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# 2. ABBREVATIONS

| | |
|---|---|
| AHR | airway hyperresponsivnes |
| APC | antigen presenting cell |
| BAL | bronchoalveolar lavage |
| BALF | bronchoalveolar lavage fluid |
| CD | cluster of differentiation |
| DAPI | 4',6-diamidino-2-phenylindole |
| DC | dendritic cell |
| FITC | fluorescein isothiocyanate |
| GFP | green fluorescence protein |
| GM-CSF | Granulocyte Macrophage Colony Stimulating Factor |
| i.n. | internasal |
| i.p. | interperitoneal |
| ICOS | inducible co-stimulator |
| ICOS-L | ligand |
| Ig | immunoglobulin |
| IL | interleukin |
| INF | interferon |
| LNs | lymph nodes |
| MHC | major histocompatibility complex |
| OVA | ovalbumin |
| OVA 594 | ovalbumin conjugated to AlexaFluor594 |
| PDL-1 | ligand of programmed death-1 (PD-1) |
| PGE2 | prostaglandin E2 |
| TCR | T – cell receptors |
| Th | T helper lymphocytes |
| TNF | tumor necrosis factor |

# 3. INTRODUCTION

Charakterizace antigen presentujících buněk v alergickém astma

Astma bronchiale je chronické zánětlivé onemocnění, jehož výskyt za posledních dvacet let prudce vzrostl, především v západních zemí. Postihuje 5% dětské populace a 10% dospělé populace. Astma je onemocnění charakterizované zvýšenou citlivostí dýchacích cest na podněty z vnějšího prostředí, jež se projevuje opakovaným bronchospasmem – zúžením dýchacích cest.

Alergické onemocnění je schopnost rozeznat alergen a reagovat specifickou imunologickou reakcí zprostředkovanou IgE protilátkami, jejich zvýšenou tvorbou IgE, zvýšeným množství buněk zánětu a hyperaktivitou cílových tkání.

Astma se projevuje širokým spektrem příznaků. Hlavním příznakem je dušnost způsobená neprůchodností dýchacích cest kontrakcí hladkého svalstva, otokem sliznice, nadprodukcí hlenu. U nedostatečně léčeného astmatu dochází k trvalým změnám struktury plicní tkáně.

V patogenezi alergického astmatu hrají důležitou roli dendritické buňky a T lymfocyty. Při remisi onemocnění dochází ke zvýšení množství jak Th memory buněk, tak i efektorových Th 2. Inhibice Th 2, či jejich efektorů, je velmi důležitá pro zamezení rozvoje astmatických záchvatů. Lymfocyty interagují s antigen presentujícími buňkami (APC), teprve tehdy nastává jejich konečná diferenciace. Pomocné Th-helper T lymfocyty podporují schopnost B lymfocytů vytvářet protilátky a usnadňují jejich vyzrávání, zvyšují funkce cytotoxických lymfocytů, T3 regulačních lymfocytů, dendritických buněk, mikrofágů a NK buněk.

Tato práce je zaměřena především na popis dendritických buněk, které hrají v patogenezi astmatu důležitou roli. Dendritické buňky, B lymfocyty a makrofágy jsou antigen presentujícími buňkami reagujícími s Th lymfocyty.

Detailní popis dendritických buněk by umožnil určit, zda Th lymfocyty reagují více s DC či makrofágy v plicní tkáni. Na základě tohoto zjištění by bylo možné přerušit vazbu mezi Th lymfocyty a DC, omezit tím zánětlivou kaskádu reakcí a snížit výskyt alergického astmatu. K charakterizaci DC a makrofágu byly použity protilátky specifické pro povrchové antigeny těchto buněk.

Předpokládáme, že Th2 paměťové buňky hrají centrální roli v iniciaci a následných remisích alergického astmatu a regulaci antigen presentujících buněk. Teorie, že paměťové CD4+T buňky přežívají v závislosti na Ag a APC je kontroverzní. Různé studie předpokládají interakce mezi MHC II a T paměťovými buňkami (Tm), a to takové, že Tm jsou schopny přežívat i bez přítomnosti MHCII. Nejdůležitějšími zástupci APC jsou DC. DC rychle migrují z plic do lymfatických uzlin. U pacientů s alergickým astmatem je charakteristická akumulace DC v bronchiální mucose a zvýšení množství DC. U pacientů, kteří jsou léčeni inhalačními steroidy, došlo ke snížení DC v plicní tkáni.

U DC v plicní tkáni se předpokládá, že se jedná o dlouho žijící APC, a mohou být popsány na základě povrchových markrů CD11b, B220, CD8a.

# 4. THEORETICAL PART

## *4.1. Allergic Diseases*

Allergic reactions occur when an individual encounter with the allergen and produce IgE antibodies in response to antigen, or allergen. Ig E is a substance which is occurred naturally in the body, normally in small amount, but plays important role in allergic asthma. If someone has asthma, the number of Ig E is increased. It can cause several chemicals reaction, which are called allergic- inflammatory processes. Ig E becomes constriction and inflammation of the airway. On type of allergic disease is allergic asthma.

## *4.2. Allergic Asthma*

Allergic asthma is characterized by an inflammatory process in the mucosa of the airways. It is one of the most common chronic diseases in western society, characterized by variable airway obstruction; mucus hypersecretion and infiltration of the airway wall with T-helper type 2 (Th2) cells, eosinophils and mast cells. Between 9 and10% of the world population suffers from asthma. It can cause of morbidity and mortality. There is significant increase of occurrence in the last 20 years. Especially in children population and youth aged 20 years or less increase allergic asthma. Allergic asthma is a complex disease characterized by local and systemic allergic inflammation associated with remodelling and reversible airway obstruction, increasing serum Ig E levels causing mast cell activation

The basic feature of asthma is airways hyper responsiveness, inflammatory response to allergens with mucus accumulation resulting in wheezing, shortness of breath and airway obstruction. Inflammatory cells (monocytes, lymphocytes, eosinophils, antigen-presenting cells) are recruited to the mucosa; they sensitize immune system by initial contact with an allergen. The main cells in allergen response are dendritic cells (DC). Inflammatory cells entrance into the airways and provoke immune reaction and cascade of

inflammatory process. Allergens activate a specific type of lymphocyte, called a $CD4^+$ Th2 cell. Different groups of triggers (allergens) may cause an asthma attack and exposure to irritants.

