration of project: 48 months

Period of funding: from 12-11-2012 to 12-11-2016

Grant: € 250,000 (zie ook accountantsverklaring)

Short description of the project for public information (in Dutch, see guidelines) (max. 250 words):

Veel symptomen van allergisch astma, zoals verhoogde slijmproductie, benauwdheid en luchtweg-onsteking worden veroorzaakt door de ontstekingsbevorderende factoren IL-4, IL-5 en IL-13. Tot voor kort werd verondersteld dat deze factoren vooral door T helper 2 (Th2) cellen worden geproduceerd. Maar recent werd een nieuw type afweercel ontdekt, de ILC2 (group 2 innate lymphoid cell), die ook grote hoeveelheden IL-5 en IL-13 kan produceren. In dit project hebben we de rol van ILC2s in astma onderzocht. We hebben ILC2s in de muis specifiek zichtbaar kunnen maken in de longen en hun localisatie vastgesteld, net onder de epitheelcellen die de bekleding van de luchtwegen vormen. We hebben in een huisstofmijt-geïnduceerde astma model gevonden dat ILC2 activatie afhankelijk is van T cellen. Dit is opmerkelijk, omdat in modellen gebaseerd op andere allergenen is gevonden dat T cel activatie nu juist afhankelijk is van ILC2s. In huisstofmijt-geïnduceerde astma blijken ILC2s dus geen vroege bron van Th2 cytokines, maar dragen ze in belangrijke mate bij aan een Th2-gemedieerde ontsteking. We vonden ook dat niet ILC2s maar Th2 cellen een verhoogde activiteit hebben na influenza virus infectie en kunnen bijdragen aan verergering van astma symptomen. Tenslotte vonden we d.m.v. epigenetische analyses dat in circulerende ILC2s ~80% van alle met astma geassocieerde genen actief is, hetgeen een rol van ILC2s in astma bij de mens ondersteunt.

2. Report

2.1 Summary:

Title: Innate lymphoid cells: identification of their role in allergic asthma and exacerbations upon respiratory tract infection

Authors: R.W. Hendriks and W.S. Li

Dept./Institute(s): Department of Pulmonary Medicine, Erasmus MC Rotterdam

Keywords (max. 6): Asthma, epigenetics, exacerbation, house dust mite, influenza, innate lymphoid cell type 2

Abstract (max. 250 words):

Many hallmarks of asthma, including increased mucus production, airway obstruction and inflammation are caused by the pro-inflammatory cytokines IL-4, IL-5 and IL-13. Classically, these cytokines are thought to be mainly produced by T helper 2 (Th2) cells. However, a novel immune cell type, named group 2 innate lymphoid cells (ILC2), producing high amounts of IL-5/IL-13, was recently identified. In this project, we explored the role of ILC2s in asthma pathogenesis. Using a reporter mouse to visualize ILC2s, we observed that ILC2s are preferentially located close to the airway epithelium or near lymphoid clusters cells that arise after chronic exposure to house dust mite (HDM). In HDM-induced allergic airway inflammation, we found that ILC2 activation required T cells. This is remarkable, because in asthma models based on other allergens the opposite was shown: T cell activation required ILC2s. We also identified a novel ILC2 subpopulation characterized...
by low IL-2R expression. We conclude that in HDM-mediated airway inflammation in mice ILC2 do not provide an early innate source of IL-5/IL-13, but contribute to the Th2-mediated inflammation. Furthermore, the activity of Th2 cells (but not ILC2s) was increased following influenza virus infection in mice, thereby likely contributing to inflammation-induced asthma exacerbation. When we quantified ILC2s in peripheral blood of asthma patients, we found that their frequency was not increased, although a correlation with Th2 cell frequencies was observed. Finally, epigenetic analyses indicated that in circulating ILC2s ~80% of asthma-associated genes from GWAS were active, supporting a role for ILC2 in human asthma.

2.2 Description of original question/aim (max. 150 words):

In this project, we aimed to test the hypothesis that ILC2s play an essential role in asthma pathogenesis and may comprise the long sought for link between respiratory tract infection and asthma exacerbation.

