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A mouse model for allergic asthma based on NOD-scid IL2Rγnull mice reconstituted with peripheral blood mononuclear cells from affected individuals

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Summary

Asthma is a chronic disease of the lower respiratory tract and affects patients all over the world. The symptoms are varying from increased wheezing, or coughing up to breathlessness and is usually associated with airway hyperresponsiveness and airway inflammation. Approximately 300 million people worldwide are suffering from asthma. A lot of fundamental research was performed during the last years in order to develop alternatives to the currently available standard therapie that is mainly focused on symptom control of asthma. For instance patients with severe allergic asthma can now be treated with an IgE antibody (omalizumab). In most cases new and experimental agents were tested in animal models in regard to efficacy and toxicity. However, many animal models poorly reflect the human conditions and numerous promising therapeutics failed to reach an effect in humans. Therefore, it is pivotal to generate new and enhanced animal models for the purpose of new drug development.

In the present work a model for allergic asthma in an immune deficient mouse strain was validated and characterized. Thereby NOD-*scid IL2Rγ*^{null} mice were engrafted with human leucocytes from patients affected by allergic asthma, or healthy donors. The NOD-*scid IL2Rγ*^{null} mice are incapable of T-, B-, and NK-cell production due to a mutation of the IL-2 receptor. To induce the asthmatic response ovalbumin (OVA), or house dust mite extract (HDM) were introduced intranasally. Thereby a small droplet of allergen was placed on the nose and consequently inhaled by anaesthetised mice.

Mice treated with HDM showed an increased influx of macrophages into the lung, an elevated clinical score that measured asthma related symptoms as well as general behaviour of the mice and elevated numbers of Th17 cells in the lung. OVA challenged mice revealed similar clinical and histological symptoms, but clearly differed in the composition of the immune cell subtypes, indicating a different immune response compared to HDM. Moreover, immune cells from allergic asthmatic patients showed an organ-specific homing to lung tissue of mice, which was not observed with cells from healthy donors. Furthermore, this asthma model was dominated by macrophages and dendritic cells. Finally, in our mouse model the allergic response was suppressed with prednisolone, while the experimental IL-4 and IL-13 receptor antagonist pitrakinra showed no beneficial effects.

In summary we suggest the NOD-*scid IL2Rγ*^{null} mouse asthma model as highly suitable for preclinical testing of newly developed anti-asthma drugs.

Zusammenfassung

Asthma ist eine chronische Erkrankung der unteren Atemwege und betrifft Patienten auf der ganzen Welt. Die Symptome reichen von vermehrtem Giemen und Husten bis hin zur Atemnot und ist in der Regel mit einer Entzündung, sowie einer Überempfindlichkeit der Atemwege assoziiert. Weltweit leiden etwa 300 Millionen Menschen an Asthma. In den letzten Jahren wurde viel Grundlagenforschung betrieben, um Alternativen zur derzeitigen Standardtherapie, die sich hauptsächlich auf die Kontrolle der Symptome konzentriert, zu entwickeln. Beispielsweise ist es nun möglich Patienten mit schweren allergischem Asthma mit einem IgE Antikörper (omalizumab) zu behandeln. Meistens werden dabei neue experimentelle Wirkstoffe zuerst im Tiermodell auf Effektivität und Toxizität getestet. Viele Tiermodelle spiegeln jedoch die humanen Bedingungen nur unzureichend wieder, weshalb im Tiermodell vielversprechende Medikamente keine Wirkung im Menschen zeigen. Eine Verbesserung der Tiermodelle ist daher unerlässlich für die Generierung neuer Wirkstoffe.

Im Rahmen dieser Doktorarbeit wurde ein allergisches Asthmamodells in einem immuninkompetenten Mäusestamm hinsichtlich der Immunreaktionen untersucht. Dabei wurden NOD-*scid IL2Rγ*^{null} Mäuse mit humanen Leukozyten von allergischen asthmatischen, oder gesunden Spendern rekonstituiert. NOD-*scid IL2Rγ*^{null} Mäuse können aufgrund einer Mutation des IL-2 Rezeptors weder T- und B-Zellen noch NK Zellen ausbilden. Ein asthmatischer Schub wurde mittels intranasaler Injektion von Ovalbumin (OVA) bzw. Hausstaubmilbenallergen (HDM) ausgelöst. Dafür wurde solvatisiertes Allergen tröpfchenweise auf die Nase aufgetragen und von den anästhesierten Mäusen inhaliert.

Mäuse, die mit HDM behandelt wurden, zeigten einen erhöhten Einstrom von Makrophagen in die Lunge, einen erhöhten klinischen Score, welcher neben Asthma spezifischen Symptomen auch das auch das allgemeine Verhalten der Mäuse misst, sowie eine vermehrte Anzahl von Th17- Zellen in der Lunge. OVA-behandelte Mäuse zeigten ähnliche klinische und histologische Befunde, unterschieden sich allerdings deutlich in der Zusammensetzung der Immunzell-Subtypen. Dies lässt auf einen unterschiedlichen Wirkmechanismus von HDM und OVA schließen. Zusätzlich zeigten Immunzellen von allergischen Asthmatikern eine Organspezifität, die bei gesunden Spendern nicht zu beobachten war. Des Weiteren war dieses Asthmamodel dominiert von Makrophagen und Dendritischen Zellen. Zuletzt wurde in unserem Tiermodell die

allergische Reaktion durch Prednisolon erfolgreich unterdrückt. Im Gegensatz dazu zeigte der experimentelle IL-4 und IL-13 Rezeptorantagonist Pitrakinra keine Wirkung.

Zusammenfassend konnten wir zeigen, dass das NOD-*scid IL2Rγ*^{null} Mausmodell sich für die präklinische Testung neuer Medikamente gegen Asthma eignet.

Abbreviations

AD	Atopic Dermatitis
AHR	Airway Hyperresponsiveness
APC	Antigen Presenting Cell
APC	Allophycocyanin (only in Cytometry)
BCR	B Cell Receptor
CCR3	C Chemokine Receptor
CD	Cluster of Differentiation
CD62L	L-Selektin
CRTH2	Chemoattractant Receptor-Homologous
DAMP	Damage-Associated Molecular Pattern
DC	Dendritic Cell
ECP	Eosinophil Cationic Protein
FACS	Fluorescence-Activated Cell Sorting
FCS	Fetal Calf Serum
FELASA	Federation of Laboratory Animal Science Association
FITC	Fluorescein Isothiocyanate
FOXP3	Forkhead-Box-Protein3
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
HBSS	Hanks Buffered Salt Solution
HLA-DR	Human Leucocyte Antigen D Related (Klasse II-Molekül)
IFN-γ	Interferon-γ
IL	Interleukin
LPS	Lipopolysaccharide
MHC	Major Histocompatibility Complex
NK	Natural Killer
NOD-scid	Non Obese Diabetic - Severe Combined Immunodeficiency
PAMP	Pathogen-Associated Molecular Pattern

PAR	Proteinase-Activated Receptor
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PE	Phycoerythrin
PerCP-Cy	Peridine-Chlorophyll-Protein complex Cyanine dye
PGD_2	Prostaglandin D_2
PHA	Phytohämagglutinin
PRR	Pattern Recognition Receptor
ROR	Retinoic Acid Receptor-Related Orphan Receptor
SD	Standard Deviation
TCR	T Cell Receptor
TGF-β	Transforming Growth Factor-β
Th	T Helper
TLR	Toll-Like Receptor
TNF-α	Tumor Necrosis Factor-α
TSLP	Thymic Stromal Lymphopoietin
TSLPR	Thymic Stromal Lymphopoietin Receptor
UC	Ulcerative Colitis
VCAM-1	Vascular Cell Adhesion Molecule-1

1. Introduction

1.1. Immune system:

The immune system protects our body from dangerous insults like infections with bacteria, viruses and parasites as well as from tumours. It consists of two major branches: the innate and the adaptive immune system. While the innate immune response is the first, quick response to evolutionary conserved pathogenic structures (PAMPs), mediated by granulocytes and monocytes/macrophages, mast cells as well as complement molecules, the adaptive immunity is a highly specific immune reaction, mediated by T- and B cells as well as antibodies and able to differentially respond to various pathogens with different types of T lymphocytes and various antibody isotypes with different effector functions.

1.2. Innate immunity:

Epithelial cells form the first line of defence in our bodies. They are connected via tight junctions and can usually not be penetrated by pathogens [82]. In lung tissue the epithelial cells are interspersed by mucus producing goblet cells [82,84]. Numerous environmental factors, especially proteases like Derp 1 can disrupt the tight junctions and damage the epithelial layer [110- 113]. As a first reaction of the innate immune system, the damaged epithelial cells release danger signals like thymic stromal lymphopoietin (TSLP), IL-25, or IL-33, which leads to a dilation of the blood vessels and the subsequent recruitment of granulocytes, mast cells and macrophages [82]. Additionally IL-33 levels are associated with the severity of asthma and treatment with anti IL-33 showed beneficial effects in rhinovirus induced forms of asthma [106 108].

1.2.1. Granulocytes

Granulocytes can be divided into neutrophils, the largest population and necessary for the fight against bacteria via phagocytosis and NETosis, and eosinophils and basophils, which are mainly important for parasite clearance. Neutrophils are the first cells entering damaged or infected tissue, while eosinophils and basophils play major roles in allergic responses. It is also the eosinophils that play an important role in the pathomechanism of asthma (see also chapter 1.4).

1.2.2. Eosinophils

Eosinophil precursors differentiate from CD34+ pluripotent stem cells by GM-CSF and G-CSF in the bone marrow [1,2,3]. They are mainly activated by IL-5 [1-4], which, among others, also plays an essential role in the survival and migration of eosinophils. Once in the tissue, eosinophils are active for 18 hours up to 2 weeks [1-7]. They have numerous effector functions, varying from defence against bacteria, viruses, fungi and parasites to immune regulation [1,8,9]. One important factor secreted by eosinophils is the eosinophil cationic protein (ECP). ECP is antibacterial, toxic to helminths, can promote the proliferation of T and B cells and cause mast cell degranulation [1,8,9]. Moreover, eosinophils can release IL-4, IL-5 and IL-13, which are necessary for a T helper (Th)2 associated immune response, inflammatory and Th1 associated cytokines as interferon γ (IFN-γ), together with tumour necrosis factor α (TNFα). Additionally they can distribute transforming growth factor β (TGFβ), which also has airway remodelling capacity [1,3,10,11]. Eosinophils express multiple receptors, like pattern recognition receptors (PRR), Toll like receptors (TLR) or proteinase-activated receptors (PAR) on their cell surface [1,3]. These receptors typical for cells of the innate immune system are essential for pathogen recognition. Additionally, they express the IgE receptor FcεRI and CC chemokine receptor 3 (CCR3), which are - to some extent - eosinophil-specific [1,3]. Interestingly, eosinophils can process and present antigen to T cells, which emphasizes their importance in linking innate and adaptive immunity [1,3]. Eosinophils play a key role in asthma because of their numerous effector roles and their remodelling capacity on bronchial tissue cells [1,10].

1.2.3. Mast cells

In addition to granulocytes, mast cells are among the first reacting cells after encounter of pathogens. Mast cell invasion into the lung is highly dependent on vascular cell adhesion molecule (VCAM)-1 expression on the endothelium [12]. Mast cells express TLRs and a highly specific IgE receptor (FcεRI). TLRs are activated by pathogen-associated molecular patterns (PAMPs) mainly introduced by viruses and bacteria. Upon activation mast cells release the contents of their inflammatory granula. These granula contain cysteinyl leucotrienes and proteases, the latter damage the invaded pathogen. A mix of cytokines and chemokines, also released by the granula, attracts macrophages and lymphocytes. One example of such a chemokine is Prostaglandin D_2 (PGD_2), which binds to the chemoattractant receptor-homologous molecule (CRTH2 or CD294) mainly expressed on Th2 cells [13]. Like eosinophils mast cells play a pivotal role in allergic asthma [107].

1.2.4. Antigen Presenting Cells (APC)

T cells only recognize antigen presented as peptides on MHC molecules on the cell surface. CD8+ cytotoxic T cells see antigen peptides of pathogens presented on MHC class I molecules, while CD4+ T helper cells need their antigen peptides presented on MHC class II molecules. While all nucleated cells express MHC class I molecules, MHC class II molecules are normally only expressed by "professional" antigen-presenting cells (APC), which are dendritic cells, monocytes/macrophages and neutrophils but also by B cells and activated human and rat T cells [104]. Under inflammatory condition, many tissue cells can aberrantly express MHC class II molecules and thus perpetuate auto reactive T cell responses. B and T cells can only present antigen to activated T cells, while presentation of antigen by professional APC is mandatory for the primary activation of naive T cells.

Decoyed by chemokines and cytokines, phagocytes support the clearance of bacteria. Circulating monocytes, the precursors of macrophages, as well as pre classical DC (cDC) and plasmacytoid DC (pDC) enter the target tissue and harvest antigen [105] Then they migrate via the lymphatic vessels to the next draining lymph node to present the antigen on their major histocompatibility complex (MHC) class II molecules on the cell surface and thus function as antigen-presenting cells for T lymphocytes. Moreover, they can collect antibodies with bound antigen via their Fc receptors and present those native molecules to B cells.

It is still difficult to clearly differentiate monocytes/macrophages and DC [14], since they are sharing surface markers. One important surface marker of both is CD86 (also known as B7.2), which is an activation marker, highly expressed after inflammatory stimuli and serves as "second signal" for the activation of T cells [15,16].

One way to activate macrophages is by binding TSLP, which is among others secreted by epithelial cells, via the TSLP-receptor (TSLPR) [86]. Another way how macrophages can be activated and which needs CD1a, were discovered more recently. Most bacterial membranes are coated with lipopolysaccharide (LPS), which are structural peptides and belong to the PAMPs. LPS can bind to CD1a on macrophages and consequently leads to their activation [17,18]. Worth mentioning is the recent finding that CD1a-bearing macrophages are significantly more frequent in patients suffering from ulcerative colitis (UC) than in healthy humans, suggesting a key role in allergic diseases [19].

Some surface markers are tissue-specifically (and species-specifically) expressed by DC. The classical DCs are sub grouped into cDC1 and cDC2, and both are found in the lung, together with monocyte-derived (moDC) and plasmacytoid DC. Lung cDC1 and 2 express CCR7 and are migratory, while for moDC and pDC it is not clear whether they can migrate. All subgroups express different markers, and they also differ between mice and man [105].

After activation macrophages undergo a maturation processes with up- or downregulation of various surface markers. For example CD64, a marker for inflammatory macrophages, is highly upregulated in inflamed tissue [20], also E-Cadherin, a single span transmembrane glycoprotein, and main component of the intercellular tight junctions, is upregulated upon activation [21]. E-Cadherin also serves as the only ligand of CD103 and marks an inflammatory subset of DC which is characterized by an increased expression of TLR and CD40, an increased production of IL-6, TNF-α, IL-23 and consequently the induction of an enhanced Th17 response [21].

1.3. Adaptive Immunity:

1.3.1. B cells

Apart from DC, B cells are also able to present antigen to T cells. In addition, B cells can exchange the constant region of their antibody heavy chain genes (class switch) and thus generate a new isotype of their antibodies. This allows the production of antibodies with the same antigen-specificity but different effector functions, e.g. fixation of complement, placental passage (IgGs), transport through mucosal epithelia (IgAs) or mediation of mast cell degranulation (IgE). This is dependent on certain cytokines and mediated by T cells [79]. In lymphoid organs like lymph nodes or spleen activation of B cells takes place in the B cell zone, where naïve B cells are organised in B cell follicles [79]. In addition to B cells, these follicles consist of DC, macrophages and antigens, which are bound and presented by FDC (follicular dendritic cells), or soluble in lymph [79,80,81]. When B cell receptors (BCR) are cross-linked by antigen it is taken up by receptor-mediated endocytosis, processed and finally presented on the MHC-class II molecules on the surface of the B cell [79]. Additionally CXCR5 is down regulated, which induces an emigration from the B cell zone, and simultaneously CCR7 is up regulated, leading to a migration towards the T cell zone inside the lymph node [73-75,79]. T cells recognizing their antigen presented by the B cells can induce their differentiation and clonal expansion. Depending on the cytokines, which are secreted by the Th cells, B cells can develop into memory or plasma cells [72]. Plasma cells are the main producers of antibodies and can be divided, according to their heavy chain, into immunoglobulin (Ig) M, IgD, IgG (4 subclasses: IgG1, 2, 3 and 4), IgE and IgA [73,76,78]. Interestingly, IgE-antibodies can bind to the Fcε receptors (FcεR) without prior binding to an antigen, therefore mast cells are always "decorated" with IgE antibodies and just need allergen contact for crosslinking the Fcε receptors and subsequent induction of degranulation. This is the main pathomechanism of the type 1 hypersensitivity reaction [78]. IgE is also associated with allergic asthma [77].

1.3.2. T-cells

The second major subgroup of lymphocytes are T cells. T cells also derive from multipotent hematopoietic precursor cells in the bone marrow. But in contrast to B cells, these precursor cells migrate via the blood stream into the thymus, where they start to mature and undergo positive and negative selection for proper binding to self-MHC molecules and recognition of auto antigens. In the thymus T cells also decide for the expression of CD4 or CD8 molecules on their surface, which determines the major effector function of T cells.

Maturated and naive T cells migrate to the peripheral lymph nodes where they can be activated by antigen-presenting APCs. One important surface molecule for lymph node homing is L-Selectin (CD62L) on naive T cells. CD62L is important for the first contact of T cells with the endothelial cells of peripheral lymph nodes and is therefore highly expressed on the surface of naive T cells [22]. In the lymph nodes APCs, especially DCs, present their antigen on MHC molecules to the T cell receptors (TCR). In a next step CD80 and CD86, which are expressed on DC, bind to CD28 on naive T cells. This "second signal" induces the production of IL-2 and the upregulation of CD25, the α chain of the IL-2 receptor, in T cells [23]. Additionally, more costimulatory molecules like CD134 (OX40) or CD40L are expressed on the surface, and the activated T cell starts to proliferate. Last but not least, in order to differentiate properly into the respective T helper (Th) subset, T cells must be stimulated by certain cytokines. Depending on the cytokine cocktail, CD4+ T cells can differentiate into one of the major Th subpopulations Th1, Th2, Th17 or regulatory T cells (Treg).

Th1

Th1 cells are necessary for the defence against viruses and intracellular bacteria. They release cytokines like IL-2, IFN-γ, TNFα and granulocyte macrophage colony stimulating factor (GM-CSF) [97].

Th17

Th17-cells are needed for the protection against extracellular pathogens, like fungi, or bacteria. They produce primarily IL-17, IL-21, IL-22, IL-26 and TNFα [24] and are regulated by the transcription factor retinoic acid receptor-related orphan receptor-γt (RORγt) and RORα [24-26]. Studies suggests that targeting RORγt might be a promising approach for the treatment of asthma [109], but to date there are controversial data concerning the role of Th17 cells in asthma, with evidence for their worsening [24,27-29] and protective impact [24,30-32].

Th2

Th2-cells are the prime producers of IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. IL-4 is important for allergic sensitization, activation B cells and promoting the switch to IgE and IgA and suppressing Th1 responses, while IL-13 plays a role in tissue remodelling and airway hyperresponsiveness (AHR) [24,33]. As mentioned above IL-5 is the major regulator of eosinophils [1-4,24,34]. In addition to IL-4, also IL-25, IL-33 and TSLP, which is released in consequence of epithelial damage, are necessary for the stimulation of Th2 cells [24]. Just as IgE, Th2 cells play a key role in type 1 hypersensitivity reactions and asthma.

Treg

Treg cells are necessary for counterbalancing the inflammatory response of Th cells. They suppress the cytokine production of inflammatory T cells and DCs especially by secreting anti-inflammatory cytokines like IL-10 or TGF-β and consequently ameliorate asthmatic responses [24,35-39]. Tregs are characterized by the expression of intracellular forkhead-box-protein (Foxp3), which is an essential transcription factor and necessary for cytokine and surface marker expression [23]. Beside Foxp3, expression of CD127, which is also known as the α chain of the IL-7 receptor, and CD25, the α chain of the IL-2 receptor, are also characteristic surface markers for Tregs [23]. Because of their protective role the amplification of the Treg response is currently a novel strategy for asthma therapy [24,40,41].

However, the maturation into the different T cell subsets is dependent on the micro milieu, and the fate of a certain T-cell is not definitely committed, which can consequently led to re-differentiation based on the exposed cytokines [24,42,43]. This enables the high plasticity of T-cells in order to quickly induce or downregulate a specific immune response.

1.4. Pathomechanism of asthma:

In 2014 approximately 334 million people worldwide suffered from the physical, psychological and social restriction of asthma [45]. Asthma is characterized by airway hyperresponsiveness (AHR), production of allergen-specific IgE and mast cell infiltration in the lung [46,47, 115]. In the USA, the estimated costs of asthma, composed of medical cost and loss of productivity, were $18 billion per year [45]. Albeit the immunological mechanisms causing the asthmatic symptoms, like wheezing, coughing, chest tightness or breathlessness [44,45,67, 115] are well described [45,48], no cure is available until now.

