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Research Visit Institute: VIB (Vlaams Instituut voor Biotechnologie), Gent, Belgium
Sponsor: Drs. Bart Lambrecht and Hamida Hammad
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AllerGen contribution: $5,700

Through the AllerGen NCE *International Trainee Research Visit Award* I had the opportunity to spend 9 weeks in the laboratory of Dr. Bart Lambrecht at the VIB in Gent, Belgium between June 18th, 2015 and August 24th, 2015. Dr. Lambrecht is the head of the Inflammation Research Center at the VIB and his research group is particularly interested in how dendritic cells, along with other innate immune cells such as basophils and the epithelium can influence the adaptive T cell response. The lab has a general focus on Th2 immune responses such as those associated with allergic asthma. Dr. Lambrecht and his group leader Dr. Hamida Hammad are both internationally known for their research in the field, and their trainees and support staff were excellent and provided a great research environment together with the exceptional facilities at the VIB.

The primary goal of my research exchange was to gain additional expertise in the influence of dendritic cells on modulating the polarization and activation of Th2 cells. Dr. Lambrecht's group has a unique research tool, transgenic mice with T cell receptors (TCRs) specific for an allergen component of dust mites (Der p1 allergen), the resulting mice being named 1Der ("wonder"). While initially it was planned to utilize these 1Der mice to complete ongoing work in Dr. McNagny's lab with SHIP1-deficient DCs, that manuscript was completed before starting my placement at the VIB. Instead, we decided to develop an *in vivo* model utilizing the adoptive transfer of *in vitro* polarized $CD4^+$ 1Der T cells initially as a proof of concept prior to further experiments looking at the role of IL-21 signaling in effector Th2 responses.

Firstly, we optimized *in vitro* polarization conditions to direct naïve $CD4^+$ T cells into a Th2 phenotype. $CD4^+$ T cells were isolated from the spleens and lymph nodes of naïve 1Der mice and were cultured in media containing the cytokines IL-2 and IL-4 and antibodies to IFNγ and IL-12p40. Cells were cultured in plates either with immobilized antibodies to CD3 and CD28 to act as a polyclonal TCR activation signal, or with freshly isolated splenic $CD11c^+$ DCs pulsed with the peptide derived from dust mites that is recognized specifically by 1Der TCRs. Through the testing of various concentrations of cytokines, antibodies and ratios between T cell and APC (or αCD3/CD28 concentration) were able to optimize both the Th2 polarization and expansion of 1Der T cells (Figure 1). Polarization utilizing immobilized antibodies to CD3 and CD28 offered the most robust Th2 polarization, as measured by GATA-3^+ cells, however the fold expansion over the culture period was reduced by 20% compared to DC-T cell co-culture polarizations.