To identify the role of ILC2s in asthma pathogenesis, we planned to investigate:

1. How ILC2s collaborate with Th2 cells in allergic airway inflammation in mice.
2. How an earlier influenza virus infection affects ILC2 activity and HDM-induced asthma.
3. The involvement of ILC2s in *M. pneumoniae* infection and subsequent HDM-induced asthma.
4. Whether ILC2s are increased in bronchial mucosal biopsies or the peripheral blood of asthma patients, compared with healthy controls.

2.3 Results (max. 2500 words, please submit a maximum of 4 figures and diagrams separately):

**Aim 1: Collaboration between ILC2s and Th2 cells in allergic airway inflammation in mice.**

**ILC2 activation in HDM-mediated airway inflammation critically depends on T cells**

Allergic asthma is a chronic inflammation of the airways mediated by an adaptive type 2 immune response. Upon allergen exposure, group 2 innate lymphoid cells (ILC2) can be rapidly activated and represent an early innate source of IL-5 and IL-13. Previous studies using e.g. papain or *Alternaria alternata* to trigger allergic airway inflammation have established that: (i) ILC2 induction precedes T cell activation, (ii) ILC2s play a critical role in initiating the T cell response, and (iii) ILC2 induction is strongly dependent on IL-33 signaling (reviewed in Li BW et al., Immunology 2013, **Publ. 1**).

In this project, we used a house dust mite (HDM)-driven asthma mouse model to study the induction of ILC2s in allergic airway inflammation. In broncho-alveolar lavage (BAL) fluid, lungs, and draining mediastinal lymph nodes (MLN), ILC2 activation was critically dependent on prior sensitization with HDM, indicating that adaptive immunity must be triggered before ILC2 activation (**Fig. 1A-E**). We employed mice in which T cells were unable to become activated and produce cytokines (conditional knock-out of the transcription factor CTCF). We found that in the absence of functional T cells, ILC2s were not induced by HDM exposure. In contrast, B cell-deficient mice developed normal ILC2 responses to HDM. In a kinetic analysis of BAL fluid, we found that T cell accumulation preceded ILC2 accumulation (**Fig. 1H-I**). From these findings, we concluded that T cells are required for ILC2 induction, whereby T-cell activation precedes ILC2 induction. During HDM-driven allergic airway inflammation ILC2 accumulation in BAL fluid is IL-33 independent, although infiltrating ILC2s produce less cytokines in *Il33⁻/⁻* mice (**Fig. 1F-G**). Transfer of *in vitro* polarized OVA-specific OT-II Th2 cells alone or in combination with Th17 cells followed by OVA and HDM challenge was not sufficient to induce ILC2s, despite significant eosinophilic inflammation and T-cell activation. In this asthma model, ILC2s are therefore not an early source of Th2 cytokines, but rather contribute to type 2 inflammation in which Th2 cells play a key role. Taken together, ILC2 induction in HDM-mediated allergic airway inflammation in mice critically depends on T cell activation. These findings were published in Li BW *et al.*, Eur J Immunol. 2016 **Publ. 2**.

We also found that shortly after birth ILC2s, eosinophils, basophils, and mast cells spontaneously accumulated in developing lungs in an IL-33-dependent manner. During the phase of postnatal lung alveolarization, HDM exposure further increased IL-33, which boosted cytokine production in ILC2s and activated CD11b+DCs. Decoy sST2 (soluble IL-33R) had a strong preventive effect on asthma in the neonatal period, less so in adulthood (De Kleer *et al.*, Immunity, **Publ. 3**).

**Differences in ILC2 phenotype between T cell-dependent or T cell–independent activation pathways**

Due to a lack of unique markers, the accurate phenotypic characterization and quantification of ILC2 requires a comprehensive panel of fluorescently labeled antibodies. The markers that are currently used to characterize ILC2 have not been standardized and often vary between research groups, which poses significant challenges when comparing data. Intranasal administration of the pro-inflammatory cytokine IL-33 in mice is associated with strong, Th2 cell-independent ILC2 activation.
ILC2s are also activated in mouse models of allergic asthma based on the physiologically relevant house dust mite (HDM) allergen, which parallel eosinophilic airway inflammation observed in asthma patients. We have described the analysis of ILC2 by flow cytometry in these two commonly used allergic airway inflammation models in the mouse in Li BW et al., Methods Mol Biol. 1559:169-183 (Publ. 4).