Asthma is caused by an allergic airway inflammation, in which lymphocytes, Th2-cytokines, DCs and granulocytes are the main effector cells [45,48]. Thereby the allergic inflammation can be divided in an early and a late phase reaction. In severe cases even a chronic reaction is possible [48]. As in other "classical" Th2 mediated diseases like ulcerative colitis (intestinal wall) or atopic dermatitis (epidermis), asthma can be triggered by damaged lung epithelial cells [82]. The damage of lung epithelial cells can be elicited by oxidative stress, air pollutants like tobacco smoke or pathogens [45,48,67]. As mentioned in the chapter "innate immunity" the consequence is the release of damage associated molecule patterns (DAMPs) including TSLP [82]. Especially proteases like Der p1, p3 and p9, the allergens from house dust mites (HDM) cause the increased release of TSLP by activating the protease activated receptor (PAR)-2 [67,82,83|. TSLP binds preferentially to monocytes and DC, which finally promote the asthma-triggering Th2 response [82,84,85]. On the other hand, airway DCs are capable of taking up the antigen (for example ovalbumin (OVA)) directly from the airway lumen and migrate to the draining lymph node, in order to present the antigen to the lymphocytes [67,87-89]. Furthermore, IgE, which is bound to Fcε receptors on the membrane of mast cells, can also bind the allergen [48], which leads to a crosslinking of the Fcε receptors followed by the activation of mast cells. Activated mast cells release preformed mediators like histamine, proteoglycans, neutral proteases and TNF-alpha [48,90,91]. These mediators give rise to the early phase reaction, which is characterized by bronchoconstriction, vasodilatation, increased mucus production and increased vascular permeability [34,48,90]. In addition, these mediators can also stimulate sensory nerves of the nose or skin, ultimately leading to sneezing, coughing or itching [48,92-95].

IL-4 and IL-13 secreted by mast cells, not only drive the early phase reaction, but also the recruitment of lymphocytes into lung tissue. This directed migration of T cells is supported by the DCs, which, on the one hand had migrated to the lymph node to present their antigen to T-cells,

but on the other hand express various co-stimulatory molecules like CD80, CD86, CD40, or OX40 and thereby regulating the immune response of T cells, B cells, mast cells, basophils and eosinophils.

Finally, the late phase response, which normally starts 2-6 hours and peaks at 6-9 hours after allergen contact, is triggered by the recruitment of circulating immune cells [48]. T helper cells, which release Th2-associated cytokines, induce an increased mucus production by the goblet cells [82,83]. Additionally, they induce the secretion of basic protein from eosinophils, which can cause further epithelial damage, and activate mast cells via IL-9 [96]. Last but not least, secretion of IL-4 and IL-13 by Th2 cells triggers IgE production by plasma cells [45]. In severe cases or in chronic stages IL-13 release by Th2 cells together with histamine, TNFα and IL-13 from mast cells, causes goblet cell metaplasia [82,83].

Because of their numerous effector functions T-cells are a promising target for future therapeutic treatments [40, 70]. The standard therapies of asthma up to date are beta 2 agonists, which relax the bronchi and are often used in combination with immunosuppressive glucocorticoids [41,49,50]. The use of inhaled glucocorticoids is currently the most effective asthma treatment [50].

1.5. Animal model

As already mentioned, it is not yet possible to cure asthmatic patients. Because of the complexity of the disease, simple cell cultures are insufficient to test the potential of new therapeutics. In order to investigate the (toxic) effects of new drugs, asthma models are needed that mirror the conditions and immunologic mechanisms of the human disease. Due to their high similarity to the human disease, chimeric animal models engrafted with human lymphocytes have gained more popularity during the last years. A recently described immunodeficient mouse strain exhibited a high engraftment level without the development of a graft versus host disease.

The non-obese diabetic - severe combined immune deficient, IL2 Receptor γ null (NOD-*scid* *IL2Rγ*^{null}) mice, which lack the IL-2R common γ chain and therefore cannot generate any B-, T- and natural killer (NK) cells have become a standard model in stem cell research [51]. In order to generate the X-linked *IL2Rγ*^{null} mutation NOD-scid females were crossed with B6.129S4-IL2Rγ$^{tmWjl/J}$ males. After that the (NOD X B6)F1+scid IL2Rγ- chainnull hemizygous descendants were backcrossed with NOD-*scid* females. The offspring were backcrossed two times and the heterozygote *IL2Rγ*^{null} female were identified via Polymerase chain reaction (PCR). After that the *IL2Rγ*^{null} allele were backcrossed with the NOD-*scid* background for eight further generations and finally the NOD.Cg-Prkdcscid Il2RγTm1Wjl/Sz females were crossed with NOD-*scid IL2Rγ*^{null} males. The NOD-*scid IL2Rγ*^{null} positive descendants were identified via quantitative PCR and double checked via flow cytometry (for more detailed information see also reference 51, 58, 59).

The major advantages of NOD-*scid IL2Rγ*^{null} (abbreviated NSG) mice when compared to NOD-*scid* or comparable mice strains are the higher lifespans of beyond 16 months, the highest engraftment levels of human lymphocytes and the high resistance to thymic lymphomas [51]. In addition to stem cell research, NSG mice have already been successfully used in Th2 associated disease models like ulcerative colitis [52-55]. Even first investigations of an asthmatic model were performed in this mouse strain by Martin and colleagues [121]. Therefore we used the NOD-*scid* *IL2Rγ*^{null} mouse strain for engraftment with human peripheral blood mononuclear cells (hPBMCs) from asthma patients in order to generate an allergic asthma mouse model.

1.6. Aim of this thesis

Animal models, which use reconstituted NSG mice, are already successfully being used for investigations in different Th2 associated diseases. However a detailed and extensive investigation regarding a NSG asthma model was never performed. The development of new and efficient asthmatic therapeutics is a huge challenge for modern medicine and pharmacy. Therefore it is necessary to get new insights in the immunological processes during an asthma attack and to establish a powerful asthma model to test new targets and consequently new drugs.

With this thesis we wanted to investigate the complex interplay between the various immune cells in order to get a better understanding of the on-going inflammation during an asthmatic exacerbation. Furthermore the translatability of the NSG asthma model to the human conditions was tested by using different donors with distinct immunologic status. Thus the influence of the donor background, which was asthmatic, or non-asthmatic (healthy) on the immunological responses of reconstituted NSG mice was investigated. Human leucocytes were isolated from spleen and lung tissue and examined in respect of activation or inhibition. Additionally the impact of human leucocytes on histological-, and clinical score in the NSG asthma model was analysed.

Furthermore the differences between ovalbumin, a protein which was formerly used as a classical allergen and house dust mite extract, which is one major asthmatic episode inducing allergen, regarding their immunological effects were examined.

Moreover pitrakinra, an IL-4 and IL-13 receptor antagonist was tested in respect of efficacy, safety and immunological role and compared to prednisolone, a glucocorticoid and the gold standard in asthma therapy.

Taking together following hypothesis were investigated:

Hypothesis 1: In vitro cell culture assays expose cells to artificial conditions and insufficiently reproduce donor specific immune profiles that are relevant *in vivo*.
Examination: PBMCs from asthmatic and healthy donors were cultivated and incubated with HDM, OVA and PHA. Differences in activation or inhibition of T-cells, B-cells and macrophages were investigated in respect of donor background and incubated antigen.

Hypothesis 2: HDM and OVA induce different immunological responses in NSG mice.
Examination: Leucocytes were isolated from mouse lung and spleen and stained for different immune cell populations. Maturation state of T-cells, B-cells, monocytes, macrophages and eosinophils was assessed by FACS. The results obtained were correlated for the exposure to HDM or OVA antigen. Additionally, the correlation between histological- and clinical scored with the different antigens was examined.

Hypothesis 3: The immunological status of the donor can be modelled in NSG mice and is essential for the immune responses.
Examination: PBMCs from asthmatic and healthy donors were transferred into NSG mice. The phenotype of the asthmatic response was assessed under the different antigens HDM and OVA. Asthmatic donors exhibited an allergic response against HDM and mice engrafted with asthmatic donor revealed an increased reaction after HDM challenge when compared to OVA. Furthermore, the correlations between histological- and clinical score and the immunological background were examined. Additionally, the maturation and the localisation of T-cells, B-cells, monocytes, macrophages and eosinophils were analysed with regard to the donor background of NSG mice.

Hypothesis 4: Preventive treatment with prednisolone and pitrakinra mitigate asthma symptoms after HDM-challenge
Examination: NSG mice were challenged with HDM and treated with prednisolone or pitrakinra or NaCl solution as a control. The histological and clinical scores as well as the inhibition of inflammatory T- and B-cells and macrophages were analysed in order to examine the preventive effect of prednisolone and pitrakinra.

2. Materials and Methods

2.1. Human PBMC Isolation

For the reconstitution of the NSG mice blood samples were collected from asthmatic and non-asthmatic (healthy) donors. All donors gave informed written consent and the study was approved by the Institutional Review Board (IRB) of the Medical Faculty at the University of Munich (2015-22).

Asthmatic donors were recruited via facebook and had to fulfill the following criteria:

positive asthma diagnosis by a clinician, positive skin prick test against HDM by a clinician, no medical treatment during the last 3 month, no allergic response against OVA (eggs), no further diseases like dermatitis and ulcerative colitis.

All asthmatic donors had a controlled form of asthma and a severity level of intermittent or light persistent. As a control sample leucocytes were also collected from healthy donors with a negative medical history for asthma and no clinical symptoms of allergies or asthma. These donors were named “Non-Asthma”.

Additionally for a detailed characterization all asthmatic donors with allergic response to house dust mite (HDM) had to complete a questionnaire (see also supplement).

After finishing the questionnaire, 80 ml blood was drawn from the arm vein of patients suffering from asthma and healthy volunteers. In order to avoid any contamination, or infections, all solutions and working steps were performed under sterile conditions. Approximately 80 ml of blood in trisodium citrate solution (S-Monovette, Sarstedt, Nürnberg, Germany), which were used as an anti coagulant, were diluted with 2 parts Hank’s balanced salt solution (HBSS, Sigma Aldrich, Deisenhofen, Germany) a buffer, which enables working at stable pH and ideal salt conditions. A maximum of 30 ml of the suspension was loaded onto Leukosep tubes (Greiner Bio One, Frickenhausen, Germany). The Leukosep tubes were filled with leucocyte separation medium (LSM, Carl Roth, Karlsruhe, Germany) and were necessary to segregate the leucocytes apart from the remaining blood components like erythrocytes. Leucocytes were isolated by centrifugation at 400 g for 30 min and no acceleration. The peripheral blood mononuclear cells (PBMC) containing interphase was collected, diluted with phosphate buffered saline (PBS, Carl Roth, Karlsruhe, Germany), which is a buffer similar to HBSS but more tolerated from NSG mice, to a final volume of 40 ml and centrifuged with 1400 g for 5 minutes. The cell number was

determined after resuspension in PBS at a concentration of 40 x 10^6 cells/ ml using a Neubauer Zählkammer (Carl Roth, Karlsruhe, Germany).

Six to twelve week old NOD IL-2Rγ^{null} mice were intravenously (tail vein) engrafted with 100 µl (4 x 10^6 cells) cell suspension.

The number of the experiments and the quantity of used animals can be seen in tab 1

Tab 1: Experimental summary. Numbers of experiment with different donor backgrounds. The quantity of animals in the different experimental groups are depicted in regard to the donor background of the NSG-mice. Abbreviations: OVA = Ovalbumin; HDM = House dust mite

Donor	Control	OVA	HDM	Pitrakinra	Prednisolone
Asthmatic Donor 1	4	4			
Asthmatic Donor 1	4		6		
Asthmatic Donor 1			5		
Asthmatic Donor 1	4		4	4	4
Asthmatic Donor 2	4	4	4		
Asthmatic Donor 3	4	4	4		
Asthmatic Donor 4	4		4	4	4
Healthy Donor 1	6	5			
Healthy Donor 2	4	5	5		
Healthy Donor 3	4	4	4		

2.2. Study protocol

Animal studies were approved by the animal welfare committee of the government of Upper Bavaria, Germany (55.2-1-54-2532-68-2014) and performed in compliance with German Animal Welfare Laws.

NOD.cg-Prkdc^{SCID} Il2rg^{tm1Wjl}/Szj mice (abbreviated as NOD IL-2Rγ^{null} or NSG) were obtained from Charles River Laboratories (Sulzfeld, Germany) and kept under specific pathogen free conditions in individually ventilated cages with unlimited access to water and food. The facility is under surveillance of the Federation of Laboratory Animal Science Association (FELASA) guidelines.

On day 3 and day 16 mice were presensitized by intraperitoneal application of 200 µl of 10 µg ovalbumin (OVA, Sigma, Deisenhofen, Germany), or 200 µl of 10 µg house dust mite (HDM, Dr. Weyers, Aachen, Germany) dissolved in a PBS/Alu Gel S (Serva Electrophoresis GmbH, Heidelberg) emulsion (1:4). Thereby Alu Gel S worked as an adjuvant and boosted the immunologic reaction. For triggering the asthmatic response mice were challenged on day 24 and at 2 consecutive days with intranasal injection of 50 µg OVA (respectively HDM) dissolved in PBS only under general anaesthesia using 4% isofluran via a 100 µl Pipette (Carl Roth, Karlsruhe, Germany) with an 100 µl tip (Starlab, Hamburg, Germany). For injection the tip and consequently the small allergen extract was placed under the nostrils and inhaled by the mice themselves and thereby reaching the lower respiratory tract. Mice were sacrificed at day 30 by anaesthesia with 2 mg Ketamin (Pfizer, Berlin, Germany) and 0.4 mg Xylazin (Bayer, Leverkusen, Germany).

For therapeutic tests prednisolone [60 µg in PBS (Prednisolut®, Mibe, Brehna, Germany) and pitrakinra (10 µg (sponsored by Prof. Dr. Thomas Müller, university Würzburg, Germany) in 0.5% Methylcellulose, 0.05% TWEEN 80 (Carl Roth, Karlsruhe, Germany) in PBS), a new therapeutic IL-4/IL-13 receptor antagonist, were used, while sterile NaCl (0.9 %, Braun, Melsungen, Germany) served as control. All treatments were applied intraperitoneally and applied on day 23 – 30 (Fig1).

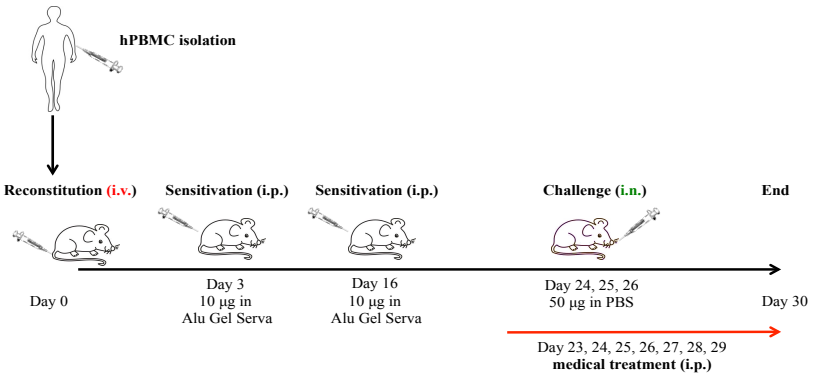


Fig.1: Schematic scheme of the study protocol. NSG mice were reconstituted with $4x10^6$ leucocytes isolated from human donors. On day 3 and 16 mice were sensitized with 10 µg OVA, or HDM dissolved in PBS/ Alu Gel S. On day 24, 25, 25 mice were challenged with 50 µg allergen. Medical treatment was performed daily starting on day 23 (red arrow). Abbreviation: hPBMC = human peripheral blood mononuclear cells; i.v. = intravenous; i.p. = intraperitoneal; i.n. = intranasal

2.3. Clinical activity score of asthma in mice

Daily assessment of asthma-severity was performed according to the following scoring system.

Tab. 2. Detailed clinical activity score

Monitoring		**Score**	**Termination criteria**
Process of body weight (1 to 4 points)	no weight loss or increasing weight	0	
	weight loss 0-5 %	1	
	weight loss 5-10 %	2	
	weight loss 10-15 %	3	
	weight loss 15-20 %	4	
	weight loss ≥ 20 %		x
General Condition			
Behaviour (1 to 3 Points)	lively/normal	0	
	calm	1	
	apathy or isolation	3	x
	ruffled fur oder abnormal posture or missing body care (max. 1 additional point)	1	
Posture			
1-4 Points	normal	0	
	intermediately hunched posture	1	
	permanently hunched posture	4	x
Respiration			
1-6 Points	no change	0	
	accelerated breathing	1	
	frequently sneezing	2	
	crackling breathing sounds	2	
	frequent thoracic respiration	4	
	dyspnea	6	x
Valuations, provisions			
No burden		0	
Minor burden, Score=3, careful monitoring 1 x daily.		3	
Moderate burden, Score =5, lasting longer than 72 h is seen as high burden		5	
High burden, Score = 7, animal will be euthanized		7	x

2.4. Bronchoalveolar lavage (BAL)

In order to isolate cells from the respiratory tract a bronchoalveolar lavage was performed. The trachea of euthanized mice was opened and a 1mm cat catheter (Henry Schein, Hamburg, Deutschland) was installed. Cells were extracted by flushing the alveoli 5 times with 0.5 ml PBS and used for flow cytometry analysis.

2.5. Isolation of human leucocytes from organic tissue

For isolation of human leucocytes from murine spleen and lungs, organs were minced and cells filtrated through a 70 µm cell strainer followed by centrifugation at 1400 g for 5 minutes and resuspended in FACS buffer. For FACS buffer PBS with 1 % FCS (Thermo Fisher Scientific, Waltham, MA, USA) were used and intensified the Antibody binding. Additionally cells were filtrated one more time using a 35 µm cell strainer for further purification before labelling for flow cytometry analysis. The antibody staining was conducted as suggested by the manufacturers (Biolegend). In order to avoid capping, or internalisation of the antibody all working steps were performed on ice. Approximately one million leucocytes were dissolved 200 µl FACS-buffer and stained with 1 µl antibody. Afterwards cells were incubated for 30 min at 4° C in the dark to guarantee a proper antibody binding to the surface. Subsequently cells were washed one time by adding 300 µl FACS-buffer and centrifuged at 1400 g for 5 minutes. Immune cells which were stained with biotin ligated antibodies were resuspended in 200 µl FACS-buffer, stained with 0,5 µl secondary antibody (streptavidin) and incubated for 30 min at 4° C in the dark again. All colours and surface markers including the secondary antibody are shown in tab 3. Leucocytes were washed one more time by adding 300 µl FACS-buffer and centrifuged at 1400 g for 5 minutes The leucocyte pellet was resuspended with 300 µl FAC-buffer and ready for flow cytometric analysis.

2.6. Flow cytometry analysis

Labelling of human leucocytes was performed with the monoclonal antibodies (anti-human) listed in table 3. Unspecific antibody binding was controlled by isotype controls (data not shown).

All antibodies were purchased from Biolegend (San Diego, USA) and used according to manufacturer's instructions. Samples were measured using a BD FACS Canto II™ and analysed with FlowJo 10.1-Software (FlowJo LLC, Oregon, USA).

Tab. 3 List of antibodies to surface markers [117, 118]

Surface marker (anti human)	Fluorochrome	Clone	Expression on	Function
CD19	Peridine-chlorophyll-protein complex cyanine dye (PerCP-Cy™ 5.5)	HIB19	B-cells	B-cell coreceptor
CD38	Phycoerythrin (PE)	HB-7	T-, B-cells	NAD-glycohydrolase
CD4	Allophycocyanin (APC)- Cy7	OKT4	T-cell, Monocytes, Macrophages	MHC-II Coreceptor
CD62L	FITC	DREG-56	T-,B-,NK-cells, monocytes,	Leucocyteadhesion, Binding of CD34
CD8	PerCP-Cy™ 5.5	HIT8a	T-cell	MHC-I Coreceptor
CD294 (CRTH2)X	APC	BM16	T-cells, Eosinophils, Basophil	Chemotaxis and prevention of apoptosis in TH2 cells
CD134	PE	ACT35	Activated T-cells	Co-stimulator, adhesion- molecule
CD14	APC-Cy7	HCD14	Myelomonocytic cells	LPS-receptor
CCR2	PE-Cy7	K036C2	Monocytes, Macrophages, DC, T-Cells	Adhesion and chemotaxis
CD80/86	PerCP-Cy™ 5.5	IT2.2	Monocytes, B-cells, DC	Ligand for CD28 and CTLA
TSLPRX	APC	1B4	DC, activated monocytes	Binding of TSLP in order to stimulate cell proliferation
CD1a (biotin) linked with secondary Ab streptavidin	FITC	HI149	Cortical thymocytes, dendritic cells, smooth muscle cells	Similar to MHC-I
CD64	PerCP-Cy™ 5.5	10.1	Monocytes, Macrophages	FcγRI receptor, IgG binding, phagocytosis
CD163	FITC	GHI/61	Monocytes, Macrophages	unknown
CD16	PE	3G8	Neutrophils, NK-cells, macrophages	FcγRIII; Necessary for phagocytosis and Ab- dependent cytotoxicity
CD11b	APC-Cy7	M1/70	Myeloid cells, NK cells	Binding of CD54, complementfactors and matrixmolecules
CD11c	PE-Cy7	3.9	Myeloid cells	Binding of fibrinogen
CD45RO	PE	UCHL1	T- and B-cells,	Isoform of CD45

			Macrophages, Monocytes	
CCR7 X	APC	G043H7	T-,B-, NK-cells, DC	Binding of CCL19 and CCL21
CD45RA	PE-Cy7	HI100	T- and B-cells, Monocytes	Isoform of CD45
CXCR3	FITC	G025H7	Maligned B-cells	Chemotaxis
CCR4 X	PE	L291H4	T-,B-,NK-cells, Basophils, Monocytes,	Binding of CCL17 and CCL22
CCR6 X	PE-Cy7	G034E3	T-, B-cells	Binding of CCL19 and CCL21
CCR10 X	APC	6588-5	T-memory cells	Binding of CCL27 and CCL28, T-cell homing
FcεRIα X	FITC	CRA-1	Eosinophils, Mast cells, Basophils, DC, Monocytes	IgE-receptor
CCR3 X	PE	5E8	Eosinophils, Mast cells, Basophils	Binding of eotaxin
CD117	PE-Cy7	104D2	Hämatopoeitic precursor cells	Stem cell factor receptor
HLADRX	APC	L243	T-, B-cells, APC	Peptide presentation to CD4+ T-cells
CD123 ((biotin) linked with secondary Ab streptavidin	APC-Cy7	6H6	Bone marrow stem cells, Monocytes, Granulocytes	IL-3 receptor α chain

2.7. Histological analysis

Lungs were fixed in 4% formaldehyde (Carl Roth, Karlsruhe, Germany) for 24 hours and then transferred to 70% ethanol (Carl Roth, Karlsruhe, Germany), followed by paraffin embedding using an embedding machine (Leica, Wetzlar, Germany). The program is listed in tab. 4

Tab. 4 Protocol for paraffin embedding

Solution	Time
70% Ethanol	1 x 2h 1 x 2h 1x 1h
96% Ethanol	2 x 1,5 h
100 % Ethanol	3 x 1,5 h 1x 30min
Xylene	1 x 1,5 h 1 x 1h
Paraffin	2 x 3h

Samples were cut into 6 µm sections utilizing a sliding microtom (Leica, Wetzlar, Germany) and stained with haematoxylin (Carl Roth, Karlsruhe, Germany) and eosin (Carl Roth, Karlsruhe, Germany) (H&E staining) using the following protocol:

Tab. 5 Protocol for haematoxilin and eosin staining

• Eosin – Solution: 2g Eosin dissolved in 200 ml 96% Ethanol	
Process:	**estimated Time (min)**
<u>Deparaffination and Rehydration</u>	
• 2 x10' Xylene	20’
• 2 x 5' Abs. Ethanol	10’
• 2 x 5'96% Ethanol	10’
• 2 x 5' 70% Ethanol	10’
• 1 x 5' distilled water	5’
<u>Progressive staining</u>	
• shortly rinse in tap water	
• hematoxilin: Tissue 4’ (= alkaline nuclear staining)	4’
• Shortly submerge in tap water, discard	10
• incubate for at least 10’ under running tap water	
<u>Regressive staining</u>	4
• Shortly rinse in distilled water	
• Eosin: Tissue 4’	
<u>Dehydrierung</u>	
• 80% Ethanol rinse very shortly	
• 96% Ethanol rinse very shortly	
• 100% Ethanol rinse shortly	
• Xylene 2 x 5’	

H&E sections were mounted with malinol (Carl Roth, Karlsruhe, Germany) and cover slides (24 x 60 mm, Carl Roth, Karlsruhe, Germany). One slide per mouse containing 3 lung sections were scored using following scoring system:

cellular infiltration: none (0 point), mild (1 - 2 layers of leucocytes surrounding blood vessels, alveoli, or bronchioles, 1 point), moderate (2 - 5 layers of leucocytes surrounding blood vessels, alveoli, or bronchioles, 2 points), severe (> 5 layers of leucocytes surrounding blood vessels, alveoli, or bronchioles, 3 points). Scoring was performed independently 2 times from an expert in animal pathology in a blinded manner.