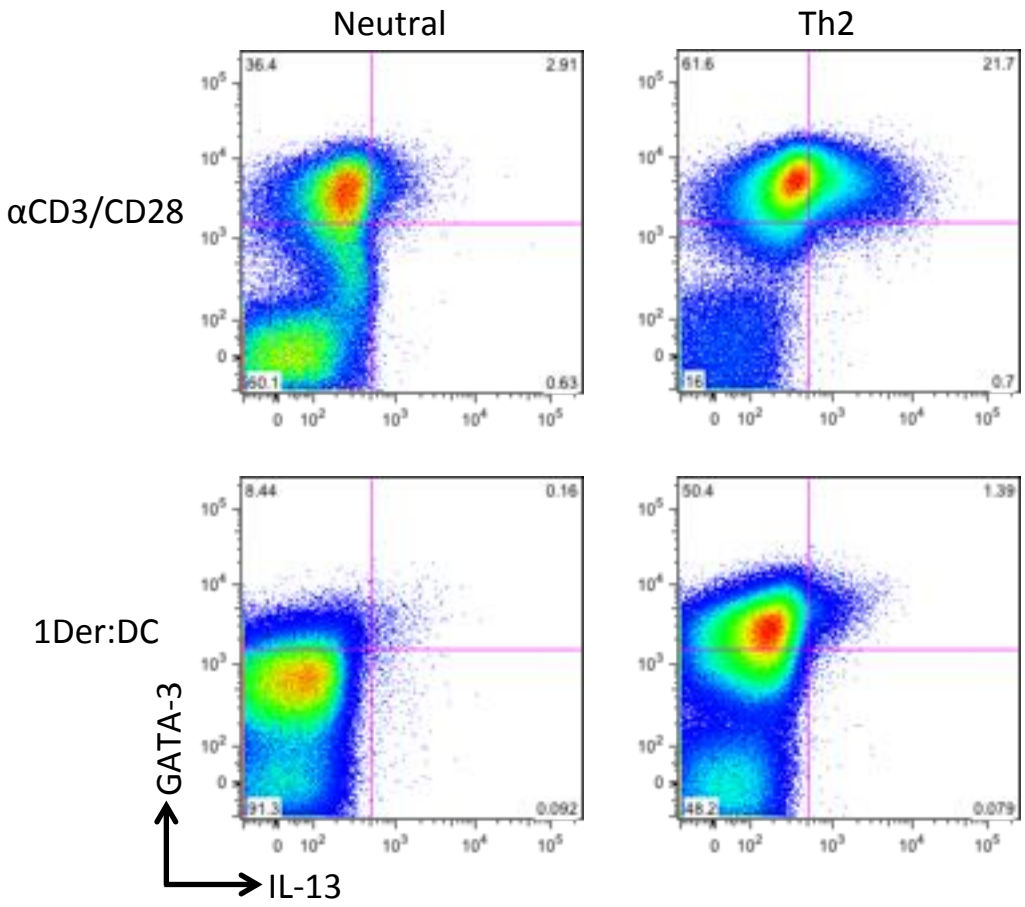

Figure 1: Isolated CD4+ T cells from 1Der mice were polarized *in vitro* in conditions promoting either neutral (IL-2 alone) or Th2 (IL-2, IL-4, αIFNγ and αIL-12p40) polarization using either immobilized αCD3/CD28 or Der p1-pulsed DCs as the TCR stimulation source. On day 6 cells were collected and expression of GATA-3 and production of IL-13 was analyzed by flow cytometry.

We next sought to examine whether adoptively transferred, *in vitro* polarized 1Der cells could induce symptoms associated with allergic asthma, namely airway eosinophilia. We transferred $1x10^6$ T cells intra-venously into naïve C57Bl/6 recipient mice, followed by five intranasal challenges with house dust mite (HDM) antigen. Bronchoalveolar lavages were collected 96 hours after the final HDM challenge, and infiltrating airway leukocytes were counted and differentiated by flow cytometry (Figure 2). As expected, mice that did not receive adoptively transferred T cells did not exhibit airway eosinophilia following the HDM challenges, as robust eosinophilia requires an adaptive immune response not elicited at this early timepoint. However, adoptive transfer of Th2-polarized T cells from the DC-co cultures induced significant infiltration of eosinophils compared to mice receiving neutral T cells(Figure 3). Surprisingly, Th2-polarized T cells from the αCD3/CD28 conditions had an attenuated effect compared to DC-co cultures, despite displaying increased GATA-3 and IL-13 staining following the *in vitro* polarization prior to their transfer. This could be due to poor proliferation and

survival of these cells once removed from the strong activation signals associated with CD3/CD28-induced TCR activation.