The immunological basis of asthma is supported by an importance of Th2 cells in asthma. In lung tissue and in broncho-alveolar lavages (BAL) have been identified $CD4^+$ Th2 cells producing the cytokines IL-4, IL-5, IL-9 and IL-13. In mice, studies have shown that Th2 antigen specific cells involve characteristic features of asthma. The cytokines IL-4, IL -5, IL- 9,IL-13 promote inflammation and the over-production of IgE ( Epstein, 2005).

Histologically is characterized by eosinophilic airway inflammation, and goblet cell hyperplasia.

The symptoms associated with chronic asthma are a result of structural changes in the lung in response to chronic inflammation and remodeling of the airway wall, leading to airway obstruction. The most common factor for developing asthma is Th2 sensitization to inhaled allergens. Another important risk factor for developing asthma is the occurrence of childhood respiratory infections.

### 4.2.1. Symptoms

Asthma **symptoms,** especially shortness of breath, increased airway hyperresponsiveness (AHR), and mucus hypersecretion are principal causes of airway obstruction observed in asthma patients. In allergic asthma are differentiated tiggers and inducers. Although asthma usually begins in childhood it can also occur at any age. Tendency for asthma can by family problem. The typical symptoms of asthma include wheezing, a dry cough, and tightness of the chest and shortness of breath. This is due to narrowing of the tubes in the lungs and inflammation.

Asthma symptoms are different from person to person, but mostly they have similar reactions. Main reactions are:

* coughing, wheezing, shortness of breath, tightness in chest

**Tiggers** initiate the airway bronchoconstriction but they not cause inflammation. The most common tigger are: cold air, emotion, smoke, inhaled irritans**.**

**Inducers** result in symptoms, their response is delayed. Between inducers belong: pollen, animal secretion, molds, and dust.

The allergen triggers activate IgE-binding cells in the exposed tissue. Activation of IgE leads to a series of responses that are characteristic of an allergy.

### 4.2.2. Pathology of allergic asthma

The **pathology** associated with asthma is mediated by Th2 cells with important roles for the cytokines IL-4 and IL-5. Not only IL-4 and IL-5 are important in allergic asthma but also elevated expression of IL-13, produced by Th2 cells and activated mast cells. IL-13 is a key factor in the asthmatic phenotype (Lambrecht et al., 2000).

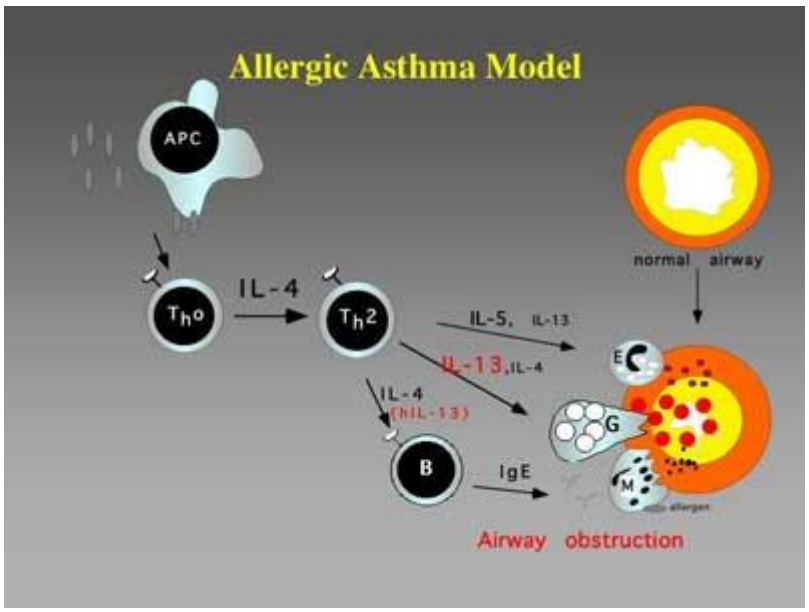
Asthma is a "complex" disease. Not only environmental triggers but also genes strongly contribute towards to the disease. Asthma is known to be strongly familial disease, 75% of asthma disease to heredity. The roles of genes in asthma predisposition are not clear.

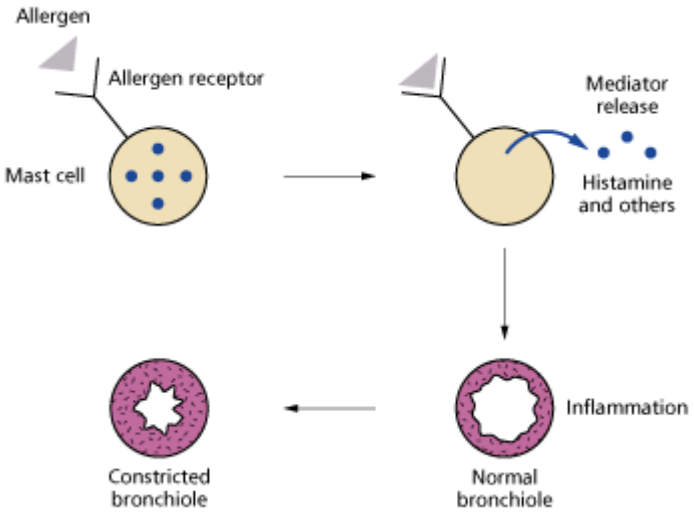
Increased occurrence of allergy and asthma may be related to the decreased exposure to mycobacterial infection, because of an absence of infection.

Dendritic cells (DCs) are the most important antigen-presenting cells in the lung and are recognized for their potential to generate a primary immune response and sensitization to inhaled allergens.

When allergens enter the lungs, they will be taken up by DCs. DCs move to the draining lymph nodes, presenting the allergen to naive T lymphocytes. Upon this interaction, the naive T lymphocytes mature into memory cells. These memory cells have the capacity to generate a much more rapid than effectors Th cells. DCs are essential also for the development of the secondary immune response.

At young age, some sensitized children develop atopic dermatitis or intermittent periods of wheezing, coughing and shortness of breath upon contact with allergens or upon non-specific stimuli, such as cold air or exercise.



The disease usually starts in early childhood about the age 2 to 6 years. Children are highly exposed to allergens, such as dust mites and tobacco smoke, and viral respiratory infections. Adult asthma occurs more often in women about the age 30 or 40 years. The triggers in this group are usually non-allergic.

Extrinsic, or allergic asthma, is more common (90% of all cases) and typically develops in childhood. Typically, there is a family history of allergies. Allergic asthma often goes into remission, 75% of cases, the asthma reappears later.

Intrinsic asthma represents about 10% of all cases. It usually develops after the age of 30 and is not typically associated with allergies. The condition can be difficult to treat and symptoms are often chronic.