2.8. cDNA synthesis

Additionally, different cytokines were analysed via quantitative real-time PCR. Therefore RNA was isolated from lung tissue and converted to cDNA. In a first step, approximately 1 cm^3 lung tissue (approximately 400 mg) was extracted from each mouse and incubated for 24 hours in RNA-later (Thermo Fisher Scientific, Waltham, MA, USA). Lung tissues were disrupted and homogenized with the TissueLyser LT (Qiagen, Hilden, Germany) followed by total RNA extraction according to the manufacturer's instruction using RNeasy Plus Universal Mini Kit (Qiagen, Hilden, Germany) and Chloroform (Sigma-Aldrich, St. Louis, MO, USA). Since gDNA Eliminator Solution is included in the kit no treatment with DNase was conducted.

For the cDNA synthesis approximately 5 µg of total RNA was used. Reverse transcription was performed in a Mastercycler gradient (Eppendorf, Hamburg, Germany) using QuantiNova Reverse Transcription Kit (Qiagen, Hilden, Germany). According to manufacturer's (Thermo Fisher Scientific, Waltham, MA, USA) guidelines all specimens were diluted with RNase free water (Qiagen, Hilden, Germany) in order to generate a cDNA concentration between 10 pg and 100 ng.

Reverse Transcription was conducted by using the following program:

Tab. 6 Setup for cDNA synthesis

Step	Time	Temperature
gDNA elimination reaction	2min	45° C
	Pause cycler	25° C
RT-transcription		
Annealing step	3 min	25° C
Reverse-transcription step	10 min	45° C
Inactivation of reaction	5 min	85° C

The cDNA purity was verified with a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Thereby 1 µl of cDNA sample were exposed to ultraviolet light at a wavelength of 260 and 280 nm and the absorbance of the sample was detected. Using the ratio of the absorbance at 260 and 280 nm the purity of cDNA can be calculated. Pure DNA has a 260/280 nm ratio of approximately 1.8.

2.9. Quantitative real-time PCR

In a next step different cytokine concentrations were determined via quantitative real-time PCR. Following the guidelines from TaqMan Fast Advanced Master Mix protocol (Thermo Fisher Scientific, Waltham, MA, USA) quantitative real-time polymerase chain reaction (PCR) was performed using the Applied Biosystems StepOnePlus real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA) with following adjustments:

Tab. 7. Setup for quantitative real time PCR

Thermal Cycling Profile				
	Incubation	Polymerase activation	PCR (40 cycles)	
Parameter	Hold	Hold	Denature	Anneal/extend
Temp. [°C]	50	95	95	60
Time [min:sec]	2:00	0:20	0.01	0.20

Single Tube TaqMan Gene Expression Assays (Thermo Fisher Scientific, Waltham, MA, USA) contained following primer:

Tab. 8 List of used Primers (including species specificity)

Gene	Species	Expression Assay
GAPDH	mouse	Mm99999915_g1
GUSB	mouse	Mm00446953_m1
CCL17/TARC	mouse	Mm01244826_g1
TSLP	mouse	Mm01157588_m1
IL-4	human	HS00174122_m1
IFNγ	human	Hs00989291_m1
HGF	human	Hs04329698_m1
TGFβ	mouse	Mm 01178820_m1

Analysis was performed using StepOnePlus™ Software v2.3

2.10. In vitro assay of human leucocytes

Isolated leucocytes from asthmatic and healthy donors were also used for *in vitro* assays and cultured for 2 days (37°C, 5 % CO_2) in 24 well plates using a concentration of 1 x 10^6 cells/well. Leucocytes were resuspended in RPMI (Thermo Fisher Scientific, Waltham, MA, USA), 10 % fetal calf serum (FCS) (Thermo Fisher Scientific, Waltham, MA, USA) and 1% Penicillin/Streptomycin (Sigma, Deisenhofen, Germany) at a total volume of 1.5 ml per well. For activation either 10 µg/ml phytohaemagglutinin (PHA) (Sigma, Deisenhofen, Germany), HDM or OVA was added.

2.11. IgE and IgG analysis

Blood was drawn from anaesthetized mice via the retro-bulbar-plexus. Approximately 1 ml whole blood was incubated for blood clotting for 1 hour at room temperature. The blood was centrifuged with 1400 g for 5 minutes and 100 µl supernatant (serum) were sent to the clinical routine laboratory (Hospital of the LMU) for IgE and IgG determination.

2.12. Statistical analysis

Statistical analysis was performed with R a language and environment for statistical computing and graphics [119]. The software was developed by the Foundation for Statistical Computing, Vienna, Austria and can be downloaded at https://www.R-project.org/ for free. Variables were normally distributed and represented with mean, standard deviation, median, and IQR values. A two-sided t-test and a confidence level of 0.95 was used to compare binary groups. For more than two groups ANOVA followed by Tukey HSD was conducted.

3. Results:

3.1. Hypothesis 1: Effect of OVA and HDM on PBMC in vitro

In order to compare the effects of OVA and HDM, 1 x 10^6 lymphocytes were incubated in the presence or absence of 15 µg of HDM or OVA for 48 hours as described in material and methods. PHA served as a positive control. The experiments were performed with 8 different donors (asthma n= 3, non-asthma n=5).

OVA, HDM and PHA evoked different and specific responses (figure 2). Surprisingly no difference was observed between PBMC from asthmatic and from non-asthmatic donors. Since PHA is known for the unspecific activation of T-cells [56] the increased frequencies of activated CD4 T cells as shown by increased numbers of CD4+ CD69+ (control versus PHA) and CD4+ CD134+ T-cells (control versus PHA) were expected, but failed to reach significance. In contrast, frequencies of these cell populations decreased significantly in the presence of OVA and HDM (CD4+ CD69+: PHA versus OVA: Non-Asthma $p < 0.001$, Asthma $p < 0.01$; PHA versus HDM: Non-Asthma $p < 0.001$, Asthma $p < 0.01$; CD4+ CD134+: PHA versus OVA: Non-Asthma $p < 0.01$, Asthma $p < 0.05$; PHA versus HDM: Non-Asthma $p < 0.001$, Asthma $p < 0.01$). In addition, PHA induced an increase in Th1 and Th2 cells (Th1: not significant, Th2: control versus PHA: Non-asthma $p < 0.05$) in contrast to HDM, which increased the frequencies of Th17 cells (control versus HDM; Non-Asthma $p < 0.05$, Asthma $p < 0.05$). These opposing responses were also reflected by the changes of frequencies of regulatory T cells (Treg, CD4+ CD25+ CD127-). The frequencies of this population were reduced after stimulation with HDM and OVA (Non Asthma: control versus OVA $p < 0.01$, control versus HDM $p = 0.01$; Asthma not significant), but unaffected in the PHA-treated group. Additionally, incubation with PHA caused a decrease in TSLPR expressing CD14+ cells (control versus PHA: Asthma $p < 0.05$), In contrast to OVA, both HDM and PHA elicited an increase of frequencies of CD1a expressing CD14+ monocytes (Non-Asthma: Control versus HDM $p < 0.05$).

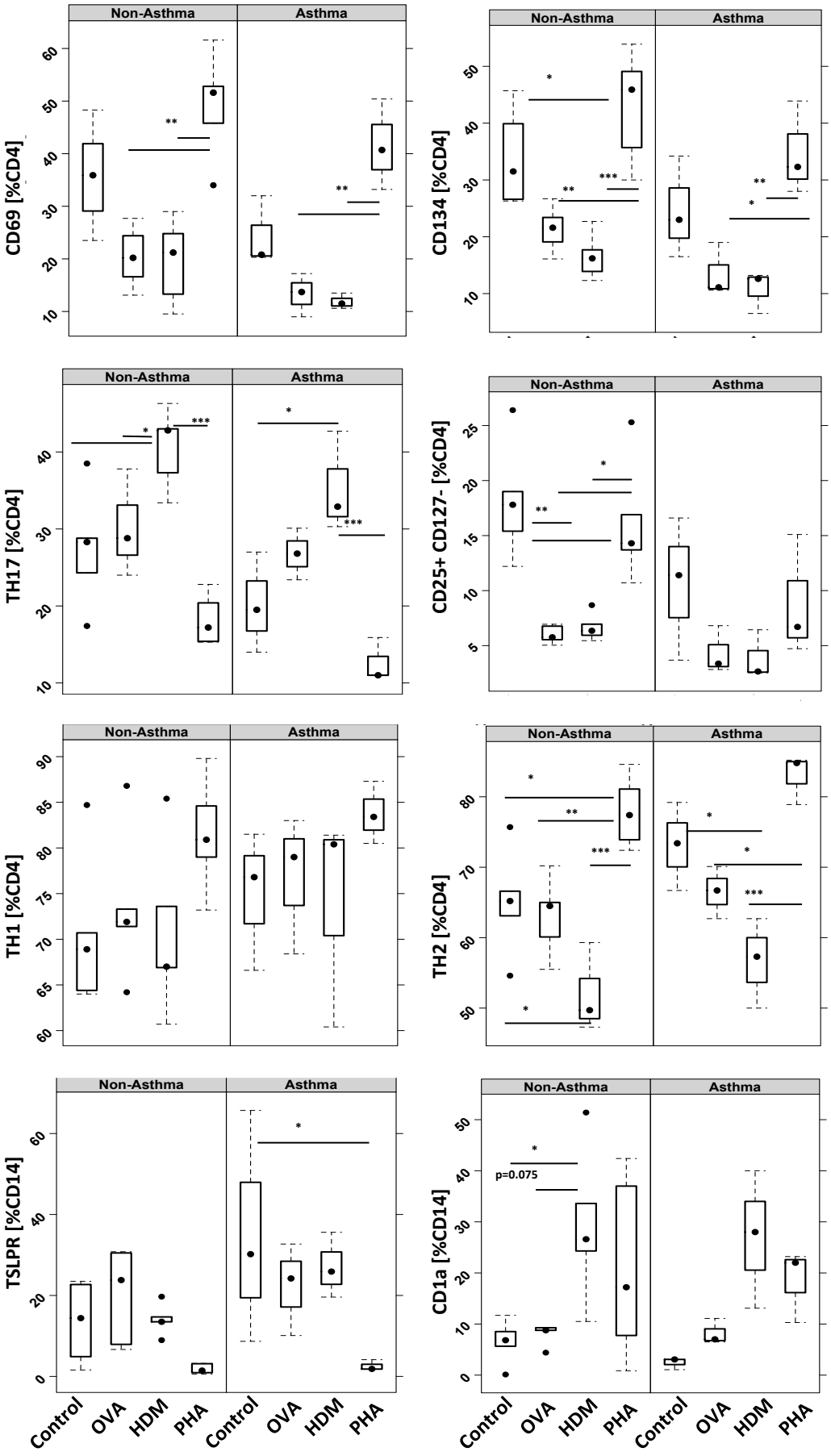

Non-Asthma
Asthma
CD69 [%CD4]
CD134 [%CD4]
TH17 [%CD4]
CD25+ CD127- [%CD4]
TH1 [%CD4]
TH2 [%CD4]
TSLPR [%CD14]
CD1a [%CD14]
p=0.075
Control
OVA
HDM
PHA

Fig. 2: *In vitro* stimulation of PBMC from human asthmatic and non-asthmatic donors with OVA, HDM and PHA. Culture medium only was used as a control. Immunofluorescence staining and gating for CD4+ cells (mainly T helper, upper panel and lower left panel) or CD14+ cells (monocytes) shows the differences of T cell and macrophage subpopulations after stimulation with different antigens.

Frequencies of leucocytes incubated with antigens or PHA depicted as conditional Tukey´s boxplots. PBMC were incubated for 48 h in RPMI in the presence or absence (control: culture medium only) of 10 µg antigen / ml (OVA, HDM or PHA) and stained for CD4, CD69, CD134, CD25, CD127; CCR4, CXCR3, CCR10, CCR6; CD14, CD1a, TSLPR, and finally subjected to flow cytometric analysis. Sample sizes: asthma n = 3, non-asthma n = 5. For comparison of groups, ANOVA followed by Tukey's HSD was conducted. Labels given on x-axes on the bottom row apply to all charts (for more detailed data set see Table S1, 2).

$p < 0.05$ *; $p < 0.01$ **, $p < 0.001$ ***

Th1: CD4+, CCR4-, CXCR3+, CCR10-, CCR6-

Th2: CD4+, CCR4+, CXCR3-, CCR10-, CCR6-

Th17: CD4+, CCR4+, CXCR3-, CCR10-, CCR6+

3.2. Hypothesis 2 and 3: Effects of allergen and donor background in vivo

Previous studies have shown that NSG mice reconstituted with PBMC derived from affected individuals and challenged with the respective agent developed symptoms of ulcerative colitis (UC) or atopic dermatitis (AD), respectively [53,55]. Hence, we wanted to examine whether this model could be also translated to allergic asthma. NSG mice were reconstituted with 4 x 10^6 PBMC derived from allergic asthmatic donors (n = 4), or non-asthmatic donors (n = 3). Engraftment levels were determined by analysing hCD45 expressing cells isolated from spleen. The reconstitution level was 21.8 ± 15.75 hCD45+ cells [% leucocytes] (mean ± SD). To support the assumption that hPBMC are driving the inflammatory responses in this model an additional control was added, using non-reconstituted mice challenged with HDM or OVA. Post reconstitution, mice were divided into three groups: unchallenged control, HDM- and OVA-challenged group. Mice were sensitized by intraperitoneal injection of 10 µg OVA or HDM dissolved in Alu-Gel-Serva as adjuvant on days 3 and 16, followed by a daily challenge with nasal application of 50 µg of allergen on days 24 – 26. Mice were monitored during the experiment on a daily basis and the clinical score including symptoms like wheezing, or coughing was determined. Immediately upon intranasal challenge with OVA or HDM mice developed asthma-related symptoms like accelerated breathing or cracking breathing sounds, independent from the donor background. As shown in figure 4 the clinical score was significantly increased in the OVA and HDM challenged groups as compared to the unchallenged control group. No significant difference between the two allergens was observed and between mice reconstituted with PMBC from healthy or diseased donors. Approximately 2 hours post challenge, asthmatic symptoms ceased and mice exhibited no disease symptoms until the next challenge. Mice were sacrificed on day 30, serum was collected for determination of total hIgG and total hIgE levels (figure 3) and lung tissue was collected for histological analysis. Mice reconstituted with PBMC from healthy donors exhibited higher levels of IgG (n=30 mice, 11.22 ± 11.67 [IU/ml] mean ± SD) as compared to IgG levels of mice reconstituted with PBMC from asthmatic donors (n = 24 mice, 1.86 ± 4.2, p = 0.02 [IU/ml]). This difference was independent of the antigen challenge (data not shown). In contrast, the total IgE levels in mice reconstituted with PBMC from asthmatic donors (n=27, 60.43 ± 136.20 [IU/ml]) were significantly higher than those of mice reconstituted with PBMC from healthy donors (n=12, 0.53 ±1.14 [IU/ml], p=0.03), irrespective of the antigen used for challenge (data not shown).

However, as shown in fig. 3, mice challenged with HDM and engrafted with PBMCs from asthmatic donors had significant enhanced levels of human IgE when compared to HDM challenged NSG mice transferred with healthy donor lymphocytes.

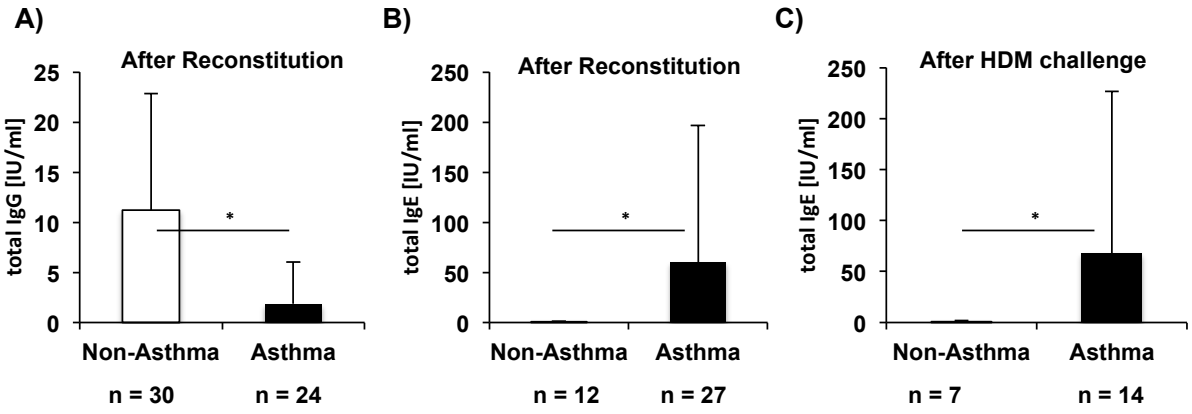
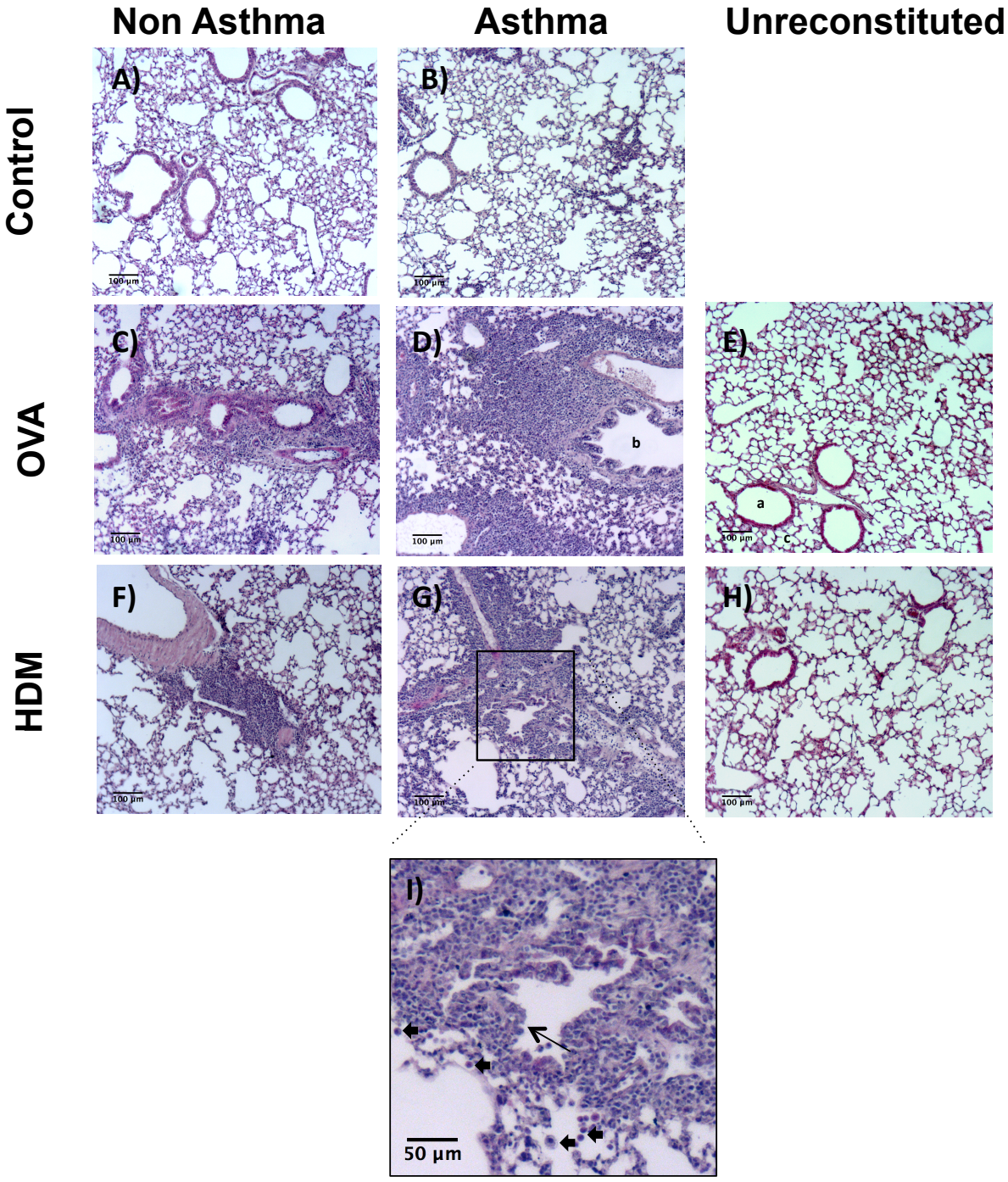


Fig. 3: Total levels of human IgG and IgE in the serum of mice engrafted with human PBL
Immune globulin levels in the serum of engrafted mice were measured 4 days after the last HDM-challenge. n = Number of analysed mice. Barplots represent mean values with standard deviation (sd).
For comparison of control versus challenged, ANOVA followed by Tukey's honest significant difference (HSD) test was performed.
$p < 0.05$ *; $p < 0.01$ **, $p < 0.001$ ***

Challenge with allergens resulted in increased influx of leucocytes accumulating around alveoli, bronchioles and blood vessels (fig. 4). In severe cases the challenge resulted in the damage of the epithelial layer, and according to the morphology of the in fluxing cells, macrophages presented as the dominant population (fig. 4). Lungs from mice reconstituted with PBMC from healthy or asthmatic donors and challenged with antigens showed a similar picture. However, a difference became obvious when the non-challenged groups were compared. Leucocytes from asthmatic donors seemed to migrate spontaneously to the lung whereas lungs from mice reconstituted with leucocytes from non-asthmatic donors appeared unaffected in the absence of challenge (fig. 6). This observation was corroborated by the histological score, which was mainly based on the number of leucocyte-layers as described in the methods part. The group of mice reconstituted with PBMC from non-asthmatic donors responded to the intranasal challenge and formed multi layers around the bronchioles and blood vessels. Mice engrafted with PBMCs from asthmatic donors revealed a similar pattern. However the response to challenge in this group seemed to be more pronounced and when compared to the control group of mice engrafted with non-asthmatic PBMCs yet started from a higher level (not significant). In the non-reconstituted group no influx of leucocytes or epithelial cell damage was observed (fig. 4) indicating a human origin of the infiltrating cells.