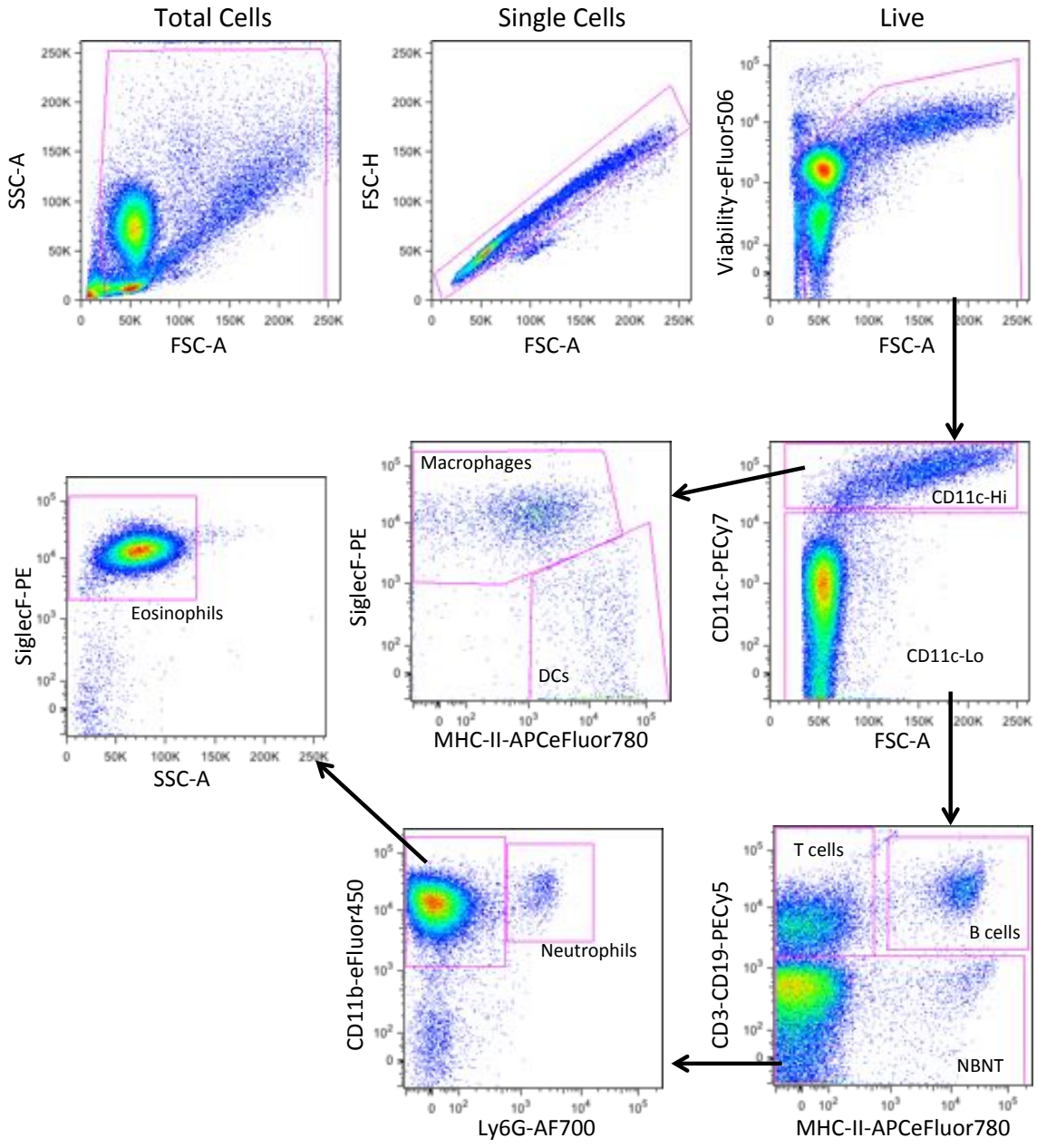

Figure 2: Leukocytes collected in the bronchoalveolar lavage (BAL) were differentiated by flow cytometry using fluorescently labeled antibodies to CD3, CD11b, CD11c, CD19, Ly6G and SiglecF and dead cells were excluded using fixable viability dye (eFluor 506).