## 4.3. *Dendritic cells*

Dendritic cells (DC) play a key role in the immunological reactions and responses. DC are highly specialized antigen-presenting cells (APC) located in the respiratory endothelium, lymphoid tissues as well as non- lymphoid tissue. DC play a main role in the first contact with allergen.

Dendritic cells that have matured from monocytes and also express very high levels of surface MHC class II and CD86, all process of maturation takes 48 hours. The immature DCs have many MHC II molecules but require a maturation stimulus.

Dendritic cells start as immature dendritic cells. They are characterized by high endocytic activity and low T-cell activation potential. They have come into contact with pathogens, become activated into mature dendritic cells. Immature dendritic cells phagocytose pathogens and degrade its proteins into small pieces (small peptides) and upon maturation present those fragments at their cell surface MHC molecules. In the same time they upregulate cell-surface receptors, co-receptors in T-cell activation such as CD80 and CD86 and ability to activate T-cells. They also upregulate CCR7, a chemotactic receptor, that induces the dendritic cell to travel through the blood stream to the spleen or through the lymphatic system to a lymph node. There they act as antigen-presenting cells: they activate helper T-cells and killer T-cells as well as B-cells. The cells become typical mature DCs.

### 4.3.1. Dendritic cells in lymph nodes

Dendritic cells have been identified in different human tissues and are in absence in the cornea and central nervous system. DCs are derived from circulating blood precursors, which bind to the endothelial receptors ICAM-1, V-CAM-1 and E-selectin through the expression of CD11a/CD18 and CD49d (Epstein, 2005). The recruitment of DCs to tissue is mediated by the local production of cytokines such as GM-CSF and by systemic signals such as bacterial lipopolysaccharide (LPS). DCs reside in an intermediate stage of

maturity as cells specialized for Ag uptake and processing. Tissue DCs take up Ag either in the fluid phase (by macropinocytosis) or by receptor-mediated endocytosis using the mannose receptor to ingest glycosylated Ag, and by the FcgRII (CD32) take up antibody-bounding ( Uphan J.W. 2003).

After capturing Ag, they process antigen, increase expression of the co-stimulatory molecules CD40, CD54, CD80 and CD86. With processing Ag migrate from the tissues to the lymph nodes and spleen. Changes in DC maturity and function, production of cytokines and products of bacterial and cellular degradation cause the activation of the immune response. DCs migrate to the secondary lymphoid tissues by the afferent lymphatic's vessels. The mechanisms of homing to the LNs and spleen are not fully understood. In LNs, DCs are occurred in T cell regions. In the spleen they are located in the marginal zones at the periphery of the periarterial sheaths.

There are at least two different types of cells involved with immunity that are called dendritic cells. The non-stimulated DC can be divided by FACS into plasmacytoid DC (pDC; CD123+CD11c-) and myeloid DC (mDC, CD123-CD11c+) (de Heer et al. 2005).

### 4.3.2. Subsets of dendritic cells

There are several Dc subpopulations, identified in human and mice. They can be divided into myeloid and plasmocytoid(lymphoid) DC. DC precursor's population is described in peripheral blood. Myeloid and plasmocytoid DC are characterized by different phenotype. Myeloid DC express surfaces marker such as CD 11c, CD 33,CD23, percentage of myeloid cells in circulation is about 0,5 – 1,0%. Plasmocytoid cells express CD123, these cells are dependent on IL-3 for their growth and survival.plasmocytoid cells are an important source of interferon – alfa. Play important role in defense against viral infection (de Heer et al., 2005).

The number of plasmocytoid cells is lower, it is about 0, 3% in blood circulation.

Myeloid are divided into two subsets: the more common MDC-1 and the extremely rare MDC-2( Lambrecht et al., 2000). Plasmacytoid dendritic cells look like plasma cells; they produce high amounts of interferon-alpha and that s why they are called interferon-producing cells. Several DC subsets have different surface markers, functions, localizations.

Compared to the mDC, the pDC have shorter dendrites, express lower level of CD1a (11.8%) and mDC (66.8%).The pDC also showed increased levels of CD86 surface marker. Several in vitro studies have shown that CD14-derived DCs prime T cells to preferentially activate Th1 responses and IL-12 implicates in this process (Epstein 2001).

Murine CD8$\alpha^+$ DCs represent as lymphoid-derived DC lineage, thymic and splenic CD8$\alpha^+$ DCs could be produced in recipient mice on transfer of a thymic lymphoid precursor population with little capacity to form myeloid cells. The DCs that lacked expression of CD8$\alpha$ but expressed the myeloid marker CD11b were represented as myeloid-derived DCs.

Murine DCs are more heterogeneous than just this CD8$\alpha^+$ lymphoid-related, and CD8$\alpha^-$ myeloid-related. There exist totally 5 subpopulations of mature DCs. These cells can be identified in mouse lymph nodes (Lambrecht et al., 2000, de Heer et al., 2005).

DC subtypes activate either Th1 or Th2 responses. The thymic DCs expressing CD8a+ appear to be functionally different from CD8a- DCs. They express Fas-L and induce T cell apoptosis. Thymic DCs are also efficient at inducing T cell IL-2 cytokine production. Thus, CD8a+ DCs may have regulatory properties, whereas CD8a- DCs seem to exert T cell stimulatory function. The myeloid pathway of differentiation gives rise to DCs that home to peripheral tissues to take up and process exogenous Ags prior to migrating to the secondary lymphoid tissues to present Ags to naïve T cells.

Thymic DCs perform a very different function. DCs, produce high levels of IL-12 and prime Th1 T cell response. The immunoregulatory potential of DCs may depend less on ontology than on recent activatory or downregulatory

stimuli (Wallet M.A. 2004). Myeloid DCs are derived from precursor's cells (especially monocytes). They migrate to and reside as immature DCs at body surfaces. Immature DCs have abundant MHC II products within intracellular compartment and respond rapidly to inflammatory cytokines and microbial products to produce mature T cell stimulatory DCs with abundant surface MHC II proteins.

### 4.3.3. *Dendritic cells in peripheral blood*

Human PB peripheral blood DCs were first isolated in 1982. The surface markers have been poorly defined. New techniques, such as immunohistochemistry and fluorescence flow cytometry are able to better characterize DCs and to distinguish the subpopulations of cells.

The morphology of these two subtypes has been described, the CD11c- population possesses a lymphoid morphplogy and the CD11c+ population possesses monocytoid morphology. There are some studies that one subset may be more mature by virtue and migrating to lymph nodes or spleen and the less mature is directly derived from the bone marrow.