Next, we aimed to further investigate whether phenotypic differences would exist between ILC2s that are activated in a T cell-dependent or T cell-independent fashion. To this end, we compared ILC2 cell surface phenotype and localization in IL-33- and HDM-driven airway inflammation (Fig. 2A). We employed mice harboring a yellow fluorescent protein (YFP) reporter for Gata3 (GATIR mice), a transcription factor essential for ILC2 development and function (we reviewed GATA3 function in innate and adaptive immunity in Tindemans I et al., Immunity 2014; see also Tindemans et al., J Allergy Clin Immunol, Publ. 5). This GATIR mouse model, in which an IRES-YFP sequence was inserted at the 3′ end of the Gata3 gene, was obtained through collaboration with prof. H.J. Fehling (Ulm, Germany). We established that the YFP signals accurately paralleled the expression of Gata3 protein, as detected by intracellular flow cytometry using Gata3-specific antibodies, both within Lineage+ and Lineage− lymphocyte fractions. Moreover, the influx of eosinophils and ILC2s in BAL fluid after IL-33 administration or HDM exposure did not differ between GATIR mice and wildtype controls. Mean fluorescent intensity (MFI) of intracellular Gata3 and proportions of IL-5 and IL-13 producing ILC2s were also comparable between the two groups of mice in BAL fluid, lungs and mediastinal lymph nodes. Using the YFP-reporter, we have been able to localize YFP-expressing cells in the lung (Fig. 2B), and to confirm their Gata3 expression by an in situ hybridization technique. Both in IL-33-driven and in HDM-driven airway inflammation ILC2s were present in the lung submucosa close to epithelial cells, as identified by confocal microscopy (Fig. 2B). In chronic HDM-driven airway inflammation ILC2s were also found to be increased (Vroman et al., Clin Exp Allergy 2016, Publ. 6) and were inside organized cellular infiltrates in close proximity to T cells.

Flow cytometry experiments showed that upon IL-33 stimulation in vivo Gata3+ ILC2s in the broncho-alveolar lavage (BAL) fluid had a uniform CD25+CD127+T1ST2+ICOS−KLRG1+ phenotype. In contrast, HDM-induced ILC2s showed a heterogeneous surface marker phenotype, which varied between tissues (Fig. 2C). We identified a prominent population of previously undescribed CD25low ILC2s that were also low in ICOS and KLRG1 expression. In HDM-induced allergic airway inflammation, CD25low as well as CD25high ILC2s contributed to type 2 cytokine production. Collectively, our findings show that ILC2s are phenotypically more heterogeneous than previously thought, whereby their cell surface marker expression is highly variable and dependent on the way they are activated. These findings are now in a manuscript that is currently under revision (Li BW et al., J Immunol., in revision).

**Genome-wide differences between BAL and MLN ILC2s**

Finally, we noticed that ILC2s that were induced in vivo upon intranasal IL-33 exposure manifested various phenotypic differences in different compartments. Both BAL and MLN ILC2 populations consisted predominantly of IL-5 and/or IL-13-producing effector cells. However, we did notice tissue-specific differences in ILC2-associated cell surface marker (e.g. KLRG1, T1/ST2) expression (Fig. 1C). ILC2s in BAL fluid are thought to be directly activated upon exposure to IL-33, but for MLN cells, which are present in the interface between B cell follicles and the T cell zone, this is less clear. We employed the GATIR mice outlined above, greatly facilitating ILC2 purification (Lin’Sca-1-YFP+).