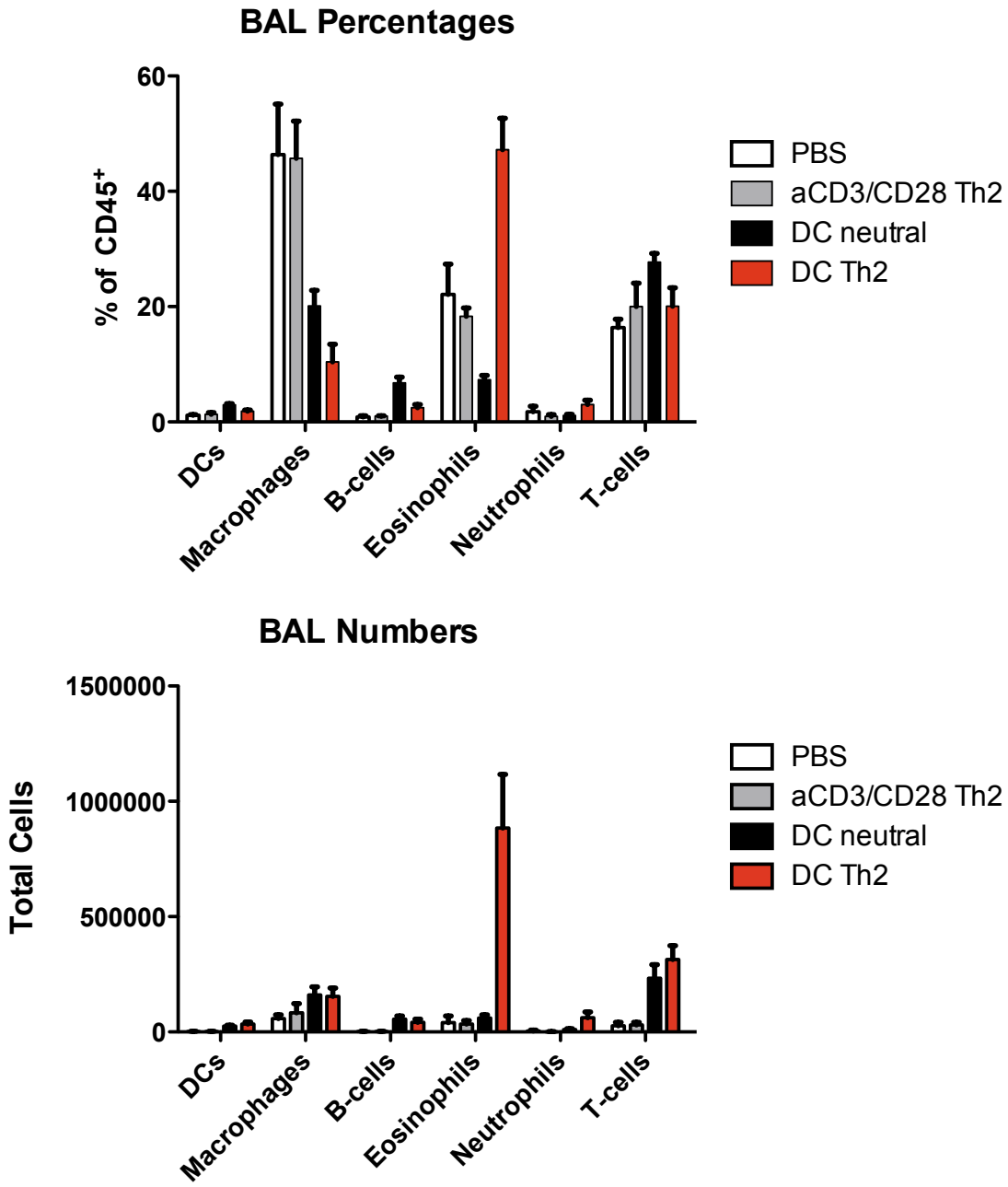

Figure 3: Naïve C57Bl/6 mice received 1x10^6 1Der CD4$^+$ T cells following *in vitro* polarization under neutral or Th2 conditions. Mice were challenged on 5 consecutive days with HDM, followed by a harvest and enumeration and differentiation of leukocytes recovered from the BAL 96 hours following the final HDM challenge.

After optimizing the model and providing a proof of concept, our next goal was to transfer Th2-polarized T cells isolated from 1Der mice and 1Der:IL-21R$^{-/-}$ mice. IL-21 is a cytokine released by follicular helper T cells (T_{FH}) and previous work from Dr. Lambrecht's group has shown an important role for IL-21 in the

development of Th2 cells (Coquet JM et al., *Immunity* 2015). We sought to explore whether IL-21 stimulation was required for the "innate" like stimulation of our Th2-polarized T cells in the short-term model we had developed. Unfortunately at this time there was a parvovirus outbreak in the animal unit at the institute and animal experiments had to be minimized. However, these experiments are continuing and I look forward to hearing their results.