### 4.3.4. *Sensitization of DC*

Particular microbial antigens can stimulate myeloid DC to induce either Th1 or Th2 immune response. Virus activates plasmocytoid DC will induce the generation of CD8+ suppressor cells.

The mechanisms by which DC differentially regulated Th1 and Th 2 immune response is incompletely understood.IL- 12 is a key cytokine responsible for initiating and maintaing interferon –gama producing Th1 cells. DC produce IL-12 in a T- cell independent manner.

Various components of fungi,bacterial toxins have ability to program DC to induce Th2 response., although the mechanism by which DC direct Th2 differentiation is unclear. IL-6 induces activation of Th2 responses.

IL-10 can inhabit both IL-12 and Th1 differentiation and immature DC are rich sources of this cytokine. Constitutive production of IL -10 by lung influence Th2 response in some animal models. Lung DC produces IL-10 and stimulates the development of regulatory T-cells. Activation of endogenous DC by GM-CSF produces airway inflammation in response to inhaled antigen, rather than tolerance (Epstein, 2005).

During sensation DC are likely to prime naïve T- cells in regional lymph nodes.

Immature DC – characterization: DC express CD1a, deficient T cell sensitization, express low level of CD40/54/58/80/86, CD25, CD83, p55, DEC-205, 2A1antigen. Responsive to GM-CSF, but not M-CSF and G-CSF and their maturation can be inhibited by IL – 10

Mature DC – characterization: are able to capture antigen by mannose receptor, DEC – 205 receptor, antigen presentation by MHC I or II expression, binding T cells, product cytokines.

## *4.4. T lymphocytes*

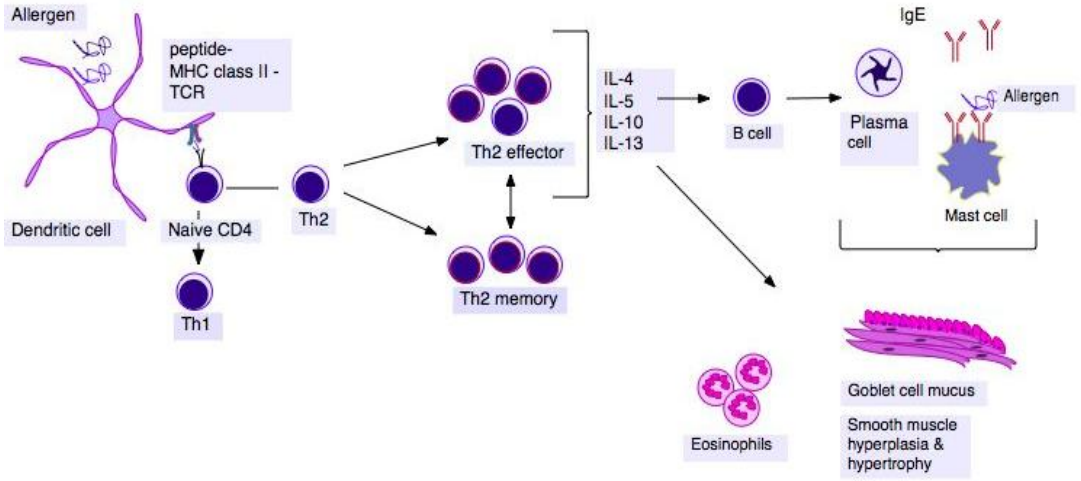
T lymphocytes are thymus-derived lymphocytes and play a central role in the coordination and regulation of the immune response. T cells are produced in higher number in childhood and decrease in maturity. Because mature T cells are long lived and circulate in the blood, about 70-80% of lymphocytes in blood and lymph they are responsible for the immunologic memory. T cells-lymphocytes play a key role in coordination of adapted immune response and in the production of soluble mediators called cytokines. T cells express antigen-specific receptor and are bone marrow-derived cells. When they arrive in the thymus, precursors do not express CD4, CD8 or T cell receptor (TCR). CD4 and CD8 are very useful markers in thymocyte development. If they not express CD4 and CD8, they are called "double negative" cells. There are two major subpopulation of T lymphocytes which are divided among effector function,MHC and co-stimulating molecules.

### 4.4.1. *T lymphocyte subpopulation*

The CD4 and CD8 have different function and MHC of the T cell. They increase the affinity of interaction between the T cell and the APC. There are occurring two subsets of thymocytes $CD4^{hi}CD8^{lo}$ and $CD4^{lo}CD8^{hi}$. A $CD4^{hi}$ cell who's TCR interacts with MHC II in the thymus, and $CD8^{hi}$, who's TCR interacts with class MHC I. TCR receptor binding is not sufficient to activate T cells. For their activation are necessary co stimulators molecules, which interact with specific ligands on APC. The CD28 molecule on T cells delivers a costimulatory signal to its ligands, B7.1 (CD80) or B7.2 (CD86).

A distinct signal is transduced by the CD40L molecule on the T cell when it is ligated to CD40. The expression of B7.1/B7.2 and CD40 distinguish specialised antigen presenting cells from other MHC positive cells.

CD4 helper T type and Th2 cells are important mediators of inflammatory diseases. T cells are found in the lung inflammation, play a critical role in coordinating the immune response to allergens. T cells have special ability to involve response to specific stimuli. They are differentiating into effectors cells, through their production of soluble factors – cytokines and chemokines. By these factors they communicate with other cells and initiate the cascade of inflammatory.




T helper type of thymocytes

Th1 – helper type of T cells, are characterized by secretion of interferon gamma and TNF (Epstein 2005). Th1 have ability to activate response against intracellular pathogens. In the lung main role of Th1 cell is a response in neutrophil-predominant inflammatory.

Th 2 – produce IL-4, IL- 5, IL – 10, IL -13 (Epstein 2005). Both play role in the pathogenesis of asthma.

Induction of Th1 or Th2 depends on many factors. Especially on genetic background, the co stimulatory signals, antigenic stimuli. IL -12 and TNF play controlling role of development of Th1 cells from naïve precursors T cells.

### 4.4.2. *Inflammation pathway*

DC are the most important antigen presenting cells for Th cells in lungs, and influence Th cells by secretion of their cytokines such as IL -12. The pathway of IL -12 cause the differentiation into Th1 or Th2 type of cells. Important for differentiation of Th cells is also important type of stimuli, the origin of DC, these all contributions play role in differentiation of Th cells and their response. Th1 produce INT gamma and participate in defense against pathogens whereas Th2 produce IL 4, IL5 and participated in allergic reactions

involving IgE, eosinophils and basophils. The most important co-stimulatory signal is CD 28 engagement by B7. Naive CD 4+ T cells require CD28-mediated signaling for IL-12production.