RNA-seq analyses revealed that the expression of ~633 genes was significantly higher in BAL ILC2s and of ~568 genes the expression was higher in MLN ILC2s (n=1201, 2-fold difference and adjusted P < 0.05) (Fig. 2E-F), with excellent reproducibility between biological replicates (R² > 0.97). In agreement with our flow cytometry analyses, Klrk1 expression was higher in MLN ILC2s while Il1rl1 (encoding T1/ST2, the IL-33 receptor) was upregulated in BAL ILC2s (Fig. 2F). Compared to ILC2s from MLN, BAL ILC2s exhibited a more inflammatory phenotype expressing high levels of the IL-33 and cysteinyl leukotriene (Cysltr1/2) activation receptors, pro-inflammatory cytokines (e.g. Il9, Il21, Il15, Il13) and chemoattractants (e.g. Cxcl6, Eotaxin2) (Fig. 2F). Besides Klrk1, MLN ILC2s also expressed higher levels of Oxl40l and MHC class II-like H2-O genes, suggesting that ILC2s in the MLN are more involved in cell-to-cell interactions with dendritic cells and/or Th2 cells. In addition, MLN ILC2s expressed high levels of Alox5, which encodes the 5-lipoxygenase enzyme involved in the production of leukotrienes - potent activators of ILC2s and allergic asthma (Fig. 2F). We also observed tissue-specific expression differences in metabolism and survival/proliferation-associated pathways. While BAL ILC2s upregulated genes involved in the lipid response (e.g. the free fatty acid receptor gene Ffar2) and cell survival (e.g. Bcl2), MLN ILC2s showed enhanced glycolysis and cell division (Fig. 2F-G). Upregulation of an aerobic glycolytic metabolic program is a hallmark of effector lymphocyte activation and therefore consistent with increased ILC2 proliferation in the MLN (e.g. upregulation of Mkl67 encoding the classic Ki67 proliferation marker). Interestingly, the absolute increase in ILC2 cellularity was comparable for both
BAL and MLN samples, suggesting that the ILC2 expansion in the MLN is a late event. Together, these data reveal location-dependent modification of the ILC2 transcriptome, indicating tissue-specific modes of action for ILC2s in AAI. These findings have been submitted for publication (Stadhouders R, Li BW et al., submitted).

Aim 2: How an earlier influenza virus infection affects ILC2 activity and HDM-induced asthma.

For this part of the project, it was important to determine the localization of ILC2s in the lungs and lymph nodes. However, visualization of ILC2s in situ is challenging because ILC2s lack the expression of a unique marker. Therefore, we have characterized the GATIR reporter mouse (collaboration with prof. H.J. Fehling, University of Ulm, Germany), allowing us to accurately determine the location of ILC2s in (i) naive mice, (ii) upon IL-33 exposure, and in an (iii) acute and (iv) chronic HDM exposure asthma model. We observed that in all these cases ILC2s were preferentially located close to the airway epithelium and – when present – within cellular infiltrates near the epithelial barrier and blood vessels of the bronchioles. ILC2s were also in and near B/T cell clusters (inducible bronchus-associated lymphoid tissue, iBALT) that arise upon chronic exposure to HDM. Within these lymphoid clusters, ILC2s were in close proximity to the T cell "zones". Although the draining lymph nodes were enlarged in HDM-treated animals, only very few ILC2s were detected (present at the B-T cell border).

Following inoculation with the X31 strain of influenza, ILC2s already appeared to accumulate in the lungs at day 4 post infection, in contrast to T cells which did not significantly increase until day 7 (Fig. 3A). However, cytokine production by ILC2s at day 4 remained low and was only initiated after T cell activation (Fig. 3B). Furthermore, confocal imaging of cellular infiltrates in the lungs showed that ILC2s were located in proximity to T cells similar to chronic HDM-induced asthma (Fig. 3C), indicating close communication between the two cell types. In order to assess the role of ILC2s in influenza-induced asthma exacerbation, we chronically exposed mice to HDM for 5 weeks and subsequently infected them with X31 influenza virus. As a result, a significant increase in cell influx in the BAL fluid was observed in mice that received both HDM and X31 (asthma exacerbation model) compared to HDM or X31 alone. Interestingly, the infiltrating granulocytes in such an exacerbation model was primarily composed of neutrophils and more importantly, ILC2 numbers were suppressed compared to HDM alone (Fig. 3D). IL-5 production in CD4+ T cells was elevated in the combination group while simultaneously IL-5+ ILC2s were significantly reduced. These results suggest that ILC2s have a limited impact on exacerbations caused by influenza infection and that instead T cells are the primary effector cells. This finding was further investigated in ICOS-DTR mice, in which ICOS-expressing cells are susceptible to deletion by diphtheria toxin. The insertion of flanking LoxP sites in the DTR construct combined with Cre recombinase behind the CD4 promotor, however rescues T cells from deletion resulting in specific ablation of ILC2s. Deletion of ILC2s in both acute and chronic HDM models generated similar levels of eosinophilic inflammation thus confirming a secondary role for ILC2s in HDM-induced asthma (Fig. 3G, 3H). These findings are currently included in a manuscript to be submitted for publication soon (Li BW et al., in preparation).