I)


Non Asthma
Asthma
Unreconstituted
Control
OVA
HDM
A)
B)
C)
D)
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F)
G)
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a
b
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100 µm
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II)

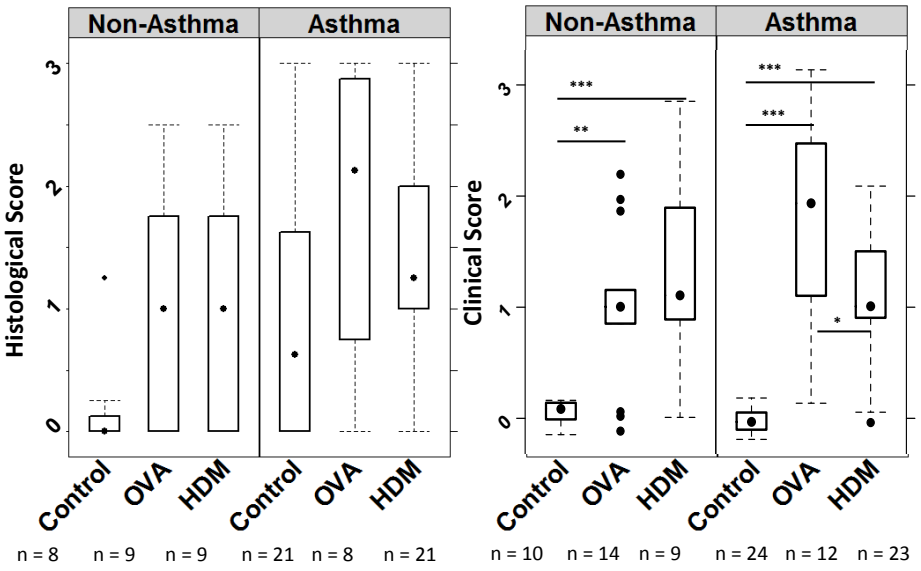

Fig. 4: Challenge with OVA or HDM induces clinical- and histological pathologies in NSG mice reconstituted with human PBMC.

Mice were reconstituted with 4 x 10^6 PBMC on day 1 followed by sensitization of 10 µg OVA or HDM dissolved in Alu-Gel-Serva as adjuvant on day 3 and 16 and challenge with nasal application of 50 µg of allergen on days 24-26.

I) A - I: H&E staining of paraffin sections from lateral parts of the right lung from sacrificed animals. Long arrow indicates infiltration of inflammatory cells, and damaged epithelial layer; bold arrow indicate morphological typical macrophages.

II) Clinical- and histological scores depicted in conditional Tukey´s boxplots (for more detailed data set see Table S4). For comparison of all groups ANOVA followed by TukeyHSD was conducted.

$p < 0.05$ *; $p < 0.01$ **, $p < 0.001$ ***

a) blood vessel, b) bronchial tube, c) alveoli

In contrast to previous studies, where development of phenotype and disease symptoms was highly dependent on the immunological background of the donor, so far, the response to allergens was similar in mice reconstituted with PBMC from diseased or non-diseased donors.

To further examine the impact of challenge and disease background human leucocytes isolated from spleen and lung were subjected to flow cytometric analysis as described in material and methods. (For complete set of data see Table S2-11). Regardless of the donor, HDM driven inflammation seemed characterized by CD11b+ macrophages (HDM versus control $p < 0.05$, HDM versus OVA $p < 0.05$), CCR2- (HDM versus control $p < 0.05$) and CD1a-expressing monocytes (HDM versus OVA $p < 0.05$). The difference was significant either to the unstimulated control group or the OVA-challenged group. In contrast to results obtained in the *in vitro* experiment, HDM evoked a strong activation of CD4+ T cells (CD4+ CD134+), however, only in the group that had been reconstituted with PBMC from a healthy donor, which however showed no symptoms of sickness, or allergy. In contrast to our expectations frequencies of CD14+ TSLPR+ cells declined in the group of mice reconstituted with PBMC from non-asthmatic donors, and remained unaffected in the other group.

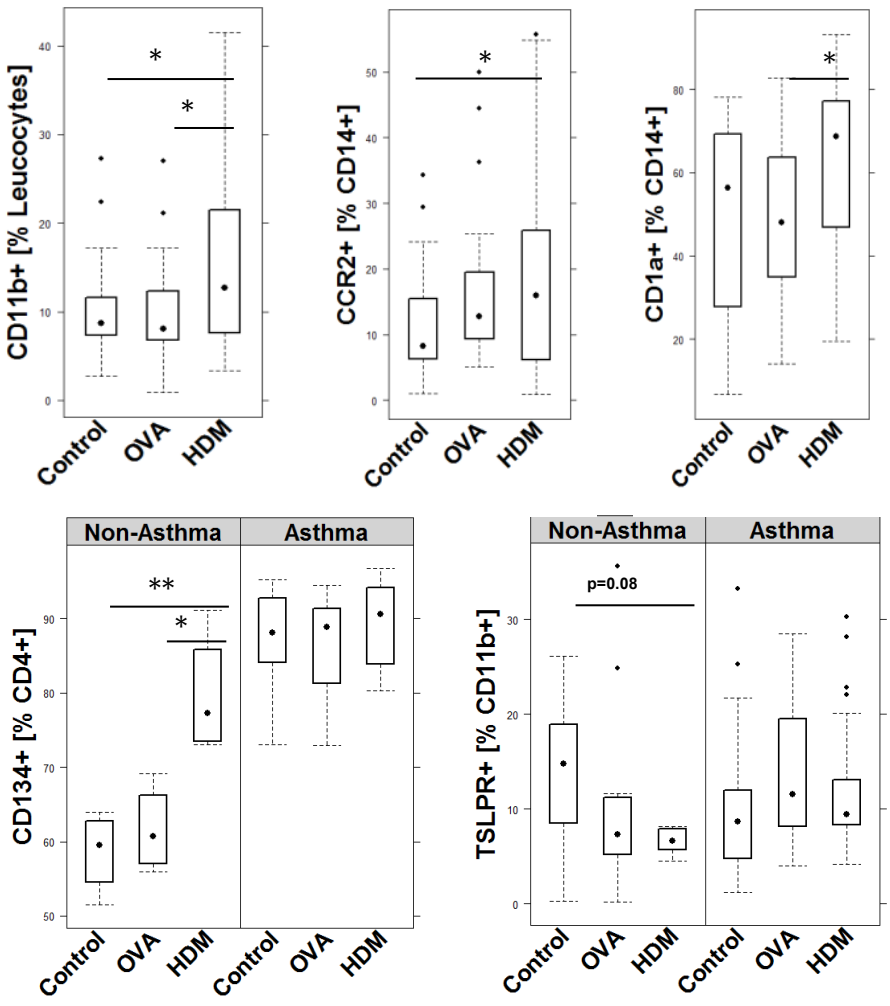

Fig. 5: Frequencies of human leucocytes isolated from spleens of reconstituted mice challenged with OVA or HDM. NSG mice were challenged as described in figure 4. Frequencies of leucocyte subpopulations are depicted as conditional Tukey´s boxplots (for complete data set see supplement table S3-5). For comparison of all groups ANOVA followed by TukeyHSD was performed.
$p < 0.05$ *; $p < 0.01$ **, $p < 0.001$ ***

When leucocyte populations isolated from lung were analysed (figure 6) the difference between mice reconstituted with PBMC from asthmatic and non-asthmatic donors became more obvious and corroborated the results obtained from histological analysis. Even without challenge (control) the frequencies of almost all cell types in lungs of mice reconstituted with PBMC from an asthmatic donor were higher than after reconstitution with cells from a non-asthmatic individual, corroborating the results obtained by histological analysis. This effect was most pronounced for CD4+ and CD8+ T-cells, CD11b+ macrophages, CD14+ monocytes, eosinophils (FceRαI+/CCR3+), mast cells (FceRαI+/CD117+) and basophils (HLA-DR+/CD123+), indicating that the immune cells of the asthmatic donors are predominantly homing to the lung (figure 6). In contrast to the cells from a non-asthmatic donor, the cells from an asthmatic donor isolated from challenged mice could not be stimulated with OVA or HDM, potentially indicating an already highly activated status or a stage of exhaustion.

This applies especially to mast cells and eosinophils. The difference in frequencies of M2 monocytes (CD14+/CD163+) and plasma cells (CD19+/CD38+) was significant when the OVA or HDM challenged group were compared to the control group. Plasma cells (CD19+/CD38+) increased in both groups after stimulation with OVA, but the difference to the non-challenged control group was only significant with cells from the non-asthmatic donor.

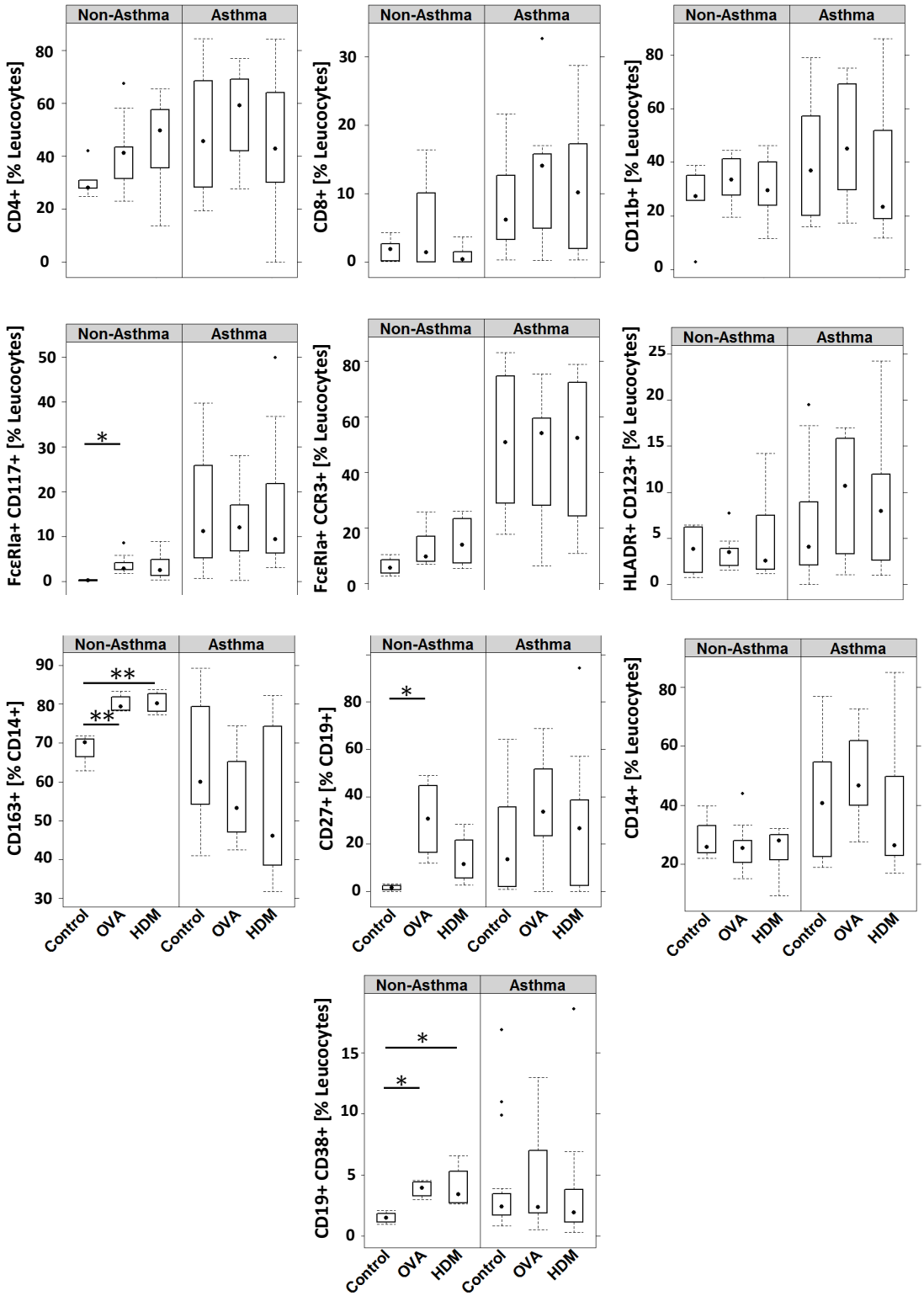

Fig. 6 Frequencies of human leucocytes in the lungs of mice challenged with OVA or HDM. NSG mice were challenged as described in figure 2. Frequencies of leucocytes isolated from lung and analysed by flow cytometry are depicted as conditional Tukey´s boxplots (for complete data set see supplement table S6, 7).

$p < 0.05$ *; $p < 0.01$ **, $p < 0.001$ ***

In order to examine the difference in cytokine responses to OVA and HDM challenge, RNA was isolated from lung tissue and analysed by RT-PCR. TGF-β1, TSLP, IL-4, IFN-γ and the chemokine TARC were selected as crucial cytokines in inflammation, and HGF as marker for epithelial healing. HGF, TARC, TGF-β1 and IFN-γ had been identified as hallmarks of inflammation in the NSG-UC mouse model. For analysis we selected a cohort of mice reconstituted with PBMC from an asthmatic donor. As shown in figure 7 the challenge with OVA (n=4) or HDM (n=12) resulted in different responses in comparison to the control group (n=11) and thus corroborated the analysis of cellular populations from the lung. In both groups challenging resulted in an increased, strong expression of IFN-γ. Levels of IFN-γ mRNA exceeded those of IL-4 in all groups by a factor of approximately 10.000, indicating that IFN-γ is the main driving cytokine in this model. However challenge with OVA resulted in significantly increased expression of TARC - the chemokine attracting Th2 cells, Tregs, and monocytes - and IL-4 - the cytokine associated with a Th2 response. Levels of TSLP mRNA were also increased in the OVA challenged group further corroborating the Th2 response. HGF mRNA expression declined in response to challenge with OVA or HDM, suggesting a severe change in the lung architecture. The most pronounced difference in the HDM challenged group was the observed decline in TGF-β1 mRNA expression, suggesting an impaired immune regulation finally favouring a Th1 response.

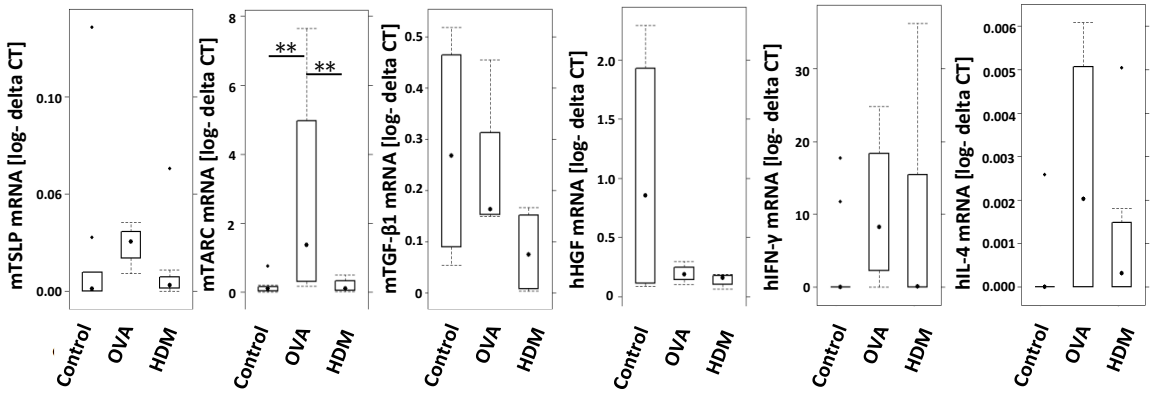

Fig. 7 mRNA expression levels of leucocytes from asthmatic donors after challenge with OVA or HDM. NSG mice were challenged as described in figure 2. Multiple Tukey´s boxplot analysis shows mTSLP, mTGF-β1, mTARC, hHGF, hIFN-γ and hIL-4 expression in the lung of NSG mice. RNA was isolated from parts of the lung and subjected to RT PCR analysis. Log - delta CT, logarithmic delta cycle threshold. For comparison of all groups ANOVA followed by TukeyHSD was performed (for detailed data set see supplement table S8).
$p < 0.05$ *; $p < 0.01$ **, $p < 0.001$ ***

3.3. Hypothesis 4: Effect of pitrakinra or prednisolone on symptoms and phenotype.

In order to validate this model and to test whether and to what extent it represents the human disease mice reconstituted with PBMC from an asthmatic donor were challenged with HDM and treated with prednisolone or pitrakinra. Pitrakinra is an IL-4 antagonist, which inhibits the activation of IL-4 type I and II receptor complexes and was therefore a promising new therapeutic for Th2 associated diseases like asthma [52]. Pitrakinra has been tested in a Phase IIa clinical study in asthma patients and has shown limited efficacy [70]. In contrast glucocorticoids like prednisolone are well known for their immunosuppressive function and the gold standard in asthma treatment. In this study prednisolone served as positive control.

For this experiment we selected asthmatic patients as donors (n=2) and mice were challenged with HDM. Reconstitution and challenge was performed according to the protocol described in the previous experiment. Following reconstitution mice were separated in four different groups: unchallenged control (control), challenged control (HDM), study group 1 (HDM + pitrakinra) and study group 2 (HDM + prednisolone) were treated on days 23-30 by intraperitoneal injection of 60 µg prednisolone or 10 µg pitrakinra as described in material and methods. Mice in the HDM group were treated with the carrier NaCl.

As shown in figure 8 challenge with HDM led to an increase of the clinical score, and treatment with both therapeutic did not ameliorate the immediate response. In contrast to treatment with prednisolone, which resulted in reduced influx of inflammatory cells, pitrakinra had no effect on the invasion of inflammatory cells into the lungs (figure 8).

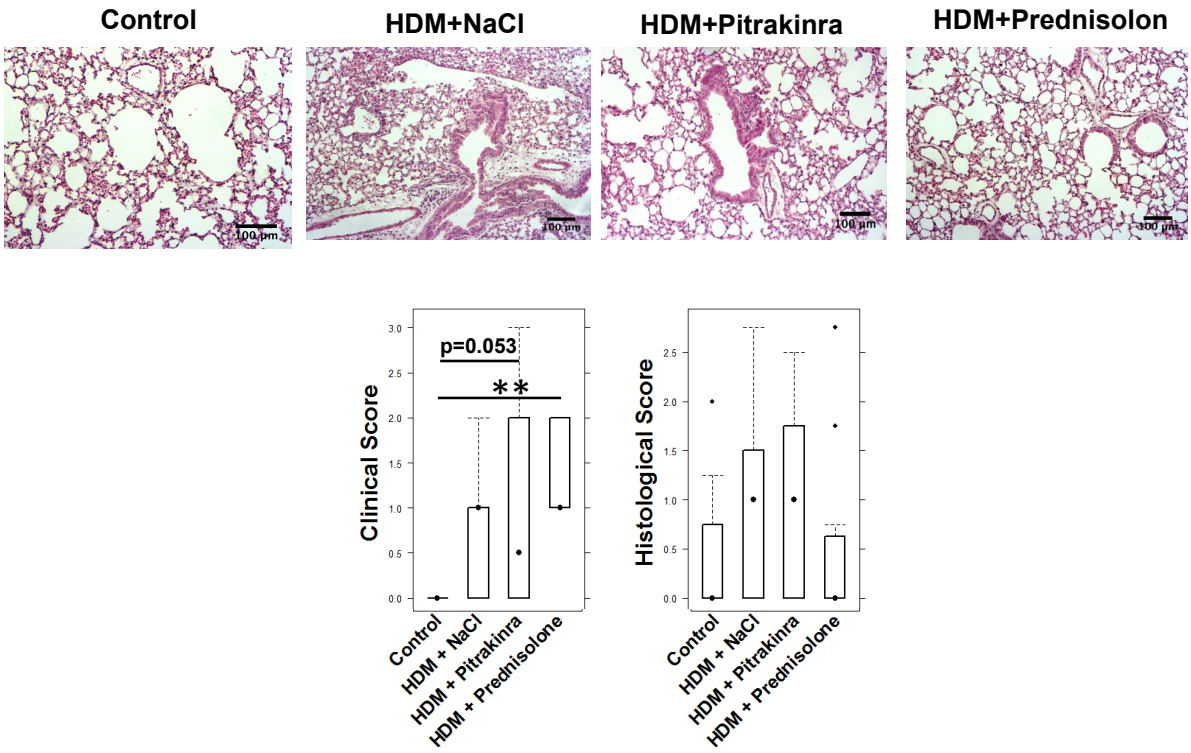

Fig. 8: Challenge with allergen results in asthma typical symptoms in NOG mice engrafted with hPBMC from asthmatic and non asthmatic Donors.