In respiratory tract are DC with immature phenotype expressing. They have low level of MHC class II on the surface; produce lower number of IL -10 and very low number of IL-12.They mostly stimulate Th2 cells. Induction of Th cells depends on the maturation state DC and ability to produce IL -12. On the Th cells is a differential expression of chemokine receptors. Th1 cells express mostly CCR5 and CXCR3, whereas Th2 cells express CCR3, CCR4 and CCR8.

Naïve T lymphocytes travel to T cell areas of secondary lymphoid organs, where they encounter antigens presented by DC. Because of encountered antigens Th precursor (naïve cell) become activated. For activation is necessary connection, contact between TCR and MHC class II molecules and also play co-stimulating molecules-signals. Activated Th effectors migrate either to B cells areas or to inflamed tissue. The migration is under the influence of cytokines. T cells are differentiated into 2 effectors groups under their cytokines production, surface markers, phenotype and functions.

### 4.4.3. *Maturation of T cells*

The maturation of T cells takes place in the thymus and is characterized by differentiation cell surface molecules. The first surface molecules are appeared to be CD3, T cell receptors (TcR), CD 4 and CD8. Thus immature thymocytes are $CD3^{+}CD4^{+}CD8^{+}$.Mature T cells lose either CD4 or CD8 molecules and become $CD3^{+}TcR^{+}CD4^{+}$ or $CD3^{+}TcR^{+}CD8^{+}$ (Kuipers H. et al)

Mature T cells migrate to the medulla of the thymus from where they circulate into the systemic circulation.

The TCR recognizes antigen determinants that are presented by MHC molecules. The major function of MHC molecules is to present antigens to T cells. Lymphocytes in the thymus are exposed to various endogenous proteins, also self proteins products by MHC. The mature T cell recognizes its specific antigen only if that antigen is presented by the correct MHC molecule.

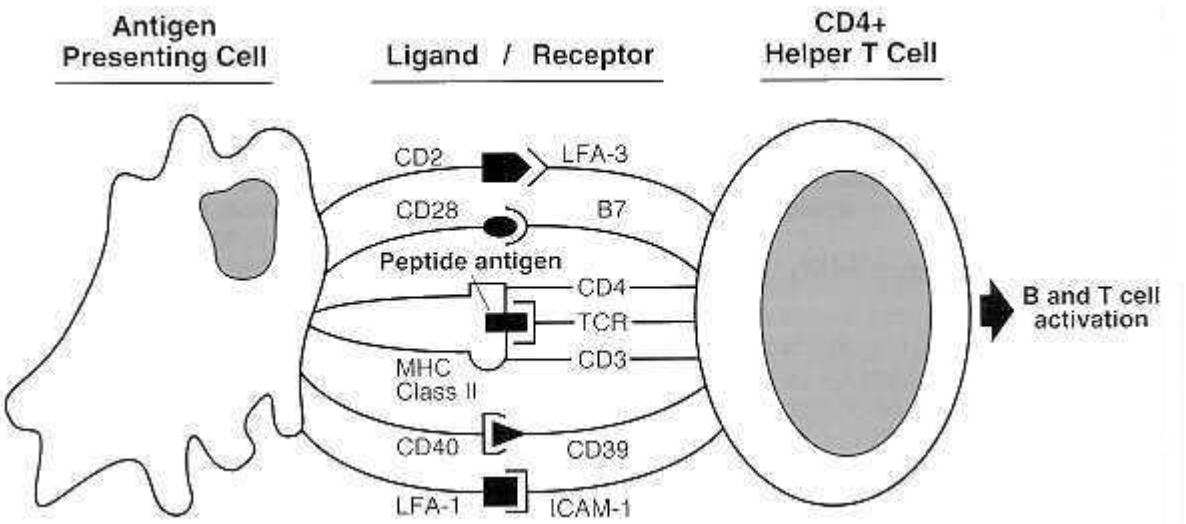
Two kinds of MHC class I and class II are involved in the development of T cells. T cells learn to recognize foreign antigens. MHC class I restricted T cells express CD8 molecules that bind MHC class I, whereas MHC class II restricted T cells express the CD4 molecule that binds to MHC class II molecule. Mature T lymphocytes leaving the thymus are either $CD4^+$ or $CD8^+$ and express CD3 and TCR molecules.

$CD4^+$ Th cells are divided into two subsets, Th1 cells that promote cellular immunity and Th2 cells that help antibody production.

$CD8^+$ T cells are cytotoxic/suppressor cells which participate in cell-mediated immunity against viruses, fungi, bacteria, and against certain tumors and play a role in immune regulation.

T Helper cells involve B cell- mediated immune responses. IgG, IgA, and IgE antibody responses against T-dependent antigens require Th2 cells. In the first the antigen is taken up and processed by accessory cells, such as macrophages or B cells that present the Ag/MHC complex to Th2 cells. Activated T cells then produce cytokines and activate B cells to produce antibodies. Antigen-specific Th2 cells that bind the Ag/MHC complex on the antigen-presenting cells become activated and produce helper factors for activation B cells. B cells bind the antigen by specific antibodies, then they internalize and process the antigen (Ag), and express a fragment of it bound to MHC class II molecules on the cell surface in the context of MHC class II molecules.

The presentation of antigen of MHC molecules is essential for T cell recognition of peptide antigens and for their activation. Interactions between the MHC and TCR and the MHC molecules is not sufficient to activate T cells. Important are ligands on antigen-presenting cells and their receptors on T cells are required to complete the process, for example: ICAM-1, LFA-3, and B7-1/2 on antigen-presenting cells binding to their receptors LFA-1, CD2, and CD28/CTLA-4 on T cells.




Th2 response to inhaled allergens is a critical point in the development of the persistent asthma. DCs influence not only the differentiation of CD4+ T cells, but also activation of T and B cells. The role of DCs polarization of Th1 effector T cells has been well studied, with DC-derived IL-12. Myeloid dendritic cells cause induction of Th2 sensitization in naïve mice. The inhaled allergen leads to allergen-specific Th2 cytokine synthesis, eosinophilic airway inflammation and bronchial hyperactivity. Endogenous myeloid DCs induce Th2 sensitization in response to inhaled antigen, providing that the antigen is capable of DC and provide the maturation of lung DCs.

In humans, less is known about the role of DCs in theTh2-sensitization process than in murine models (Epstein 2005).