The lung environment contains a multitude of innate immune cells, however their relative contribution to the formation of an adaptive response is unclear. It is widely accepted that dendritic cells are the primary antigen presenting cell in the lung, but basophils and ILC2s have been shown to express MHC-II and could potentially play important roles in shaping the ensuing T cell response. To test this hypothesis we derived dendritic cells (GM-CSF), basophils (TSLP) and ILC2s (IL-2, IL-25, IL-33, TSLP) from the bone marrow of wild-type mice. These cells were pulsed with house dust mite antigen and then co-cultured in various combinations with CFSE-labelled 1Der $CD4^+$ T cells. We found that DCs were essential to induce antigen-specific T cell proliferation, and the addition of ILC2s and/or basophils did not significantly magnitude of the proliferation. However, the presence of ILC2s appeared to significantly influence the activation state of the T cells. T cells from co-cultures containing ILC2s had significantly higher expression of CD40L (Figure 4), but not of T1/ST2 (IL-33R), OX40 or CD44 (data not shown). It's unclear whether cell-cell contact between ILC2s and DCs and/or T cells is required for this response, or if the high concentrations of cytokines secreted by ILC2s, such as IL-5 and IL-13 promote this increased expression.

This research exchange provided myself with an invaluable experience conducting research in a high caliber international research institute. The studies I had initiated have been continued on by other members of Bart's group and additional collaborative experiments have been suggested and are currently in progress, hopefully leading to a long lasting and fruitful collaboration between the VIB and AllerGen. I have attached some pictures of the scientific complex and research institute (Figure 5).

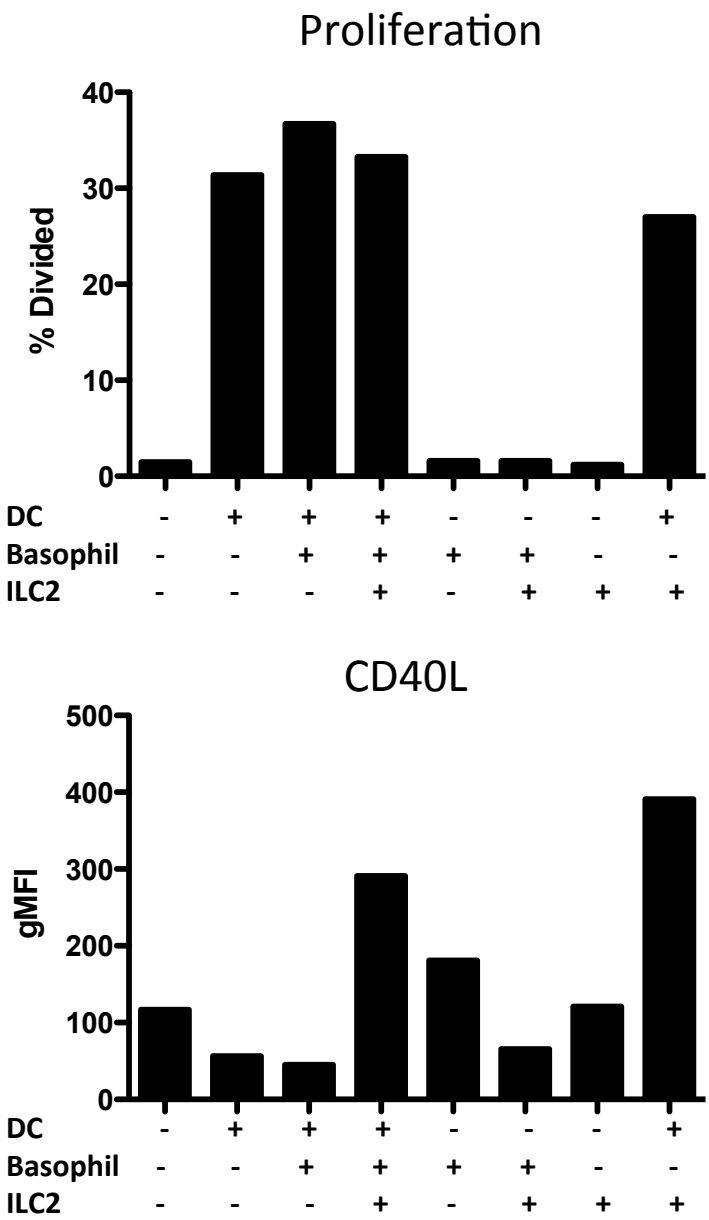

Figure 4: Dendritic cells (10,000/well), basophils (2,000/well) and/or ILC2s (2,000/well) were cultured in the presence of CFSE-labelled 1Der T cells. Proliferation and expression of CD40L was quantified by flow cytometry.

Figure 5: Technologiepark and VIB Research Center.