Lung tissues were fixed in Formalin 4 days after the last challenge, sectioned, and stained with haematoxylin and eosin (H&E) for histological analysis. Pictures display a representative H&E section from mice reconstituted with allergic donors and challenged with HDM.

Mice were challenged with 50 µg allergen for 3 consecutive days. Additionally mice remained untreated (control) challenged with HDM (HDM) treated with pitrakinra (HDM+pitrakinra), or prednisolone (HDM+prednisolone). Drugs were intraperitoneally administered on day 22 until the end of the experiments. Conditional Tukey´s boxplot analysis shows the histological and clinical score. For comparison of control versus challenged, an ANOVA followed by Tukey's honest significant difference (HSD) test was performed (for detailed data set see supplement table S9). Lines represent values without variability.

Flow cytometric analysis of human leucocytes isolated from spleen further delineated the effects of both therapeutic (see fig 9). In this analysis the panel was expanded and included Th1, Th2 and Th17 cells. In contrast to our expectations challenge with HDM resulted in an increase of frequencies of Th cells, which express both Th1 and Th17 associated markers.

Both therapeutics diminished frequencies of subtypes of CD4+ cells associated with a Th2 response (CD294+ and CCR4+) activation (CD134+) and inflammation (Th1 /Th17: CD4+, CCR4-, CXCR3+, CCR10-, CCR6+), however prednisolone seemed more effective with the exception of CD4+ CCR4+ cells.

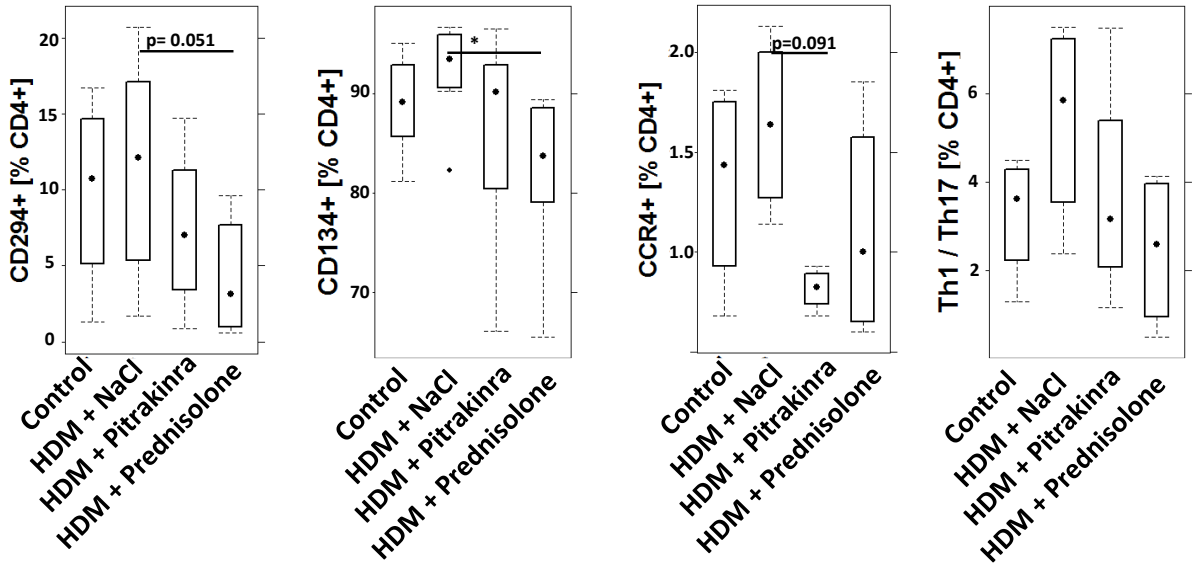

Fig. 9 . Treatment of an asthmatic NOG mouse model with prednisolone or pitrakinra: analysis of leucocyte populations in the spleen

Immunofluorescence staining for markers of T cells, DCs, macrophages and eosinophils of human leucocytes isolated from lungs of mice. Numbers on the y-axes indicate, unless specified otherwise, percentage of CD4+ cells. Mice were challenged with 50 µg allergen at day 23 for 3 consecutive days. Control mice were untreated. One day before challenge daily treatment with prednisolone or pitrakinra was initiated and continued until the end of the experiment. Boxes represent upper and lower quartiles, whiskers represent variability and outliers are plotted as individual points. For comparison of all groups ANOVA followed by TukeyHSD was performed (for detailed data set see supplement table S9).

$p < 0.05$ *; $p < 0.01$ **, $p < 0.001$ ***

Analysis of lung tissue confirmed these observations (fig. 10). Challenge with HDM resulted in increased frequencies of naive, Th17, Th1 / Th17 cells and a decline of activated CD4+ effector T cells. Of note, the increased frequency of Th17 cells has also been observed in the *in vitro* experiment. In most cases prednisolone and pitrakinra restored the cellular level to control standard, the only cellular population pitrakinra did not affect was Th17. Treatment with pitrakinra and prednisolone resulted in increased frequencies of TSLPR-expressing CD14+ monocytes, indicating a shift towards remodeling processes [82].

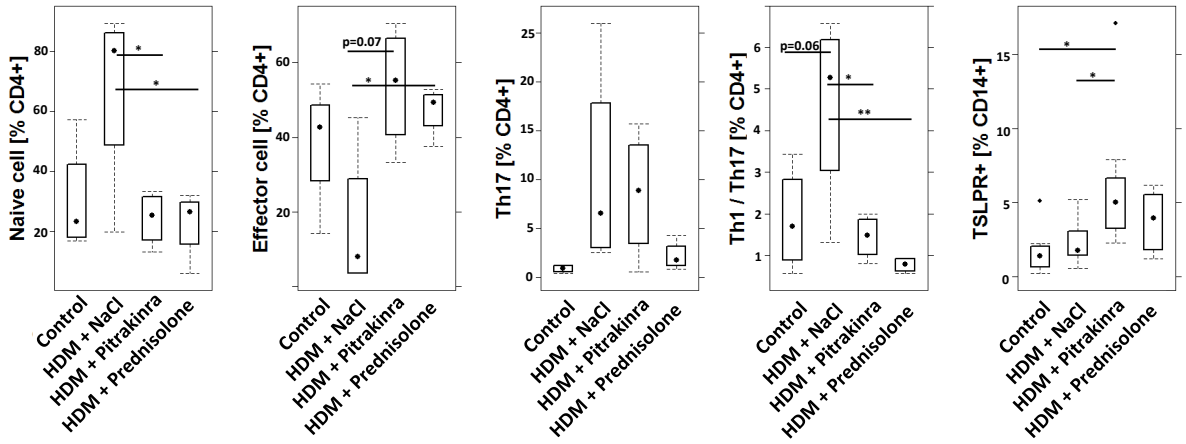

Fig. 10 . Treatment of an asthmatic NOG mouse model with prednisolone or pitrakinra: analysis of leucocyte populations in the lung

Immunofluorecence staining for markers of T cells, DCs, macrophages and eosinophils of human leucocytes isolated from lungs of mice. Numbers on the y-axes indicate, unless specified otherwise, percentage of CD4+ cells. Mice were challenged with 50 µg allergen at day 23 for 3 consecutive days. Control mice were untreated. One day before challenge daily treatment with prednisolone or pitrakinra was initiated and continued until the end of the experiment. Boxes represent upper and lower quartiles, whiskers represent variability and outliers are plotted as individual points. For comparison of all groups ANOVA followed by TukeyHSD was performed (for detailed data set see supplement table S10)

$p < 0.05$ *; $p < 0.01$ **, $p < 0.001$ ***

A similar effect can also be seen in cell populations isolated via bronchoalveolar lavage (fig. 11). In contrast to pitrakinra, prednisolone induced an anti inflammatory $CD14^{low}$ and $CD64^{low}$ population. Simultaneously inflammatory $CD14^{hi}$ CD64+ double positive cells are decreasing after treatment with prednisolone. Additionally CD19+ B cells and activated (CD86+) monocytes populations are shrinking after prednisolone but not after pitrakinra treatment.

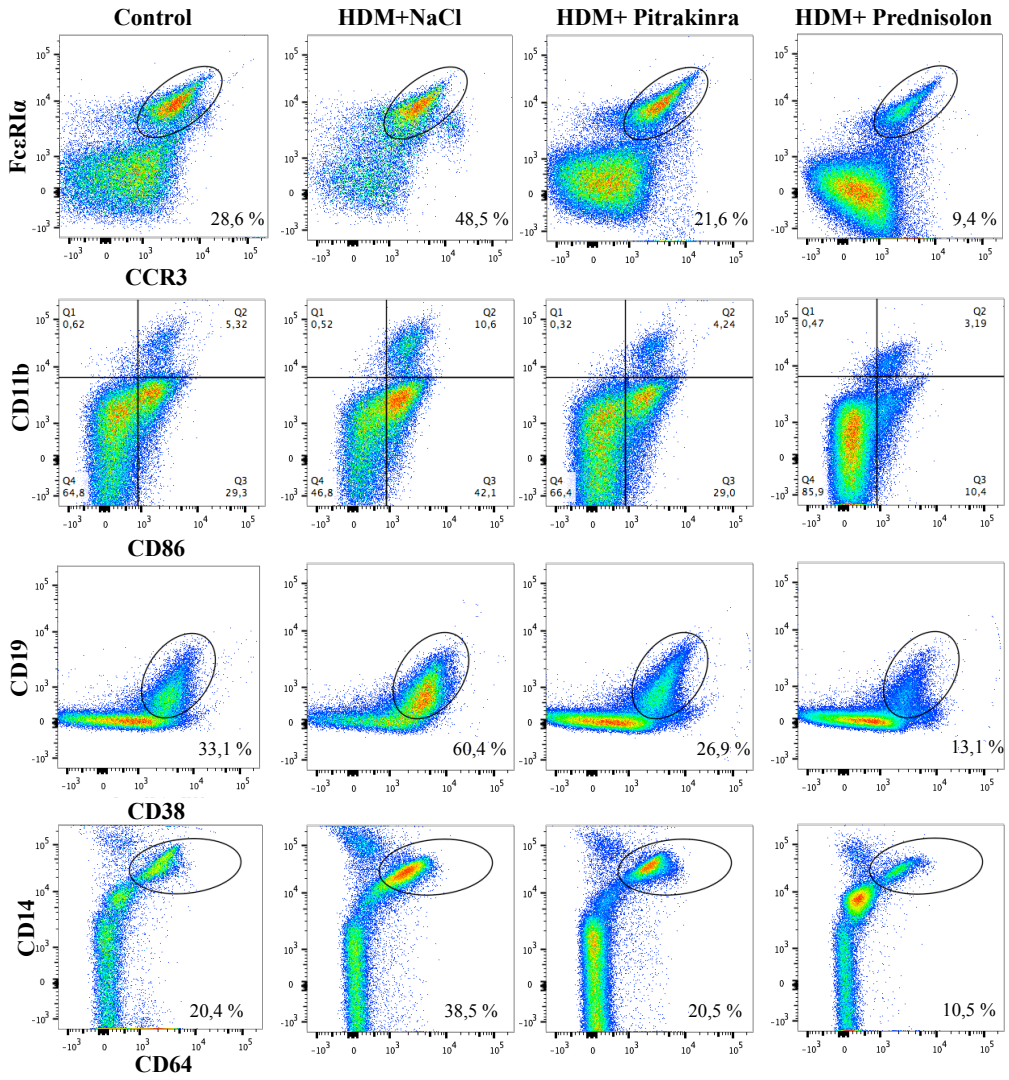

Fig. 11. FACS analysis of cells from bronchoalveolar lavage of NSG mice reconstituted with cells from an asthmatic donor, challenged with allergen and treated as indicated.
Representive fluorescence activated cell analysis (FACscan) plots of leucocytes subgroups isolated via bronchoalveolar lavage and pooled group wise (n=4). From left to right: no allergen (control), house dust mite extract (HDM), HDM + prednisolone, HDM+ pitrakinra. The axes displaying the different surface markers.

The limited efficacy of pitrakinra was also shown by the increase of TARC and TSLP expression in the murine lung tissue and the increase of IFNγ and IL-4 mRNA in the human leucocytes in the lung, indicating an ongoing inflammatory response (fig. 12).

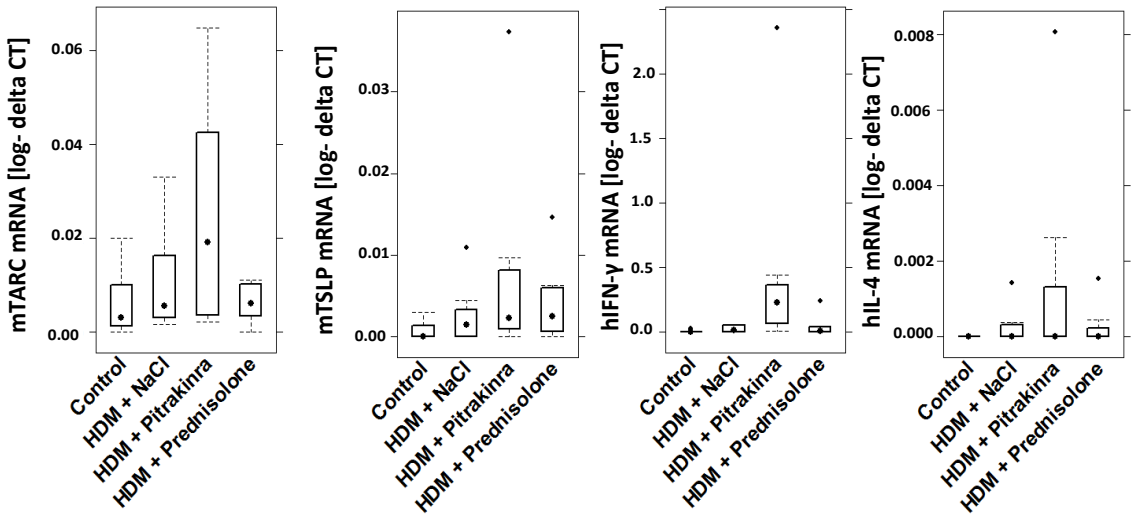

Fig. 12 . Treatment with Prednisolon or Pitrakinra has protective effects on different subgroups of human T-cells, B-cells, monocytes and macrophages in an asthmatic NOG mice model.

Conditional Tukey´s boxplot analysis of mouse (m)TARC, human (h) IL-4, human (h)IFNγ and mouse (m)TSLP mRNA expression in the lung of NSG mice. RNA was isolated from lung tissue and subjected to RT-PCR analysis. Experiments were performed with PBMCs from two allergic donors.

Comparison of control versus challenged, an ANOVA followed by Tukey's honest significant difference (HSD) test was performed. Lines represent values without variability. For more detailed date set see supplement figure S11.

4. Discussion.

By now NSG mice reconstituted with PBMC from diseased donors have been characterized as robust models, which reflect the human disease [19, 53]. This mouse model has the unique advantage to allow for the testing of therapeutics directed against human target molecules, which require high ligand, receptor, sequence, or structure homology. In our mouse model we used ovalbumin (OVA), or house dust mite (HDM) extract as allergens. OVA has often been used as model antigen. Hence, we wanted to examine whether this approach would be translatable to the disease of asthma in NSG mice reconstituted with PBMCs from asthmatic or healthy donors. However, one major disadvantage of OVA is that mice or humans would not initiate an allergic response against OVA. Quite the opposite, in most cases people would develop a natural oral tolerance against OVA as the major constituent of chicken eggs. Consequently, most mice model protocols need high doses of allergen in combination with high amounts of adjuvants, in order to provoke an allergic response. This is clearly in contrast to normal allergic reactions, which can be triggered by even the smallest doses of allergen. Because of this, recent allergic mouse models use natural occurring allergens like HDM. [47].

4.1 Hypothesis 1: Effect of OVA and HDM on PBMC *in vitro*

In a first attempt, we tested OVA and HDM *in vitro* and used phytohemagglutinin (PHA) as a positive control. As reported by Zaunders, PHA induced the expected activation of T-cells [56] and an increase in Th2 associated and CD4+ CD134+ double positive cells were detected. As anticipated, PHA had no effect on monocytes and/or macrophages. The lack of Th17 cells after PHA stimulation could be explained by the interaction with Th1 and Th2 cells. Increased expression of Th1 cells often results in suppression of Th17 cells and vice versa [118]. OVA induced no reaction in T-cells, monocytes or macrophages supporting the hypothesis that OVA is well tolerated and does not provoke an allergic response. Surprisingly, HDM also failed to activate T-cells, monocytes and macrophages. One explanation could be that *in vivo,* HDM predominantly acts via its protease activity and consequently damages epithelial cells. Due to the lack of this activity in HDM extracts HDM might have lost its full allergenic potential. Alternatively, HDM acts like other autoantigens some of which are known for their immunmodulating capacity [114]. This effect was also seen by Jodeleit et al., who tested the effect of the autoantigen CD99 in an NSG ulcerative colitis model. Like HDM, CD99 did not activate T

cells *in vitro* [120]. In contrast to results in conventional models, in which HDM induced asthma is associated with a Th2 response [46], HDM induced the frequency of Th17 cells and led to a decline of Th2 cells *in vitro*. This observation, however, would emphasize the role of Th17 cells in asthma [24,27-29]. It is noteworthy that no difference between healthy and asthmatic donors was detected indicating that control mechanisms are intact in this artificial *in vitro* situation.

Hypothesis 1: *In vitro* cell cultures assays expose cells to artificial conditions and insufficiently reproduce donor specific immune profiles that are relevant *in vivo*

Conclusion: These *in vitro* data suggest that cell culture assays are inappropriate to get specific and extensive insights into asthmatic disease.

4.2 Hypothesis 2 and 3: Effects of allergen and donor background in vivo

Next, we examined the response *in vivo*. This approach has been extensively tested using SCID mice showing that exposure to HDM induced symptoms and phenotype of asthma [46]. The further developed NSG mice, however, have the huge advantage that reconstitution is reliable due to the absence of NK cells and that leakiness is not observed [98,99].

Already in 2012 Martin and her colleagues successfully demonstrated that NOD-scid IL2Rγ^{null} (NSG) mice are suitable for an allergic asthmatic mouse model [121]. A similar approach was performed by Sonar et al in 2010 [57]. However, they were basically interested in the therapeutic potential of T-cells. A detailed characterization of symptoms and phenotype in NOD-scid IL2Rγ^{null} mice was never performed. Furthermore, a comparison of OVA and HDM was never conducted in this mouse strain. As observed by others, asthmatic symptoms and phenotype could be induced [52,57,59,61,121].

4.2.1. Clinical and histological score

Intranasally challenged NSG mice exhibited a significantly elevated clinical score in contrast to untreated control mice. As observed in conventional mouse models, challenge resulted in influx of leucocytes to form thick layers around alveoli, bronchioles and blood vessels. Infiltrating macrophages were the dominating population. Macrophages were characterized by a prominent nucleolus surrounded by a voluminous cytoplasm. This observation was unexpected as classical asthma is thought to be a Th2 mediated disease, however, recent studies revealed that macrophages and/or DC are sufficient to induce asthma [45] explaining the predominance of macrophages in this model.

Surprisingly, the histological score was not significantly different to the control group. One explanation for this result might be that the influx of cells was not evenly distributed in all parts of the lung and therefore the sections were not representative of the ongoing inflammation in the lung. Furthermore, we tried to use as little allergen as possible to reflect the human conditions, thereby accepting a lesser inflammatory response. Applying higher allergen doses might have intensified the leucocyte infiltration. Finally, the observed moderate influx of inflammatory cells could be due to the fact that lung histology was conducted four days after the last challenge. By that time inflammation might have already declined. Analysis of lung histology 24 hours post challenge at peak of the asthmatic response [45], might have resulted in a more pronounced histological score. The observation that we could not detect increased mucus production indicates

that this model lacks airway remodelling and thus reflects the early responses to allergen exposure. This observation is in accordance with previous studies, which described first airway remodelling processes 4 weeks upon persistent allergen contact and only by chronic and not brief allergen challenge [65].

Nevertheless, there was a clear tendency towards an increased infiltration of leucocytes into the lung. The infiltrating macrophages must have been of human origin as no infiltration was observed in unreconstituted mice.

4.2.2. Analysis of human leucocytes isolated from spleen.

Due to the complex interplay between innate and adaptive immunity in asthmatic disease [45,48,67] we thought that it was not sufficient to analyse one specific immune cell subtype. Therefore, we employed a more holistic approach and characterized a broad spectrum of immune cells isolated from spleen of mice. Levels of isolated leucocytes were similar to the levels described in the literature [66].