### *4.5. Macrophages*

Macrophages and monocytes play important proinflammatory roles in allergic inflammation, they are leukocytes (white blood cells), long-lived cells (but it has been shown that depending on the type of tissue, their viability ranges between 6 and 16 days) distributed to all organs of the body, where they contribute to trophic interactions and tissue homeostasis. Macrophages can be divided into normal and inflammatory macrophages. Normal macrophages include macrophages in connective tissue (histiocytes), liver (Kupffer's cells),

lung (alveolar macrophages), lymph nodes (free and fixed macrophages), spleen (free and fixed macrophages), bone marrow (fixed macrophages), serous fluids (pleural and peritoneal macrophages), skin (histiocytes, Langerhans's cell) and in other tissues. Inflammatory macrophages are present in various exudates. They may be characterized by various specific markers, e.g. peroxidase activity.

Macrophages are important producers of arachidonic acid and its metabolites. They participate in both specific immunity via antigen presentation and IL-1 production and nonspecific immunity against bacterial, viral, fungal, and neoplastic pathogens. Macrophages secrete not only cytotoxic and inflammation controlling mediators but also substances participating in tissue reorganization. They include enzymes, as hyaluronidase, elastase, and collagenase, inhibitors of some of them (antiproteases), regulatory growth factors and others. Also they secrete signalling molecules called cytokines and chemokines which cause the immune response and play a central role in acute and chronic inflammation. They secrete proteases and growth factors which are important in tissue remodelling and in wound repair after injury as well.

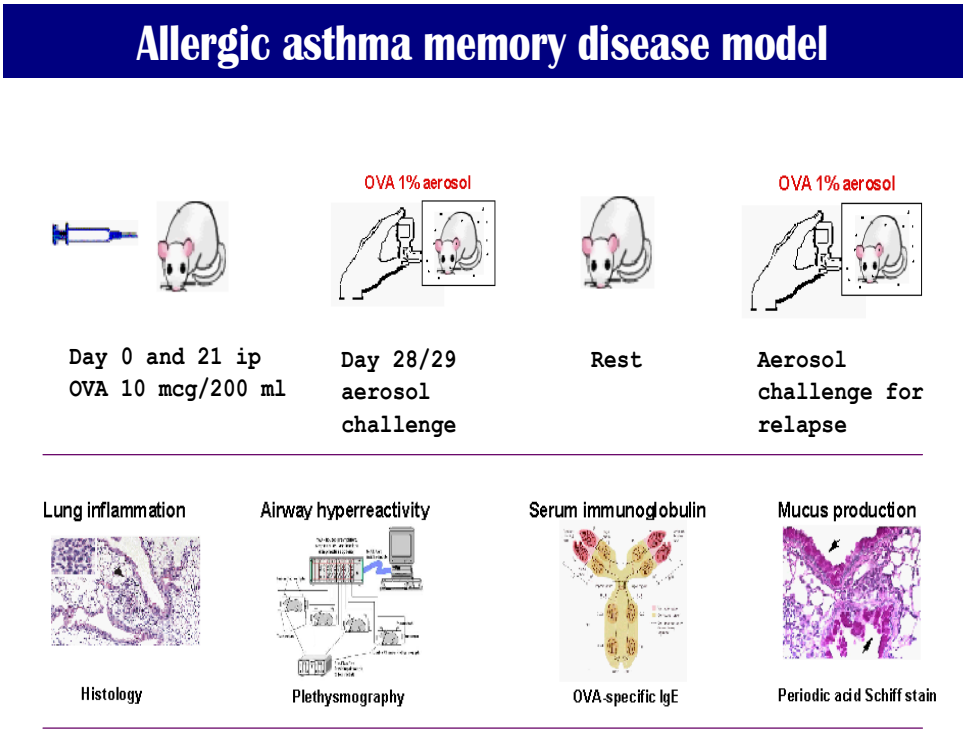
Macrophages can present processed foreign antigens to already primed T-lymphocytes allowing the enhancement or inhibition of a specific immune response.

## *4.6. Mouse models of allergic asthma*

Mice are widely used as animal models of asthma. Experiments and research in mouse model of bronchial hyper responsiveness is important for investigating the pathophysiology of asthma. Their relevance for human asthma is not clearly understood.

We used for our experiments mice sensitized and challenged with allergens. Their reaction and immune response seems to be similar to humans. Animal models of eosinophilic airway inflammation suggest a role of pulmonary DCs in the induction of regulatory T cells. With mouse model we are able to identify biological markers of asthma and discrete mechanistic phenotypes,

pathogenesis of asthma. We propose that DCs are essential for maturation allergen-specific effector Th2 and their responses in ongoing inflammation in sensitized mice.




Mouse models, using ovalbumin (OVA) as an allergen can show also the importance of these cytokines.The developments of these models help to find and improve new technologies and reagents to characterize immunological pathways and mediators, provide new opportunity and develop novel therapeutic strategies in treatment of allergic asthma.

# 5. MATERIAL AND METHODS

## *5.1. Immunoflorescence and histological analysis*

### 5.1.1. Immunohistochemistry and cutting frozen sections

4-µm lung, spleen, liver, kidney sections were cut from OCT compound-embedded blocks, air dried, and stored at -20°C until use. After thawing, sections were fixed with acetone and subsequently incubated with mAb. Sections were washed between steps with PBS and destile water. Slides were then embedded with Florosave (Molecular Probes). Immunoflorescence was visualized with a Nikon florescent microscope with a 40x objective with red, green, triple (red, green, blue) filters.

For immunohistochemical double labeling was use same protocol of staining: sections were sequentially incubated with anti hamster AF488 (Molecular Probes), anti rat AF488 (Molecular Probes), SA 594, 405 ( Molecular Probes), SA-FITC(BD Pharmingen)

### 5.1.2. Immunohistochemistry staining protocol

Slides were kept in room temperature for 10 min, fixed in acetone in 4° C for 10 min and washed in PBS for 10 min in room temperature. Then 200 un of primary antibody anti CD 11c (N418, hamster mAb, eBiosince), anti CD 11b AF488 (BD Pharmingen), anti MHC II FITC (BD Pharmingen), anti F 4/ 80 (Serotec), anti GFP ( Molecular Probes), anti B220 (BD Pharmingen), anti CD11b bio (BD Pharmingen) was add, incubated slides in 37° C for 60 min. Slides were washed in PBS again.

Add 200 un of secondary antibody, kept it in room temperature for 40 min, washed in PBS, kept in dark for 2 min and rinsed them with distilled water.