Aim 3: The involvement of ILC2 cells in M. pneumoniae infection and subsequent HDM-induced asthma.

We analysed recruitment and activation of B, T and innate lymphocytes as well as various myeloid cell populations. In this acute infection model, in which Mycoplasma was rapidly cleared, eosinophilia and concomitant activation and induction of ILC2 was limited (Fig. 3F). We decided to focus first on influenza and perform influenza infections in chronic HDM-asthma and to focus on our human studies (Aim 2 and Aim 4).

Aim 4: Investigation of ILC2s in bronchial mucosal biopsies and peripheral blood of asthma patients and healthy controls.

Our human study exploring the role of ILC2s in controlled and uncontrolled allergic asthma has been approved by the METC (approval enclosed) and we are currently including asthma patients and healthy control subjects. We have set up sensitive methods to detect ILC2 in peripheral blood and induced sputum samples by flow cytometry (Fig. 4).

ILC2s have been proposed to play an important role in the pathogenesis of asthma due to their ability to produce type 2 cytokines. It has been reported that peripheral blood ILC2s are increased or have enhanced activity in asthma patients. However, since asthma is a heterogeneous disease it is unclear whether ILC2s are important in all endotypes of asthma. In this study, we stratified asthma patients according to the Global Initiative for Asthma (GINA) 2014 classifications into the following subgroups: controlled, partly controlled and uncontrolled, based on asthma control
questionnaire (ACQ) score. We used flow cytometry to compare peripheral blood and induced sputum immune profiles of asthma patients with healthy controls and correlated these to clinical parameters. In this report, we have included 23 healthy controls and 36 asthma patients (10 controlled, 10 partly controlled and 16 uncontrolled asthma patients) in our analysis of peripheral blood samples. Blood eosinophils were significantly elevated in all asthma groups and the proportions of Th2 and Th17 within CD4+ T helper cells were increased as well (Fig. 4B). However, ILC2 frequency remained comparable to healthy controls (See Fig. 4A for gating strategy; Fig 4C).

Interestingly, ILC2 frequency was significantly correlated with Th2 and Th17 frequency, but not with that of eosinophils (Fig. 4D). For ILC2s the expression levels of the surface marker IL-2RA (CD25) and the transcription factor Gata3 were also similar in asthmatic patients and controls. Our findings suggest that peripheral blood ILC2 numbers are either not important in asthma pathogenesis or are not affected by an inflammatory status in the lung. Therefore, local ILC2s in the lung require further investigation as they may still contribute to asthma symptoms.

However, it remains possible that there are important differences in the ILC2s between asthma patients and healthy controls, regarding detailed phenotype or epigenetic marks. Therefore, we have characterized the H3K4Me epigenome of directly isolated human peripheral blood naïve ILC2 fractions, as well as in vitro activated ILC2 by IL-25 and IL-33 (Fig. 4E). Assays have been set up to perform these epigenetic analyses with as few as ~10,000 cells by ChIP-Seq (Chromatin immunoprecipitation followed by next generation sequencing). We found that in circulating naïve ILC2s ~80% of asthma-associated genes from GWAS (genome wide associated studies) were active, supporting a role for ILC2 in human asthma. An example of the epigenetic marks of ILC2s at one the loci that is most significantly associated with asthma in human (IL1RL1, encoding the IL-33R T1/ST2 component) is shown in Fig. 4E. These findings have recently been submitted for publication (Stadhouders R, Li BW et al., submitted).

In addition, we participated in a study aiming to identify how ILC precursors (ILCp) in human give rise to mature tissue-resident ILC subsets. We identified circulating and tissue ILCps in humans that fail to express the transcription factors and cytokine outputs of mature ILCs but have these signature loci in an epigenetically poised configuration. Human ILCps robustly generated all ILC subsets in vitro and in vivo. While human ILCps expressed low levels of retinoic acid receptor (RAR)-related orphan receptor C (RORC) transcripts, these cells were found in RORC-deficient patients and retained potential for natural killer (NK) cells, interferon gamma-positive (IFN-γ+) ILC1s, IL-13+ ILC2s, and for IL-22+, but not for IL-17A+ ILC3s. Our results supported a model of tissue ILC differentiation ("ILC-poiesis"), whereby diverse ILC subsets are generated in situ from systemically distributed ILCps in response to local environmental signals. These data have been published in Lim A.I. et al., Cell 2017).