As shown by histological analyses of the lung, flow cytometry (FACS) analysis of leucocytes isolated from spleen tissue also indicated an increased infiltration or proliferation of macrophages, emphasizing their role in asthma. It is noteworthy that in contrast to the NSG-UC or NSG-AD models the disease background of the donor was not required for the development of the phenotype in response to challenge with the exception of activated CD4+ cells.

As indicated by the histology analysis, HDM challenge had the greatest effect on macrophages. Similar to recent results in a related colitis mouse model [19,53] numbers of monocytes and macrophages expressing CD1a were specifically elevated in HDM challenged mice, suggesting an important role, not only in colitis but also in general allergic diseases. CD1a was originally found on Langerhans cells in the skin, which have been shown to present lipids and self-lipids to T-cells [17,18]. However, whether lipid presentation contributes to an asthmatic immune response should be further investigated. No difference between challenged and unchallenged mice could be detected with regard to CD11b+ CD86+ double positive cells. One explanation could be that CD86 has a more important role in initiating T-cell responses [15,16] and could be already down regulated at that phase of inflammation. Further experiments to investigate the maturation of macrophages at different stages of the episode could clarify if and how long APCs are activated. This also applies to TSLPR expressing macrophages. TSLP is known to be secreted by epithelial cells in response to allergen and to induce proliferation of TSLPR expressing macrophages [100].

In contrast to our expectations, frequencies of this subtype declined in response to allergen. This result could be explained by the fact murine TSLP does not bind to the human TSLPR receptor and thus fails to promote proliferation of human TSLPR expressing macrophages [100].

4.2.3. Analysis of human leucocytes and cytokines in the lung

Difference between asthmatic and non asthmatic donors:

The analysis of human leucocytes isolated from lung revealed a difference between mice in respect of the donor background. Generally, the frequency of T-cells, eosinophils, basophils, mastcells and macrophages were increased in mice reconstituted with PBMCs from asthmatic donors. One explanation could be that, PBMCs from asthmatic donors seemed to be more responsive to signals directing them spontaneously to the lung. This signal applied to most of the cells analyzed.

Secondly, an activation of plasma cells, macrophages and to some extent eosinophils, in response to challenge was only observed in mice reconstituted with PBMCs from non asthmatic donors indicating that these cells were already activated to their limits in mice engrafted with PBMCs from asthmatic donors.

In contrast to levels of eosinophils, which were significantly elevated in mice reconstituted with asthmatic donors, levels of TGFβ1, one key player for airway remodelling [10], were reduced. As eosinophils are thought be be a major source of TGFβ1 in asthma it has to be further investigated why increased levels of eosinophils did not result in elevated levels of TGFβ1. Maybe, in the acute inflammatory response charactrized by IFNγ secreting macrophages and DC, epithelial to mesenchymal transition (EMT) is still inhibited, [10].

Difference between OVA and HDM:

Challenge with OVA or HDM resulted in different responses as indicated by frequencies of M2 monocytes, memory B-cells, TSLP mRNA and TARC mRNA expression levels in the compartment of the lung.

These parameters were increased in the OVA challenged group suggesting that this model is reflective of the conventional OVA mouse model characterized by a Th2 response. The fact that a response to OVA could only be observed in mice reconstituted with PBMC from non-asthmatic donors raises concerns, how reflective this model is of relapses observed in human asthma.

This concern is further supported by the notion that asthmatic relapses have not been described in humans in response to OVA. Normally, humans would develop tolerance against OVA, which was not seen in NSG mice. One explanation could be that host and tissue specific signals are essential in order to establish tolerance. This missing communication between murine tissue and human immune cells could be the reason for the asthmatic response against OVA. The observed allergic response of NSG mice engrafted with non-asthmatic donors after OVA challenge was rather unexpected. Maybe the immunization with adjuvant was effectively priming the immune system and essential for the immune response against OVA. Further experiments where engrafted NSG mice would be challenged without immunization with adjuvant might prove this hypothesis.

Rather unexpectedly, challenge with HDM evoked a Th17 /Th1 driven inflammation *in vitro* and *in vivo*. This inflammation was independent from IgE levels *in vivo*. It is noteworthy, that no correlation between IgE serum titres and different allergen challenge in comparison with the control group could be detected (data not shown), emphasizing the poor applicability of IgE as biomarker [45,69]. The fact that in this experiment a house dust mite extract was used might explain this result. It might be that HDM evokes a dual response in humans depending on the activities of HDM as an antigen and a protease. In the HDM extract the protease activity might have been obstructed and therefore the Th2 response may be missing. This hypothesis is supported by previous results describing the higher capacity of the active Der p1 protease in evoking asthma symptoms [83].

In order to develop a highly efficient asthma therapy a detailed screening of PBMCs from asthmatic patients and consequently an individual characterization of different homing receptor and activation markers will become more important.

Hypothesis 2: HDM and OVA induce different immunological responses in NSG mice.

Hypothesis 3: The immunological status of the donor can be modelled in NSG mice and is essential for the immune responses.

Conclusion: Challenge with HDM initiated a Th1 /Th17 associated immune response, while OVA led to an increase in Th2 associated immune cells. PBMC from asthmatic donors seemed responsive to signals directing them spontaneously to the lung. Furthermore leucocytes from asthmatic donors seemed inert to further challenges.

The disease background of the donor was not required for the development of symptoms and phenotype in response to challenge.

4.3 Hypothesis 4: Effect of pitrakinra or prednisolone on symptoms and phenotype.

As these results suggested mice, reconstituted with PBMC from asthmatic donors and challenged with HDM, were more reflective of the human disease. One big advantage of this chimeric mouse model is the possibility to test new therapeutics, which address human specific targets. Therefore, we tested pitrakinra a human IL-4 and IL-13 receptor antagonist, which should inhibit Th2 associated immune responses. In addition, we tested prednisolone, an anti-inflammatory glucocorticoid, which is often used in allergic asthma therapy [49,50]. Treatment with prednisolone had a preventive effect in HDM challenged mice and restored homeostasis. Levels of the histological score, frequencies CRTH2+ CD4+, Th1 / Th17 cells isolated from spleen or naïve CD4+ cells, effector CD4+ cells, Th17 or Th1 / Th17 cells and TARC, TSLP, IFNγ expression returned to normal levels. On the cellular level, pitrakinra seemed to act similarly with one important difference: treatment with pitrakinra had no impact on frequencies of Th17 cells and this might be the reason for the ongoing inflammation as indicated by elevated TARC, TSLP, IFNγ and IL-4 expression and it might also be the reason for the observed limited efficacy in human trials [70]. In this study pitrakinra had no effect on the early phase response and showed no improvement concerning the airway hyperresponsiveness. Since the biological half-life of pitrakinra was tested in a different animal strain, we cannot exclude an insufficient plasma concentration. More tests with different working concentrations will be needed to find the ideal study protocol. Moreover small modifications of pitrakinra like additional glycosylation could increase the biological half-life of and thus improve the activity. New experiments with different variants of pitrakinra would clarify this aspect. However, pitrakinra might be efficacious in late responses responsible for alterations of the lung architecture by fibrosis.

Hypothesis 4: Preventive treatment with prednisolone and pitrakinra mitigate asthma symptoms after HDM-challenge

Conclusion: Treatment with prednisolone had a preventive effect in HDM challenged mice and restored homeostasis. Treatment with pitrakinra showed limited efficacy.

Taken together, one important observation in this study was the induction of a Th17 response *in vitro* and *in vivo* by HDM, corroborating previous results that suggested a Th17 response to initiate inflammation [102]. To date, it is disputed whether Th17 cells are beneficial or add to the pathologies in asthma [68]. The second important observation was the spontaneous influx of leucocytes from asthmatic donors to the lung and their inertness to further challenge. Thus, leucocytes from asthmatic patients seemed to have an inherent capacity to induce inflammation, suggesting that one focus of therapeutic intervention might shift from inhibiting challenge to preventing migration. In conclusion, this study has shown that the model is useful to study mechanism underlying inflammatory processes in asthma and that it can be used to test therapeutics directed against human target molecules.

4.4 Limitations of the NSG asthma model

Comparable to many animal models, this animal model does not fully reflect the human disease as for example the IgE induced response does not seem to be mounted. In addition, one has to keep in mind that the described NSG model is a chimeric model and that some of the chemokines are essential for the recruitment of the immune cells to the lung. Cytokines are essential for the type and magnitude of the immune response so that as long as the molecular patterns used in an immune response are not cross-reactive between species it is very difficult to remake or remodel the human immunity in mouse. This is one of the biggest challenge - but there is nothing one can change. It would be good to use human IL4 or human TSLP-transgenic mouse as an example but plenty of chemokines and cytokines do not show cross-reactivity between human and mouse species and they do not activate receptors of the other species as it has been shown for IL-4 and TSLP [100,101].

Furthermore, the challenge with antigens could be improved by using active proteases.

Another point is the enhancement of the clinical manifestation of asthma symptoms. Using a higher allergen concentration and more intranasal injections could address this point. Increased histological and clinical scores as well as elaborated influx of leucocytes into the lung could reveal significant correlations between different immune cells, donor backgrounds and the severity of asthma.

In Addition, in order to extend the observation some of the experiments could be ended 24 hours after the last challenge. This could increase the asthmatic response and consequently the influx of immune cells. Finally a more severe manifestation of asthma symptoms could also reveal a higher potential of experimental therapeutics.

Another limitation of the NSG asthma model is given by the number of mice per group, due to the fact that only a limited amount of leucocytes can be isolated from the donor. One solution could be to pool different blood samples, or expand donor cells *in vitro* and then transfer into the mouse in order to increase the group size, but in that case donor specific reactions cannot be analysed.

Beside of the limitations of the animal model, the characterization of the donors were also limited due to logistic and financial reasons. A lung function test of healthy and asthmatic donors and the translation into the animal model could bring new insights in the pathology of asthma. By using a skin prick test for healthy donors, hidden allergies could be detected, which could improve the donor selection.

5. Outlook

Asthma is a complex and variable disease affecting millions of people. Developing new and more efficient therapies will be one of the major challenges in asthma research.

The NOD-*scid IL2Rγ*^{null} asthmatic mouse model is a novel, powerful tool to investigate immunologic processes and eminent for drug testing. However, further experiments should be conducted in order to answer open questions.
Future tests will show, if pitrakinra can improve the clinical outcome in a Th2 driven asthmatic response. Additionally, further tests could reveal new therapeutic targets, like possible asthmatic specific migration and/or homing factors.

A lot more research is needed to fully understand asthma, but the NOD-*scid IL2Rγ*^{null} asthmatic mouse model can help to receive extensive insights in the immunological aspects of asthma with high reliability to the human disease.

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8. Supplement

	Mean				SD				IQR				n				HDM/ control	p-value	lower	upper	OVA/ control	p-value	lower	upper	OVA/ HDM	p-value	lower	upper	PHA/ Control	p-value	lower	upper	PHA/ OVA	p-value	lower	upper	PHA/ HDM	p-value	lower	upper
Leukocyte[% FoP]	**Con-trol**	**OVA**	**HDM**	**PHA**	**Con-trol**	**OVA**	**HDM**	**PHA**	**Con-trol**	**OVA**	**HDM**	**PHA**	**Con-trol**	**OVA**	**HDM**	**PHA**																										
CD4+	21.36	16.73	19.88	12.81	9.40	8.60	12.06	7.24	8.1	3.6	2.4	4	5	5	5	5																								
CD69+ [%CD4]	35.74	20.4	19.57	49.16	9.87	5.85	8.05	10.19	12.8	7.8	11.5	7	5	5	5	5																		0.0001	12.34	45.18		0.0001	13.17	46.02
CD134+ [%CD4]	34.0	21.38	16.56	42.92	8.54	4.05	4.01	9.83	13.3	4.3	3.8	13.4	5	5	5	5		0.0120	-32.10	-2.78														0.0013	6.88	36.20		0.0001	11.70	41.02
CD103+ [%CD4]	2.14	1.92	1.70	2.09	0.98	0.57	0.99	0.84	0.84	1.02	1.53	0.44	5	5	5	5																								
CD4+ CD25+ CD127lo	18.16	6.01	6.67	16.18	5.29	0.82	1.25	5.55	3.6	1.23	1.01	3.2	5	5	5	5		0.0040	-20.13	-2.84		0.0022	-20.79	-3.51										0.0134	1.53	18.81		0.0240	0.86	18.15
CCR4+ [%CD4]	14.2	12.34	13.74	6.60	3.59	3.02	2.52	1.85	5.9	4.7	3	2.77	5	5	5	5																								
CD11b+	5.44	5.17	5.69	4.96	2.26	3.72	2.95	3.00	1.5	1.52	1.46	3.63	5	5	5	5																								
CD86+ [%CD14]	64.62	32.78	24.9	37.84	14.49	3.82	5.48	14.61	21.6	2.6	6.4	17.6	5	5	5	5																								
CCR2+ [%CD14]	17.95	5.29	0.43	0.67	11.55	5.79	0.35	0.25	12.65	9.22	0.52	0.26	5	5	5	5																								
CD1a+ [%CD14]	6.57	8.11	29.28	21.04	4.26	2.09	14.94	18.09	2.86	0.5	9.3	29.24	5	5	5	5		0.0469	0.20	45.23																				
TSLPR+ [%CD14]	13.42	19.94	14.07	1.87	10.02	11.89	3.84	1.24	17.81	22.58	1.2	2.21	5	5	5	5																								
TH1	70.54	73.52	70.72	81.5	8.42	8.22	9.39	6.20	6.3	1.9	6.7	5.6	5	5	5	5																								
TH2	65.04	63.06	51.8	77.88	7.56	5.54	4.94	5.04	3.5	4.9	5.7	7.2	5	5	5	5		0.0204	-25.05	-1.43											0.0263	1.033	24.65		0.0072	3.01	26.62		0.0000	14.27	37.89
TH17	27.46	30.06	40.56	18.22	7.67	5.46	5.14	3.29	4.5	6.5	5.7	5	5	5	5	5		0.0167	1.68	24.52														0.0383	-23.26	-0.42		0.0000	-33.76	10.92

Supplement Table 1: *in vitro* analysis of leucocytes extracted from healthy (non asthma) donors and cultivated with different allergens and subjected to flow cytometric analysis. Statistical analysis was conducted by ANOVA followed by TukeyHSD test. Abbrevations: FoP = Frequent of Parent, the percentage of events (cells) in this gate out of the parent gate (one level up); SD = Standard Deviation; IQR = Interquartile Range; n = Number of used Animals; OVA = Ovalbumin; HDM =House Dust Mite; PHA = phytohemagglutinin.

	Mean				SD				IQR				n																									
Leukocyte [% FoP]	**Con-trol**	**OVA**	**HDM**	**PHA**	**Con-trol**	**OVA**	**HDM**	**PHA**	**Con-trol**	**OVA**	**HDM**	**PHA**	**Cont-rol**	**OVA**	**HDM**	**PHA**	**HDM/ con-trol**	**p-value**	**lower**	**upper**	**OVA/ con-trol**	**p-value**	**lower**	**upper**	**OVA/ HDM**	**p-value**	**lower**	**upper**	**PHA/ con-trol**	**p-value**	**lower**	**upper**	**PHA/ OVA**	**p-value**	**lower**	**upper**	**PHA/ HDM**	**p-value**	**lower**	**upper**
CD4+	20.90	17.77	19.00	15.27	2.17	2.44	3.80	2.70	1.9	2.4	3.8	2.7	3	3	3	3																								
CD69+ [%CD4]	24.37	13.30	11.87	41.43	6.62	4.12	1.48	8.62	5.85	4.11	1.45	8.6	3	3	3	3																		0.0041	6.93	49.34		0.0024	8.36	50.77
CD134+ [%CD4]	24.57	13.57	10.77	34.73	8.95	4.71	3.71	8.22	8.85	4.2	3.35	7.95	3	3	3	3																		0.0209	2.24	40.1		0.0066	5.04	42.90
CD103+ [%CD4]	1.03	1.14	1.02	0.95	0.37	0.36	0.06	0.46	0.37	0.34	0.06	0.46	3	3	3	3																								
CD25+ CD127lo [CD4%]	10.56	4.33	3.87	8.84	6.50	2.16	2.22	5.51	6.46	1.99	1.96	5.19	3	3	3	3																								
CCR4+ [%CD4]	10.56	11.36	11.63	9.38	1.21	3.37	3.11	0.07	1.05	3.37	3.11	0.01	3	3	3	3																								
CD11b+	4.86	3.92	2.61	2.51	2.31	1.32	1.42	0.60	2.18	1.205	1.31	0.60	3	3	3	3																								
CD86+ [%CD14]	62.67	45.57	33.73	55.40	34.41	5.52	5.07	14.38	32.85	4.85	4.75	12.45	3	3	3	3																								
CCR2+ [%CD14]	19.53	1.51	0.62	1.93	23.10	1.08	0.16	0.92	21.71	1.01	0.15	0.85	3	3	3	3																								
CD1a+ [%CD14]	2.40	8.20	27.03	18.50	1.16	2.52	13.48	7.13	1.01	2.3	13.45	6.45	3	3	3	3																								
TSLPR+ [%CD14]	34.86	22.33	27.03	2.59	28.80	11.42	8.06	1.34	28.52	11.3	8	1.19	3	3	3	3														0.0341	-62.92	-1.61								
TH1	74.97	76.8	74.07	83.73	7.62	7.54	11.85	3.41	7.45	7.3	10.5	3.4	3	3	3	3																								
TH2	73.10	66.5	56.67	82.97	6.26	3.70	6.37	3.53	6.25	3.7	6.35	3.15	3	3	3	3		0.0283	-31.68	-1.19															0.0278	1.22	31.71		0.0002	11.06	41.54
TH17	20.17	26.77	35.3	12.63	6.53	3.35	6.54	2.83	6.5	3.35	6.2	2.45	3	3	3	3		0.0413	0.39	29.87																		0.0007	-37.41	-7.93

Supplement Table 2: *in vitro* analysis of leucocytes extracted from asthmatic donors and cultivated with different allergens and subjected to flow cytometric analysis. Statistical analysis was conducted by ANOVA followed by TukeyHSD test. Abbrevations: FoP = Frequent of Parent, the percentage of events (cells) in this gate out of the parent gate (one level up); SD = Standard Deviation; IQR = Interquartile Range; n = Number of used Animals; OVA = Ovalbumin; HDM =House Dust Mite; PHA = phytohemagglutinin.

	Mean			SD			IQR			n														
Leukocytes [% FoP]	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**HDM/ control**	**p-value**	**lower**	**upper**	**OVA/ control**	**p-value**	**lower**	**upper**	**OVA/ HDM**	**p-value**	**lower**	**upper**
CD4+	25.09	13.70	23.69	14.79	11.96	14.62	19.75	20.11	22.58	38	26	38						0.0055	2.87	19.90		0.0172	-18.51	-1.47
CD134+ [%CD4]	81.39	78.07	87.64	13.44	13.81	7.18	14.35	23.4	11.05	19	12	20										0.0679	-19.71	0.57
CD62L+ [%CD4]	41.74	44.96	53.75	21.05	24.17	20.63	35.05	24.4	36.4	36	25	34		0.0598	-24.40	0.39								
CD294+ [%CD4]	11.31	10.33	13.51	11.56	7.88	13.40	10.27	14.26	13.34	35	20	29												
CD11b+	9.78	9.99	14.37	5.21	5.78	9.49	4.34	5.03	10.76	38	26	38		0.0182	-8.54	-0.65						0.0491	-8.77	-0.01
CD86+ [%CD11b]	37.61	35.33	37.04	22.02	24.33	21.25	39.63	37.9	35.73	36	24	34												
E-Cadherin+ [%CD11b]	33.27	35.98	38.02	27.49	22.22	23.76	55.22	42.13	43.45	36	24	34												
CD1a+ [%CD11b]	48.85	44.86	51.52	27.00	26.97	28.59	45.68	48.5	49.93	36	25	34												
TSLPR+ [%CD11b]	11.42	11.91	10.74	13.35	15.46	11.95	5.71	7.58	7.07	36	25	34												
CD86+ [%CD14]	39.66	36.27	39.37	24.47	23.72	21.31	49.15	42.1	36.58	36	25	34												
CCR2+ [%CD14]	10.76	16.97	19.10	7.66	11.52	14.89	8.65	10.22	19.17	36	25	34		0.0101	-15.00	-1.69								
CD1a+ [%CD14+]	49.66	47.44	61.28	21.85	20.67	21.55	40.7	28.8	28.95	36	25	34		0.0656	-23.84	0.59						0.0422	-27.30	-0.39
TSLPR+ [%CD14]	13.69	11.9	11.97	13.35	15.46	11.95	5.71	7.58	7.07	36	25	34												
CD206+ [%CD14]	15.24	11.16	54.91	15.52	11.63	18.32	12.62	8.35	15.81	31	19	29												
CD163+ [%CD14]	42.82	46.41	19.29	23.04	20.88	20.52	40.25	32.95	25.6	31	19	29												
CD45	15.53	15.96	19.32	14.07	13.65	12.2	8.51	9.61	14.00	19	12	20												

Supplement Table 3: Leucocytes isolated from spleen out of NSG- mice engrafted with PBMC from Asthma and Non Asthma patients and challenged with OVA, or HDM, were subjected to flow cytometric analysis. Statistical analysis was conducted by ANOVA followed by TukeyHSD test. Abbrevations: FoP = Frequent of Parent, the percentage of events (cells) in this gate out of the parent gate (one level up); SD = Standard Deviation; IQR = Interquartile Range; n = Number of used Animals; OVA = Ovalbumin; HDM =House Dust Mite.