## *5.2. Mice*

BALB/c and C57B1/6 littermate mice and transgenic mice expressing a DTR and GFP under the control of CD11c promoter. We used different stages

of immunization of allergic asthma. Mice were either naïve, Ag – primed and challenged with an i.n. installation, recovered from acute episodes of allergic asthma and rechallenged i.n. with Ag, or recovered without rechallenge. We treated them with 10 ug of OVA – 594 in 50 ul of PBS i.n. Mice were kept under clean conditions (specific pathogen free)

BALB/c and C57B1/6 littermate mice and transgenic mice expressing a DTR and GFP under the control of CD11c promoter. We used different stages of immunization of allergic asthma. For experiment were also used naïve mice as control. Mice were obtained from the lab of prof. Hoffmann, Department of pharmacology and toxicology, Faculty of pharmacy, University of Vienna. They were free of pathogens. Weight was about 40 grams. Male and female BALB/C and B6 mice were used at 6–9 wk of age.

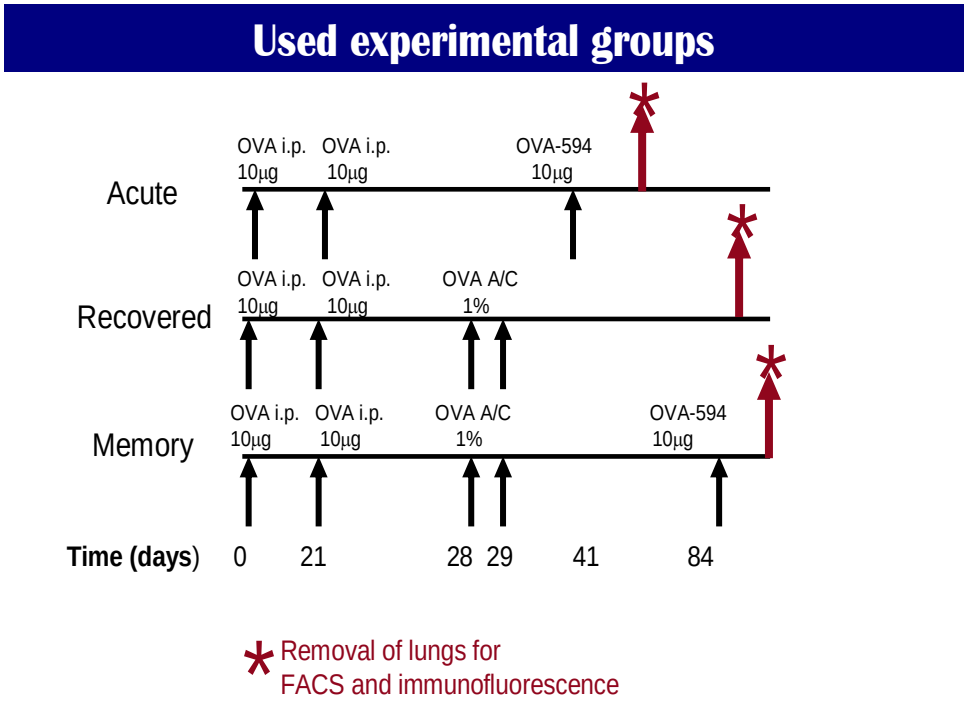
## *5.3. Organs preparation*

### 5.3.1. Lung preparation

Mice were slightly anaesthetized with 100µl of a mixture containing 2ml Rompum (Bayer), 10ml Ketanest (Pfizer) and 18ml distilled water. One and four hours after i.n. installation of OVA conjugated to the red fluorochrome AlexaFluor594 (OVA 594) (Molecular Probes) in 50µl 1x PBS mice were euthanized and took out the lungs. Then lungs were put into OCT compound-embedded blocks, air dried, and stored at -20°C until use.

### 5.3.2. Spleen, liver, kidney preparation

One, two, three and four hours after i.n. installation mice were euthanized and took out these organs. Then organs were put into OCT compound-embedded blocks, air dried, and stored at -20°C until use.




## *5.4. Immunization*

### 5.4.1. Memory (rechallenge)

Mice are sensitized with ovalbumin i.p. 10ug on the day 0 and 21, which induces symptoms of allergic asthma. Later on, the mice are challenged with ovalbumin by aerosol, which results in airway hyperresponsiveness, eosinophilic infiltration in the airways and elevated levels of antigen-specific IgE in serum. Ovalbumin-sensitized mice are repeatedly challenged with ovalbumin inhalations on the day 28 and 29. On the day 84 mice received OVA AF 594 i.n. 10ug/ 50ul

### 5.4.2. Recovered

Mice are sensitized with ovalbumin i.p. 10ug on the day 0 and 21, which induces symptoms of allergic asthma. Later on, the mice are challenged with ovalbumin by aerosol on the day 28 and 29.

### 5.4.3. Acute

Mice are sensitized with ovalbumin i.p. 10ug on the day 0 and 21, on the day 41 they received i.n. OVA AF 594 10 ug/50 ul.

### 5.4.4. Naïve

Naive mice were treated by OVA 594 AF i.n. for 1 and 4 hours, then they were euthanized and we took out the lungs

### 5.4.5. Naive control mice

Mice without treatment of OVA AF 594 i.n.

## *5.5. Animals*

už je nahoře

## *5.6. Reagents*

Primary antibodies

anti CD 11c (N418, hamster mAb, eBiosince)
anti CD 11b AF488 (BD Pharmingen),
anti MHC II FITC (BD Pharmingen)
anti F 4/ 80 (Serotec)
anti GFP ( Molecular Probes)
anti B220 (BD Pharmingen)
anti CD11b bio (BD Pharmingen)

Secondary antibodies

anti hamster AF488 (Molecular Probes)
anti rat AF488 (Molecular Probes)
SA 594, 405 (Molecular Probes)
SA-FITC (BD Pharmingen)

Ovalbumin conjugated Alexa Fluor 594(Molecular Probes)
Rompun 2% (Bazer, Veinna, 23.32 mg Xylazin hydrochlorid 1mg p-Hydroxybenzoesaure-methylester)
Ketanest S 25mg/ml 10ml ampullen (Pfizer, Vinna)
Gimsa - azur-eosin methylene blue solition, contains methylen(Merck. KGaA, Germany)

May- Grunwald, eosin methylene bleue solition (Merck, KGaA, Germany)
Lysin puffer (NBK)
Hematoxylin - Papanicolaon s solution 1a Harris hematoxylin solution (Merck, KGaA, Germany)
O.C.T Tissue-Tek, polyvinyl alcolol 11%, carbowax 5%, nonreactive ingredients 85%, (Sakura Finetek Europe B.V. Netherlands)
Lympholyte – M(CL5035, Cedarlane, Canada)
IMDM 1x( ohne NaHCO3 + L- glutamin + 25mmol HEPES, NBK)
PBS1x (NBK)
PBS 10X (NBK)

## *5.7. Instruments*

Slides cover slides, forceps, stand, pipette, tips, scissors,
centrifuge, pad, beaker,
Nikon florescent microscope with a 40x objective

# 6. RESULTS

## *6.1. Spleen stained for DAPI, naive mice treated with OVA 594 AF, i. n.*

Fig.1: on these pictures is clearly seen that number of OVA positive cells depends on time. After four hours there are more OVA positive cells than after one hour. Some OVA positive cells are DAPI positive, which means they are double positive. OVA are located around the nucleus. Some cells build clusters, distributed over the tissue.