2.4 Did the study solve the original question? yes/no (explain) (max. 250 words):

(1) **How ILC2s collaborate with Th2 cells in allergic airway inflammation in mice.**

**YES.** We thoroughly mapped the relationship between ILC2s and T cells in HDM-induced allergic airway inflammation and used multiple approaches to determine the dependency of ILC2s on T cell activation in this model. Furthermore, we showed phenotypic differences in ILC2s after T cell-dependent and T cell-independent manners of activation.

(2) **How an earlier influenza virus infection affects ILC2 activity and HDM-induced asthma.**

**YES.** We have shown that influenza infection causes ILC2 accumulation prior to T cells but that ILC2 cytokine production occurred concomitantly with T cell cytokine production. Furthermore, our influenza-induced asthma exacerbation model displayed a primarily neutrophilic infiltrate and that T cells, and not ILC2s, are the main cytokine producing cells in such an exacerbation.

(3) **The involvement of ILC2s in *M. pneumoniae* infection and subsequent HDM-induced asthma.**

**PARTIALLY.** We have determined the extent of ILC2 induction after mycoplasma infection. However, due to our focus on influenza, which is known to induce the most severe types of asthma exacerbations in humans, we have not fully developed an exacerbation model incorporating *M. pneumoniae*.

(4) **Whether ILC2s are increased in asthma patients compared with healthy controls.**

**YES.** We have compared ILC2 values in peripheral blood between asthmatics (36 instead of the originally proposed 20) and healthy controls and have found that ILC2s were not significantly elevated. Moreover, we have set up innovative epigenetic Chip-Seq methods. The study is still in progress and more asthma patients will be included.

3 Papers (see instructions)
3.1 All publications (published or submitted peer-reviewed manuscripts):


**Publ. 5.** Tindemans I, Lukkes, M., de Bruijn MJ, Li BW..... and Hendriks RW (2017). Notch signaling in T cells is essential for allergic airway inflammation, but expression of the Notch ligands Jagged 1 and Jagged 2 on dendritic cells is dispensable. J Allergy Clin Immunol. (in press).


3.2 All publications (not peer-reviewed like abstracts, newspapers, websites, etc.):

**2013**
- Li BW. NRS Young Investigator Symposium – November 2013 Amsterdam, the Netherlands. Poster presentation.

**2014**
- Li BW. EAACI Winter School on Basic Research in Allergy and Clinical Immunology 2014 (Poiana Brasov, Romania). Oral presentation.
- Li BW. International Conference on Lymphocyte Activation & Immune Regulation 2014 (Irvine, USA). Oral presentation.
4. Implementation (see instructions):

- The research is of fundamental nature and does not have immediate implementations in health care. However, we have uncovered new insights into the function of ILC2s in HDM-mediated asthma in mouse models. Most importantly, we have demonstrated that ILC2s have multiple phenotypes depending on methods of activation and are more plastic than previously thought, opening ways for further research, in particular into the field of ILC2s and steroid-resistant asthma.
- Regarding ILC2 research in human, we have found that the frequency of ILC2s was not increased in peripheral blood of asthma patients, although a correlation with Th2 cell frequencies was observed. In an ongoing research effort, together with the Sint Franciscus Gasthuis, we are currently characterizing detailed phenotypic differences in peripheral blood and sputum immune cells (eosinophils, neutrophils, B and T cell subsets, ILCp and ILC2) in patients with controlled, partially controlled and uncontrolled asthma. We plan to link these differences to clinical parameters.
- Finally, epigenetic analyses indicated that in circulating ILC2s ~80% of asthma-associated genes from GWAS were active, supporting a role for ILC2 in human asthma. We now are extending these epigenetic human studies in an NWO-VENI supported project (to Dr. Ralph Stadhouders) in order to identify epigenetic marks in ILC2s or T cell subsets that are unique to asthma patients.