	Mean			SD			IQR			n														
Leukocyte [% FoP]	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**HDM/ control**	**p-value**	**lower**	**upper**	**OVA/ control**	**p-value**	**lower**	**upper**	**OVA/ HDM**	**p-value**	**lower**	**upper**
CD4+	22.13	9.59	21.47	15.21	10.61	18.37	24.97	18	27.45	11	14	9						0.0956	-1.79	26.87				
CD134+ [%CD4]	58.65	61.62	79.67	5.45	5.85	8.33	6.05	7.18	9.53	4	4	4		0.0040	-34.18	-7.87						0.0101	-31.21	-4.89
CD62L+ [%CD4]	26.26	37.45	47.56	10.85	21.02	19.39	11.38	16.4	9.7	12	13	9		0.0263	-40.40	-2.19								
CD294+ [%CD4]	17.23	13.54	30.64	14.98	7.99	23.83	4.70	11.69	18.06	11	8	4												
CD11b+	11.94	12.03	16.59	6.86	6.61	9.38	7.04	6.35	15.82	11	14	9												
CD86+ [%CD11b]	29.12	26.84	28.07	19.94	18.40	17.31	38.55	26.5	30.2	12	12	9												
E-Cadherin + [%CD11b]	27.51	33.14	42.3	30.13	21.46	20.68	59.32	33.7	35.5	12	12	9												
CD1a+ [%CD11b]	42.84	35.49	41.29	21.78	23.47	28.92	36.4	41.3	50.4	12	13	9												
TSLPR+ [%CD11b]	13.86	9.85	6.597	7.06	9.83	1.42	9.54	6.04	2.16	12	13	9		0.0858	-0.84	15.35								
CD86+ [%CD14]	46.93	34.72	37.54	25.12	26.48	31.29	44.9	45.1	60.24	12	13	9												
CCR2+ [%CD14]	13.32	20.48	21.48	8.31	13.99	15.59	8.65	9.1	17.14	12	13	9												
CD1a+ [%CD14+]	46.08	39.27	46.33	25.55	22.75	27.62	47.05	43.6	52.4	12	13	9												
TSLPR+ [%CD14+]	21.08	11.83	4.15	20.08	20.92	3.48	19.01	7.71	6.97	12	13	9		0.0941	-2.35	36.22								
CD64+ [%CD14+]	23.23	12.43	4.39	23.23	17.58	1.39	25.36	4.59	0.84	11	7	4												
CD163+ [%CD14+]	67.75	50.26	59.42	24.89	23.54	8.47	40.1	28.5	14.28	11	7	4												
CD206+ [%CD14+]	23.26	13.49	5.84	22.70	17.75	2.56	35.54	8.68	1.62	11	7	4												
clinical Score	0	1	1.33	0	0.68	1	0	0	1	10	14	9		0.0002	-2.17	-0.50		0.0028	-1.75	-0.25				
histological Score	0.19	1.06	0.94	0.44	0.99	1.02	0.06	1.75	1.75	8	9	9												

Supplement Table 4: Leucocytes isolated from spleen out of NSG- mice engrafted with PBMC from Non Asthma patients and challenged with OVA, or HDM, were subjected to flow cytometric analysis. Statistical analysis was conducted by ANOVA followed by TukeyHSD test. Abbrevations: FoP = Frequent of Parent, the percentage of events (cells) in this gate out of the parent gate (one level up); SD = Standard Deviation; IQR = Interquartile Range; n = Number of used Animals; OVA = Ovalbumin; HDM =House Dust Mite.

	Mean			SD			IQR			n														
Leukocytes [% FoP]	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**HDM/ control**	**p-value**	**lower**	**upper**	**OVA/ control**	**p-value**	**lower**	**upper**	**OVA/ HDM**	**p-value**	**lower**	**upper**
CD4+	26.29	18.50	24.38	14.74	12.06	13.56	19.05	18.13	22.8	27	12	29												
CD134+ [%CD4]	87.46	86.29	89.63	6.22	7.30	5.50	8.7	9.33	9.7	14	8	16												
CD62L+ [%CD4]	49.48	53.11	55.98	20.74	25.56	20.98	34.55	40.35	40.9	24	12	25												
CD294+ [%CD4]	8.59	8.18	10.77	8.69	7.36	9.04	10.46	9.64	11.83	24	12	25												
CD11b+	8.90	7.60	13.69	4.22	3.56	9.58	3.56	4.93	10.53	27	12	29		0.0335	-9.27	-0.31						0.0357	-11.83	-0.33
CD86+ [%CD11b]	41.86	43.81	40.28	22.16	27.25	21.91	39.08	48.75	42.5	24	12	25												
E-Cadherin + [%CD11b]	36.15	38.82	36.47	26.27	23.54	24.98	52.19	44.5	50.16	24	12	25												
CD1a+ [%CD11b]	51.85	55.02	55.2	29.22	27.75	28.14	52.43	47.8	47.7	24	12	25												
TSLPR+ [%CD11b]	10.20	14.14	12.23	7.67	7.35	7.30	6.77	11.16	4.79	24	12	25												
CD86+ [%CD14]	36.03	37.95	40.03	23.83	21.37	17.21	48.88	33.95	22.2	24	12	25												
CCR2+ [%CD14]	9.48	13.16	18.24	7.15	6.73	14.88	9.96	9.17	19.86	24	12	25		0.0190	-16.31	-1.22								
CD1a+ [%CD14+]	51.45	56.28	66.66	20.11	14.25	16.44	34.25	17.08	27	24	12	25		0.0103	-27.33	-3.10								
TSLPR+ [%CD14+]	10.00	11.98	14.78	5.94	6.61	12.69	7.26	7.96	10.36	24	12	25												
CD64+ [%CD14+]	7.64	16.57	17.74	7.35	23.59	20.28	7.09	34.82	34.14	19	12	21												
CD163+ [%CD14+]	38.98	44.17	54.05	21.64	19.90	22.13	37.1	26.75	32.7	19	12	21		0.0732	-31.28	1.13								
CD206+ [%CD14+]	10.83	9.8	21.85	7.18	6.63	18.94	11.16	7.74	26.67	19	12	21		0.0263	-20.93	-1.09						0.0379	-23.53	-0.56
clinical Score	0	1.83	1.09	0	0.94	0.67	0	1.25	0.5	24	12	23		0.0000	-1.62	-0.56		0.0000	-2.48	-1.19		0.0144	0.10	1.39
histological Score	0.89	1.81	1.31	1.02	1.19	0.97	1.56	1.94	1	21	8	21												

Supplement Table 5: Leucocytes isolated from spleen out of NSG- mice engrafted with PBMC from Asthma patients and challenged with OVA, or HDM, were subjected to flow cytometric analysis. Statistical analysis was conducted by ANOVA followed by TukeyHSD test. Abbrevations: FoP = Frequent of Parent, the percentage of events (cells) in this gate out of the parent gate (one level up); SD = Standard Deviation; IQR = Interquartile Range; n = Number of used Animals; OVA = Ovalbumin; HDM =House Dust Mite.

	Mean			SD			IQR			n														
Leukocytes [% FoP]	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**HDM/ control**	**p-value**	**lower**	**upper**	**OVA/ control**	**p-value**	**lower**	**upper**	**OVA/ HDM**	**p-value**	**lower**	**upper**
CD4+	30.8	40.79	45.6	6.68	14.83	17.78	3.1	11.9	15.23	5	9	8												
CD134+ [%CD4]	94.5	90.08	92.22	1.68	5.54	2.16	1.6	3.38	2.58	3	4	4												
CD62L+ [%CD4]	85.2	69.78	71.83	4.26	3.98	11.79	4.25	3.53	9.13	3	4	4						0.0784	-1.85	32.70				
CD294+ [%CD4]	47.3	24.12	25.43	12.27	4.69	11.82	12.05	5.28	8.58	3	4	4		0.0478	0.23	43.52		0.0372	1.53	44.82				
CD8+	1.85	4.80	0.97	1.78	6.66	1.29	2.48	10.05	1.33	5	9	8												
CD11b+	25.96	33.32	30.63	14.03	9.42	11.87	9.4	13.5	10.9	5	9	8												
CD86+ [%CD11b]	11.48	10.27	8.91	6.82	5.27	2.11	6.79	6.66	2.68	5	9	8												
E-Cadherin+ [%CD11b]	57.94	66.31	64.61	16.71	13.50	9.49	11.6	20.4	15.4	5	9	8												
CD1a+ [%CD11b]	82.42	72.06	73.47	12.66	18.14	17.27	11.6	31.3	27.93	5	9	8												
TSLPR+ [%CD11b]	6.48	7.11	4.26	1.53	2.87	1.32	2.44	1.77	1.81	4	4	4												
CD86+ [%CD14]	8.03	5.52	7.39	7.65	3.43	3.16	13.02	4.59	4.75	4	9	8												
CCR2+ [%CD14]	2.90	6.49	9.58	1.48	3.54	7.01	1.72	4.39	7.48	4	9	8												
CD1a+ [%CD14+]	75.9	78.98	79.15	7.33	16.59	15.65	5.85	29.5	27.4	4	9	8												
TSLPR+ [%CD14]	8.7	2.29	3.55	13.88	1.62	3.08	10.42	2.68	1.99	4	9	8												
CD163+ [%CD14]	68.27	80.1	80.38	4.72	2.36	2.84	4.45	2.55	3.43	3	4	4		0.0032	-19.24	-4.97		0.0037	-18.97	-4.70				
CD206+ [%CD14]	16.6	24.8	17.62	5.06	4.38	5.18	4.65	2.95	4.73	3	4	4												
FceRIa+ CCR3+	6.04	12.81	15.02	3.26	6.73	8.31	3.33	8.86	15.14	4	9	8												
FceRIa+ CD117+	0.21	3.75	3.27	0.14	2.15	2.86	0.17	1.55	3.20	4	9	8						0.0472	-7.05	-0.04				
HLADR+ CD123+	3.73	3.46	4.83	2.90	1.95	4.76	4.59	1.86	4.44	4	9	8												
IgD+ [%CD27]	20.33	56.98	65.88	18.01	26.64	32.28	26.48	34.53	25.43	4	4	4		0.0852	-97.48	6.38								
CD27+ [%CD19]	1.57	30.58	13.62	1.27	17.13	11.02	1	23.98	11.32	4	4	4										0.0172	-52.27	-5.75
CD19+ CD38+	1.49	3.83	3.99	0.48	0.72	1.83	0.48	0.93	1.92	4	4	4		0.0350	-4.80	-0.19						0.0467	-4.65	-0.04

Supplement Table 6: Leucocytes isolated from lung out of NSG- mice engrafted with PBMC from Non Asthma patients and challenged with OVA, or HDM, were subjected to flow cytometric analysis. Statistical analysis was conducted by ANOVA followed by TukeyHSD test. Abbrevations: FoP = Frequent of Parent, the percentage of events (cells) in this gate out of the parent gate (one level up); SD = Standard Deviation; IQR = Interquartile Range; n = Number of used Animals; OVA = Ovalbumin; HDM =House Dust Mite.

	Mean			SD			IQR			n													
Leukocytes [% FoP]	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**HDM/ control**	**p-value**	**lower**	**upper**	**OVA/ control**	**p-value**	**lower**	**upper**	**OVA/ HDM**	**p-value**	**lower**	**upper**
CD4+	49.24	55.67	45.21	22.38	17.32	23.24	37.43	23.08	33.45	16	8	16												
CD134+ [%CD4]	90.28	91.71	80.58	4.30	3.83	24.13	4.78	2.25	6.83	16	8	16												
CD62L+ [%CD4]	59.3	50.17	57.02	15.71	17.21	25.49	26.78	27.95	29.88	12	8	12												
CD294+ [%CD4]	10.55	8.70	8.32	4.05	4.54	7.07	5.63	5.94	10.68	16	8	16												
CD8+	7.79	12.79	10.36	6.25	9.99	8.57	8.48	9.74	14.54	16	8	16												
CD11b+	39.14	47.54	35.67	20.45	22.26	23.87	35.9	35.95	27.9	16	8	16												
CD86+ [%CD11b]	25.04	11.26	23.62	27.12	5.58	27.04	31.17	9.32	28.70	16	8	16												
E-Cadherin+ [%CD11b]	77.97	83.88	78.11	9.73	7.81	10.46	12.53	5.93	15.5	16	8	16												
CD1a+[%CD11b]	83.12	84.75	81.99	9.48	7.72	16.05	14.55	11.98	6.85	16	8	16												
TSLPR+ [%CD11b]	8.14	9.74	9.44	4.16	4.23	10.06	6.04	8.43	6.95	16	8	16												
CD86+ [%CD14]	7.27	9.77	5.60	4.89	5.96	4.13	7.99	4.60	6.11	16	8	16												
CCR2+ [%CD14]	6.79	7.27	6.71	6.37	7.11	5.35	5.69	4.98	5.61	16	8	16												
CD1a+ [%CD14+]	77.74	78.69	67.02	11.37	13.35	26.34	17.98	14.55	20.25	16	8	16												
TSLPR+ [%CD14]	3.92	6.46	10.35	3.48	3.98	19.78	4.53	5.79	5.16	16	8	16												
CD206+ [%CD14]	15.24	19.72	19.63	10.24	14.07	14.18	16.61	20.40	22.51	11	8	12												
CD163+ [%CD14]	64.29	56.04	53.08	16.07	11.35	19.21	25.2	15.93	33.9	11	8	12												
FceRIa+ CCR3+	51.44	45.54	47.93	23.02	22.69	24.89	42.2	29.18	46.78	16	8	16												
FceRIa+ CD117+	15.04	12.44	15.51	12.77	8.55	13.47	18.71	8.77	12.82	16	8	16												
HLADR+ CD123+	6.02	9.72	8.34	5.86	6.55	6.87	5.76	11.34	9.12	16	8	16												
IgD+ [%CD27]	42.00	63.54	35.40	28.15	24.68	30.31	34.95	37.38	43.1	16	8	16												
CD27+ [%CD19]	20.78	35.78	25.78	21.68	22.12	25.95	31.52	23.75	35.79	16	8	16												
CD19+ CD38+	4.11	13.16	3.41	4.49	24.04	4.48	1.37	8.21	2.40	16	8	16												

Supplement Table 7: Leucocytes isolated from lung out of NSG- mice engrafted with PBMC from Asthma patients and challenged with OVA, or HDM were subjected to flow cytometric analysis. Statistical analysis was conducted by ANOVA followed by TukeyHSD test. Abbrevations: FoP = Frequent of Parent, the percentage of events (cells) in this gate out of the parent gate (one level up); SD = Standard Deviation; IQR = Interquartile Range; n = Number of used Animals; OVA = Ovalbumin; HDM =House Dust Mite.

	Mean			SD			IQR			n														
Cytokines	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**HDM/ control**	**p-value**	**lower**	**upper**	**OVA/ control**	**p-value**	**lower**	**upper**	**OVA/ HDM**	**p-value**	**lower**	**upper**
TGF	0.3768	0.3325	0.1798	0.2226	0.1483	0.0838	0.3295	0.0867	0.1334	4	4	4												
HGF	1.0210	0.1943	0.1407	1.0900	0.0789	0.0550	1.6230	0.0554	0.0536	4	4	4												
TARC	0.1849	2.6328	0.1812	0.2186	3.4727	0.1708	0.0744	3.2409	0.2793	9	4	12						0.0026	-4.20	-0.69		0.0016	0.76	4.14
IFNγ	3.2677	10.3283	7.5715	6.6459	10.8287	12.3432	0.0249	11.6667	13.8906	9	4	12												
IL-4	0.0003	0.0025	0.0010	0.0009	0.0030	0.0015	0	0.0045	0.0015	9	4	12												
TSLP	0.0199	0.0238	0.0079	0.0444	0.0110	0.0177	0.0098	0.0074	0.0051	9	4	12												

Supplement Table 8: mRNA cytokine levels isolated from lung out of NSG- mice engrafted with PBMC from Asthma patients and challenged with OVA, or HDM, were subjected to real-time polymerase chain reaction (RT-PCR) analysis. Statistical analysis was conducted by ANOVA followed by TukeyHSD test. Cytokine concentrations are given in logarithmic delta cycle threshold; Abbreviations: SD = Standard Deviation; IQR = Interquartile Range; n = Number of used Animals; OVA = Ovalbumin; HDM =House Dust Mite. TGF = Transforming Growth Factor β; HGF = Hepatocyte Growth Factor; TARC = Thymus and Activation Regulated Chemokine; IFNγ = Interferonγ; IL-4 = Interleukin 4; TSLP = Thymic Stromal Lymphopoietin.

	Mean				SD				IQR				n																												
Leukocyte [% FoP]	**Con-trol**	**HDM**	**HDM +Pitra kinra**	**HDM +Pred nisolo n**	**Con-trol**	**HDM**	**HDM +Pitra kinra**	**HDM +Pred nisolo n**	**Con-trol**	**HDM**	**HDM +Pitra kinra**	**HDM +Pred nisolo n**	**Con-trol**	**HDM**	**HDM +Pitra kinra**	**HDM +Pred nisolo n**	**HDM/ con-trol**	**p-value**	**lower**	**upper**	**HDM +pitra kinra/ con-trol**	**p-value**	**lower**	**upper**	**HDM + pitrak inra/ HDM**	**p-value**	**lower**	**upper**	**HDM + predni solon/ con-trol**	**p-value**	**lower**	**upper**	**HDM +pred nisolo n/ HDM +pitra kinra**	**p-value**	**lower**	**upper**	**HDM +pred nisolo n/ HDM**	**p-value**	**lower**	**upper**
CD4+	10.45	13.48	14.5	16.11	8.23	7.99	8.77	10.90	12.43	5.82	9.69	11.15	8	11	11	8																								
CD69+ [%CD4]	42.42	49.75	52.25	38.04	22.29	11.65	23.76	28.66	29.18	6.7	24.75	38.41	4	4	4	4																								
CD134+ [%CD4]	88.97	92.4	86.21	82.21	4.75	4.71	10.27	8.06	6.23	5.2	9.95	7.73	8	8	8	8																						0.0450	-20.20	-0.17
CCR4+ [%CD4]	1.34	1.64	0.82	1.11	0.52	0.45	0.10	0.58	0.67	0.6	0.1	0.76	4	4	4	4																						0.0990	-1.77	0.13
CD294+ [%CD4]	9.87	11.16	7.68	4.22	5.79	7.09	4.66	3.66	7.76	11.76	7.90	6.38	8	11	11	8																						0.0515	-13.90	0.03
CD11b+	7.60	13.47	11.91	9.55	3.04	11.74	9.83	3.58	2.54	4.65	3.55	4.88	8	11	11	8																								
CD1a+ [%CD11b]	70.97	53.57	49.29	57.9	12.26	30.01	34.92	24.85	11.5	46.75	67.55	41.18	8	11	11	8																								
TSLPR+ [%CD11b]	8.56	11.26	12.33	8.65	3.01	7.6	5.71	2.10	3.44	3.18	5.05	2.11	8	11	11	8																								
CD86+ [%CD11b]	61.67	46.49	45.24	50.51	8.90	20.37	24.34	19.47	12.25	25.45	40.5	32.28	8	11	11	8																								
CD1a+ [%CD14]	62.62	68.81	62.07	61.89	11.84	19.47	17.26	15.36	17.55	21.65	19.85	22.1	8	11	11	8																								
TSLPR+ [%CD14]	9.12	8.95	9.49	8.40	3.12	3.70	4.22	4.49	3.1	3.39	6.09	6.75	8	11	11	8																								
CD86+ [%CD14]	64.41	55.05	55.74	59.14	5.40	8.82	12.81	12.86	3.73	12.3	14.15	14.48	8	11	11	8																								
CD163+ [%CD14]	63.02	52.19	55.87	53.5	5.77	11.49	11.40	18.38	3.98	12.25	15.8	13.35	4	7	7	4																								
CD206+ [%CD14]	8.04	20.53	20.23	8.01	0.70	15.77	8.55	2.07	1.10	8	14.05	2.36	4	7	7	4																								
effector memory	42.64	41.27	66.88	61.15	26.58	24.93	17.47	12.77	16.01	18.09	14.48	18.3	4	4	4	4																								
effector cells	55.28	40.48	38.38	41.1	29.99	11.76	10.81	6.57	19.48	14.68	12.63	5.2	4	4	4	4																								
naiv	6.96	15.73	12.11	12.18	5.54	8.06	3.06	5.29	7.31	7.83	1.94	8.66	4	4	4	4																								
TH1	95.45	91.03	94.55	95.45	1.98	4.57	3.97	3.41	2.25	4.78	3.4	5.1	4	4	4	4																								
TH1/TH17	3.26	5.40	3.74	2.46	1.42	2.34	2.67	1.78	1.48	2.98	1.81	2.69	4	4	4	4																								
TH2	79.38	82.05	90.8	88	18.44	6.27	4.82	5.11	13.28	6	4.6	7.3	4	4	4	4																								
TH17	8.14	9.91	8.58	7.05	2.76	4.02	1.78	4.90	1.83	3.76	1.04	7.18	4	4	4	4																								
clincal Score	0	0.92	1.23	1.08	0	0.76	1.09	0.64	0	1	2	0	13	13	13	13						0.0536	-2.01	0.01						0.0047	-2.39	-0.36								
histological Score	0.40	0.96	1.02	0.52	0.67	0.93	0.91	0.88	0.63	1.5	1.75	0.56	13	13	13	12																								

Supplement Table 9: Leucocytes isolated from spleen out of NSG- mice engrafted with PBMC from Asthma patients and challenged with different HDM, were subjected to flow cytometric analysis. Additionally one group were treated with prednisolon or pitrakinra. Statistical analysis was conducted by ANOVA followed by TukeyHSD test. Abbrevations: FoP = Frequent of Parent, the percentage of events (cells) in this gate out of the parent gate (one level up); SD = Standard Deviation; IQR = Interquartile Range; n = Number of used Animals; OVA = Ovalbumin; HDM =House Dust Mite.

	Mean				SD				IQR				n																										
Leukocyte [% FoP]	**Con-trol**	**HDM**	**HDM +Pitra kinra**	**HDM +Pred nisolo n**	**Con-trol**	**HDM**	**HDM +Pitra kinra**	**HDM +Pred nisolo n**	**Con-trol**	**HDM**	**HDM +Pitra kinra**	**HDM +Pred nisolo n**	**Con-trol**	**HDM**	**HDM +Pitra kinra**	**HDM +Pred nisolo n**	**HDM/ con-trol**	**p-value**	**lower**	**upper**	**HDM +pitra kinra/ con-trol**	**p-value**	**lower**	**upper**	**HDM + pitrak inra/ HDM**	**p-value**	**lower**	**upper**	**HDM + predni solon/ con-trol**	**p-value**	**lower**	**upper**	**HDM +pred nisolo n/ HDM +pitra kinra**	**p-value**	**lower**	**upper**	**HDM +pred nisolo n/ HDM**	**p-value**	**lower**	**upper**
CD4+	46.04	35.75	41.27	45.23	16.11	13.80	10.89	16.21	21.7	13.4	8.98	23.8	8	8	8	8																								
CD69+ [%CD4]	15.73	18.41	15.1	10.51	2.83	8.21	4.39	2.73	4.53	9.55	3.75	3.09	4	4	4	4																								
CD134+ [%CD4]	44.48	29.28	25.93	31.33	18.43	13.50	6.96	8.79	16.53	18.23	6.1	12.23	4	4	3	4																								
CD294+ [%CD4]	6.39	6.65	7.22	5.37	2.04	4.45	6.57	2.64	3.04	7.29	3.69	1.97	8	8	7	8																								
CCR4+ [%CD4]	8.66	2.52	1.45	2.0	10.86	1.76	1.20	1.08	6.18	1.68	1.85	1.25	4	4	4	4																								
effector memory	37.89	33.1	54.73	37.96	24.76	17.80	15.50	20.50	23.72	23.65	21.53	18.34	4	4	4	4																								
effector cells	38.48	16.29	53.63	47.25	34.94	26.62	15.16	25.24	22	27.13	23.29	28.68	4	4	4	4										0.0262	4.15	70.52									0.0704	-2.23	64.14
naiv	30.03	67.3	24.15	22.55	18.62	32.10	9.09	11.46	16.28	21.3	11.8	8.36	4	4	4	4		0.0871	-79.14	4.59						0.0427	-85.02	-1.28									0.0350	-86.62	-2.89
CD86+ [%CD11b]	38.44	41.19	42.92	41.46	33.18	28.97	34.03	28.52	59.38	49.18	61.8	50.85	8	8	8	8																								
CD1a+ [%CD11b]	83.24	78.81	83.46	74	11.20	22.53	9.45	12.24	17.43	7.7	9.6	8.58	8	8	8	8																								
TSLPR+ [%CD11b]	6.35	8.69	78.67	8.55	3.79	3.43	3.51	2.79	5.38	2.63	3.75	3.42	8	8	8	8																								
CD86+ [%CD14]	6.49	6.61	6.35	6.78	4.79	4.92	1.99	3.07	6.85	7.24	1.67	4.73	8	8	8	8																								
CCR2+ [%CD14]	6.62	9.84	19	12.26	4.66	5.96	18.43	14.70	4.43	6.91	13.01	12.58	8	8	8	8																								
CD1a+ [%CD14]	3.46	56.63	81.36	73.19	13.74	33.62	14.33	12.87	16.1	45.38	23.38	8.58	8	8	8	8																								
TSLPR+ [%CD14]	3.49	5.61	15.95	9.00	2.06	4.06	11.46	5.03	2.63	4.68	14.74	3.42	8	8	8	8						0.0040	-21.74	-3.23		0.0226	1.08	19.59												
CD206 [%CD14]	8.45	8.07	9.54	11.94	1.22	2.95	2.86	5.70	1.06	2.05	2.19	8.56	4	4	4	4																								
CD163+ [%CD14]	82.72	78	79.28	80.5	4.62	4.93	1.23	7.50	4.98	5.85	0.83	11.55	4	4	4	4																								
FceRIa+ CCR3+	58.14	50.73	50.47	47.4	23.25	28.23	32.07	29.30	35.4	47.83	60.1	55	8	8	7	8																								
FceRIa+ CD117+	18.17	23.30	22.33	22.43	13.79	15.31	14.09	18.58	24.87	16.55	22.7	33.89	8	8	7	8																								
HLADR+ CD123+	3.48	3.67	4.78	5.38	3.81	2.94	2.58	3.57	3.76	3.82	2.91	5.82	8	8	7	8																								
CD19+ CD38+	1.94	1.47	2.17	1.55	0.58	0.86	1.50	1.12	1.02	0.78	2.39	1.49	8	8	7	8																								
TH1	96.63	89.73	96.9	96.3	0.99	9.43	0.65	3.66	0.88	6.23	0.6	2.6	4	4	4	4																								
TH2	88.98	85.45	93.7	96.03	14.15	5.55	5.44	2.15	8.08	8.4	3.4	1.78	4	4	4	4																								
TH1/TH17	1.85	4.61	1.44	0.77	1.25	2.32	0.53	0.17	1.49	2.10	0.68	0.26	4	4	4	4		0.0571	-5.59	0.07						0.0269	-6.01	-0.34									0.0079	-6.68	-1.01
TH17	0.89	10.43	8.51	2.2	0.38	10.85	6.52	1.50	0.42	10.49	7.57	1.26	4	4	4	4																								

Supplement Table 10: Leucocytes isolated from lung out of NSG- mice engrafted with PBMC from Asthma patients and challenged with different HDM while one group were treated with prednisolon or pitrakinra respectively, were subjected to flow cytometric analysis. Statistical analysis was conducted by ANOVA followed by TukeyHSD test. Abbrevations: FoP = Frequent of Parent, the percentage of events (cells) in this gate out of the parent gate (one level up); SD = Standard Deviation; IQR = Interquartile Range; n = Number of used Animals; OVA = Ovalbumin; HDM =House Dust Mite.

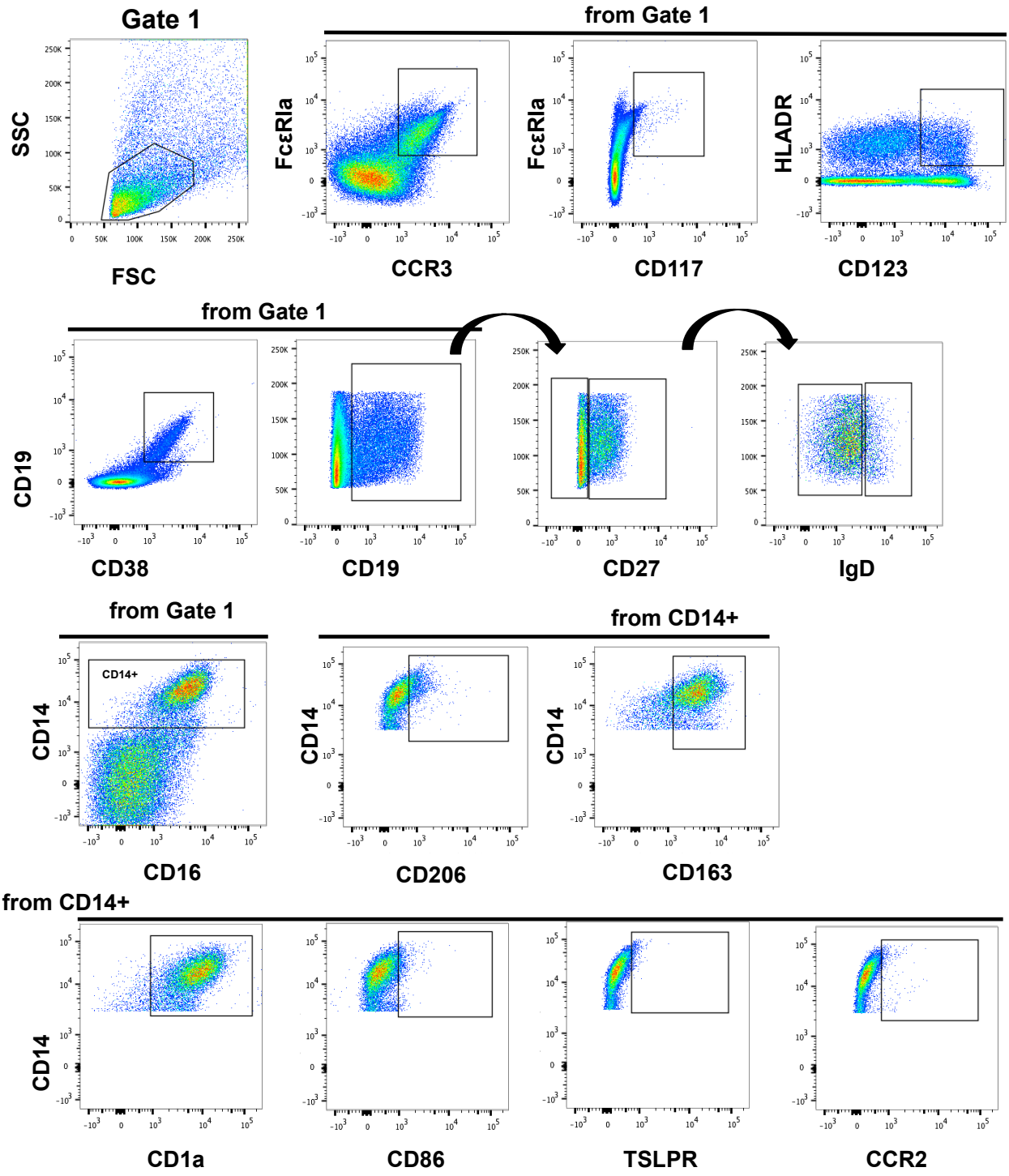
	Mean				SD				IQR				n																											
Cyto-kines	Control	HDM	HDM +Pitra-kinra	HDM +Predni solon	Control	HDM	HDM +Pitra-kinra	HDM +Predni solon	Control	HDM	HDM +Pitra-kinra	HDM +Predni solon	Control	HDM	HDM +Pitra-kinra	HDM +Predni solon	HDM/ control	p-value	lower	upper	HDM +pitra-kinra/ control	p-value	lower	upper	HDM+ pitra-kinra/ HDM	p-value	lower	upper	HDM+ predni solon/ Control	p-value	lower	upper	HDM +predni solon/ HDM +pitra-kinra	p-value	lower	upper	HDM +predni solon/ HDM	p-value	lower	upper
TARC	0.1849	0.1812	0.2470	0.06311	0.2186	0.1708	0.2486	0.0408	0.0744	0.2793	0.3118	0.0559	9	12	8	8																								
IFNγ	3.2677	7.5715	0.4610	0.0419	6.6459	12.3432	0.7794	0.0813	0.0249	13.8906	0.2423	0.0342	9	12	8	8																								
IL-4	0.0003	0.0010	0.0013	0.0002	0.0009	0.0015	0.0029	0.0005	0	0.0015	0.0007	0.0001	9	12	8	8																								
TSLP	0.0199	0.0079	0.0075	0.0041	0.0444	0.0177	0.0125	0.0049	0.0098	0.0051	0.0060	0.0048	9	12	8	8																								

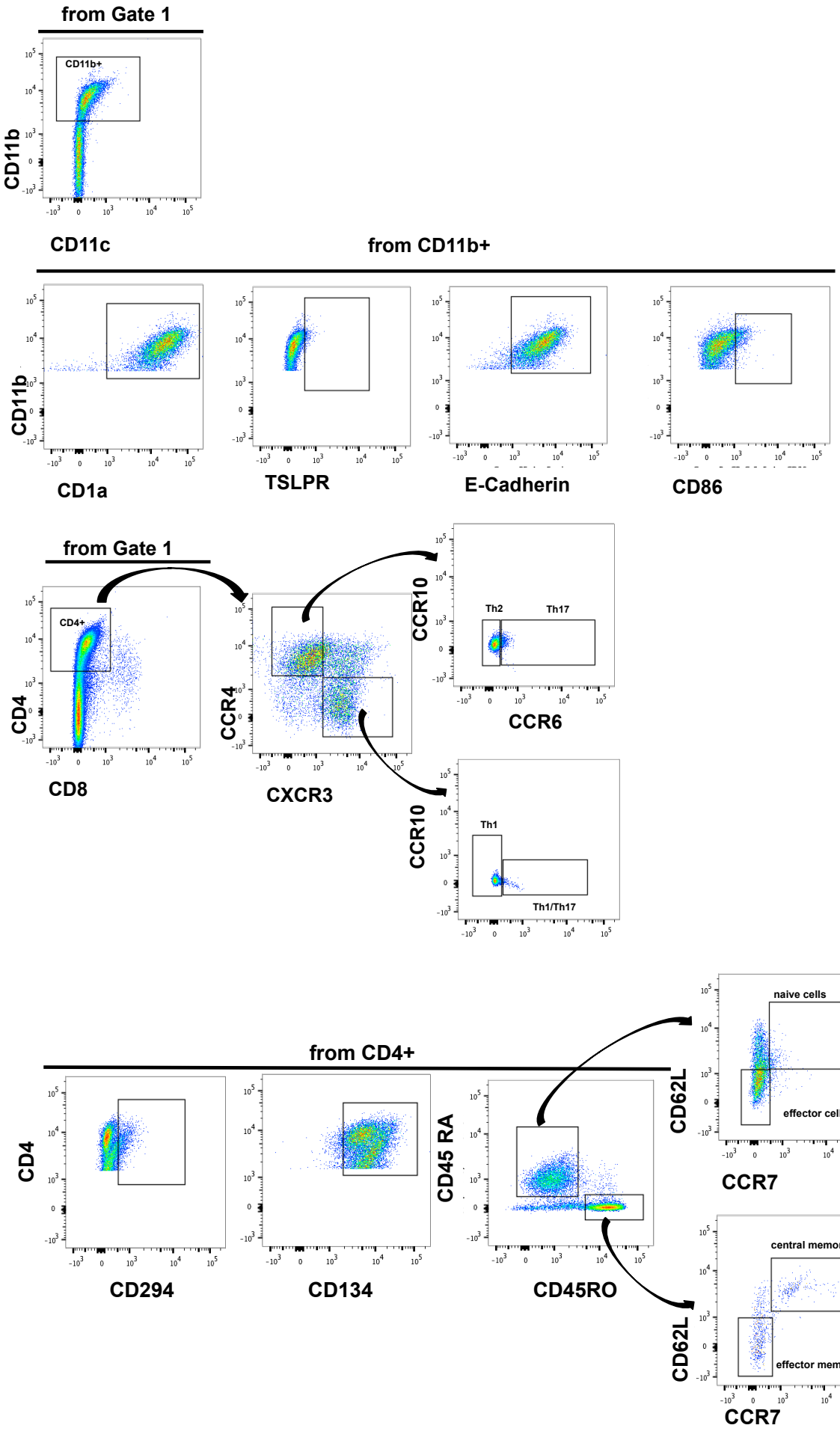
Supplement Table 11: mRNA cytokine levels isolated from lung out of NSG- mice engrafted with PBMC from Asthma patients and challenged with HDM while one group were treated with prednisolon or pitrakinra respectively, were subjected to real-time polymerase chain reaction (RT-PCR) analysis. Statistical analysis was conducted by ANOVA followed by TukeyHSD test. Cytokine concentrations are given in, logarithmic delta cycle threshold; Abbreviations: SD = Standard Deviation; IQR = Interquartile Range; n = Number of used Animals; OVA = Ovalbumin; HDM =House Dust Mite; TARC = Thymus and Activation Regulated Chemokine; IFNγ = Interferonγ; IL-4 = Interleukin 4; TSLP = Thymic Stromal Lymphopoietin.

	Mean			SD			IQR			n														
Leukocytes [% FoP]	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**Control**	**OVA**	**HDM**	**HDM/ control**	**p-value**	**lower**	**upper**	**OVA/ control**	**p-value**	**lower**	**upper**	**OVA/ HDM**	**p-value**	**lower**	**upper**
hCD45+	20.35	16.91	20.68	17.62	11.29	13.34	25.87	16.45	13.88	16	8	16												
CD134+ [%CD4]	90.94	91.17	82.91	4.27	4.28	21.98	4.05	2.15	7.8	19	12	20												
CD294+ [%CD4]	16.35	13.84	11.74	14.83	8.77	10.52	8.07	11.37	14.20	19	12	20												
CD11b+	36	40.01	33.99	19.65	17.74	20.51	25.9	16.6	23.83	21	17	24												
CD86+ [%CD11b]	21.81	10.74	18.72	24.41	5.27	22.98	13.68	6.32	9.79	21	17	24												
E-Cadherin+ [%CD11b]	73.2	74.58	73.61	14.26	14.12	11.87	21.8	26.5	16.13	21	17	24												
CD1a+ [%CD11b]	82.96	78.03	79.15	9.97	15.27	16.60	13.9	27.3	10.95	21	17	24												
TSLPR+ [%CD11b]	7.81	8.87	8.40	3.81	3.91	9.20	5.07	6.52	6.37	20	12	20												
CD86+ [%CD14]	7.43	7.52	6.19	5.31	5.12	3.86	10.85	5.08	5.83	20	17	24												
CCR2+ [%CD14]	6.01	6.86	7.67	5.91	5.34	5.96	4.84	4.78	6.88	20	17	24												
CD1a+ [%CD14]	77.38	78.84	71.06	10.54	14.68	23.69	15.45	23.8	24.3	20	17	24												
TSLPR+ [%CD14]	4.88	4.25	8.08	6.62	3.58	16.39	4.80	5.9	3.03	20	17	24												
CD206+ [CD14]	15.53	21.45	19.13	9.21	11.73	12.39	12.72	16.73	16.07	14	12	16												
CD163+ [CD14]	65.14	64.06	59.91	14.32	14.96	20.52	21.85	28.08	39.23	14	12	16												
FceRIa+ CCR3+	42.36	28.21	36.96	27.70	23.06	26.00	47.53	44.37	44.03	20	17	24												
FceRIa+ CD117+	12.07	7.84	11.43	12.88	7.36	12.47	16.84	8.31	12.23	20	17	24												
HLADR+ CD123+	5.56	6.40	7.17	5.41	5.57	6.37	4.56	7.41	8.93	20	17	24												
IgD+ [%CD27]	37.66	61.35	41.50	27.50	24.32	32.35	39.05	34.98	58.68	20	12	20						0.0730	-49.14	1.77				
CD27+ [%CD19]	16.94	34.05	23.35	20.82	19.95	24.00	25.22	23.38	31.70	20	12	20						0.0927	-36.46	2.24				
CD19+ CD38+	3.59	10.05	3.52	4.14	19.72	4.05	1.37	3.68	2.84	20	12	20												

Supplement Table 12: Leucocytes isolated from lung out of NSG- mice engrafted with PBMC from Asthma and Non Asthma patients and challenged with OVA, or HDM, were subjected to flow cytometric analysis. Statistical analysis was conducted by ANOVA followed by TukeyHSD test. Abbrevations: FoP = Frequent of Parent, the percentage of events (cells) in this gate out of the parent gate (one level up);; SD = Standard Deviation; IQR = Interquartile Range; n = Number of used Animals; OVA = Ovalbumin; HDM =House Dust Mite.

Gating Strategy [116]




from Gate 1
CD11b+
CD11b
CD11c
from CD11b+
CD11b
CD1a
TSLPR
E-Cadherin
CD86
from Gate 1
CD4+
CD4
CD8
CCR4
CXCR3
CCR10
Th2
Th17
CCR6
CCR10
Th1
Th1/Th17
from CD4+
CD4
CD294
CD134
CD45 RA
CD45RO
CD62L
naive cells
effector cells
CCR7
CD62L
central memory
effector memory
CCR7

Questionnaire

Alter?

Geschlecht?

Bestehen Allergien?

□ Pollen (Gräser, Getreide, Bäume)

□ Tiere (welche)_____

□ Insektengift (Bienen, Wespen)

□ Hausstaubmilben

□ Nahrungsmittel (Gewürze, welche)

□ Medikamente (welche)

□ Andere (Metalle, Kosmetika, Chemikalien)_____

□ Nein

Welche Beschwerden/Symptome liegen/ lagen vor

□ Heuschnupfen/Fließschnupfen

□ Augenjucken/Bindehautentzündung

□ Hautreaktionen (Ausschlag, Juckreiz, Nesselsucht, Ekzem)

□ Irritationen im Rachenraum

□ Atembeschwerden

□ Asthma bronchiale

□ chronische Bronchitis

□ andere _____

Bei welchen Gelegenheiten treten/traten die Beschwerden auf

□ während der Berufsausübung

□ bei körperlicher Anstrengung

□ nur saisonal bei Pollenflug

□ bei (Haut-)Kontakt mit gewissen Stoffen

□ andere _____

Wie oft haben Sie Atembeschwerden bzw. Asthmaanfälle?

□weniger als 2 x wöchentlich

□mehr als 2 x wöchentlich

□nur saisonal

Haben Sie auch nachts Atembeschwerden?

□ ja, mehr als 2 x wöchentlich

□ ja, weniger als 2 x wöchentlich

□ Nein

War in den letzten 2 Jahren eine Therapie erforderlich?

□ ja zuletzt; _____

□ Nein

Wenn ja hilft diese Behandlung normalerweise bei Ihnen und wie schnell?

Ja □/ Nein □

Nach _____ Minuten

Nach _____ Stunden

Nach _____ Tagen

Bestehen andere chronische Lungenerkrankungen? (z.B. chronische Bronchitis, Lungenemphysem)

□ ja; _____

□ Nein

Wann sind die Beschwerden erstmals aufgetreten?

_____

Können Sie die einzelnen allergie-/asthmafreie Phasen verlängern und was machen Sie dafür?

Ja □/ Nein □

Was sind die ersten Symptome mit denen sich ein Schub bei Ihnen ankündigt?

Kommen folgende Erkrankungen in ihrer Familie vor?

Allergien Ja □ / Nein □

Asthma Ja □ / Nein □

Darmerkrankungen Ja □ / Nein □

Hauterkrankungen Ja □ / Nein □

Vielen Dank für Ihre Teilnahme