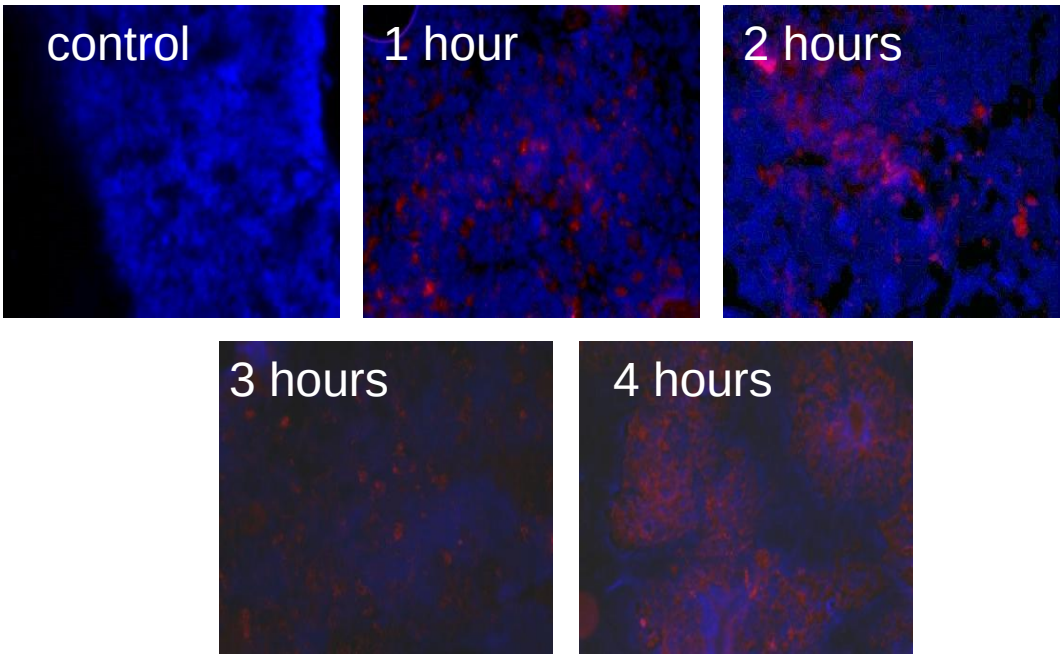



Figure 1. Spleen sections of naive mice treated with OVA 594.

The sections were stained with DAPI. The cells that picked up OVA 594 are red and cells with nucleus are blue because of DAPI staining. Frozen lung sections of naive mice (without treatment) and with i.n. administration of OVA 594 after 1, 2, 3 and 4 hours.

### 6.2. Naive mice, liver stained for DAPI, mice received OVA 594 AF, i. n.

Fig.2: we can clearly see increasing number OVA positive cells in the liver, after 4 hours comparing to one hour. Some OVA positive cells are DAPI positive. Double stained cells are distributed over the tissue. Especially after 4 hours is visible that OVA is located around the nucleus.

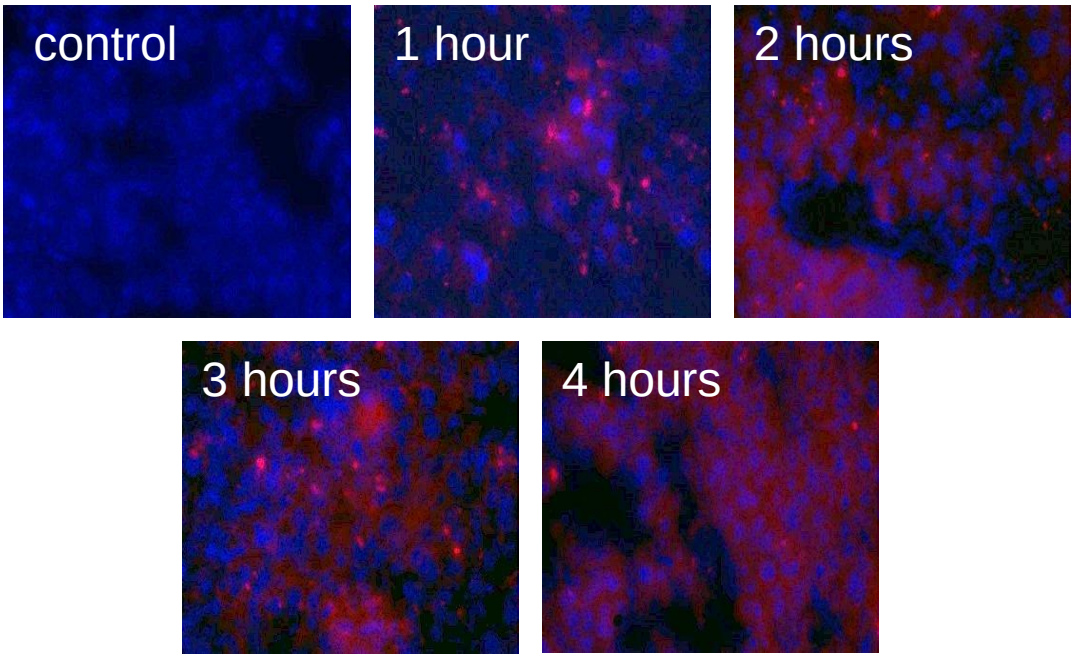



Figure 2. Liver sections of naive mice treated with OVA 594.

The sections were stained with DAPI. The cells that picked up OVA 594 are red and cells with nucleus are blue because of DAPI staining. Frozen lung sections of naive mice (without treatment) and with i.n. administration of OVA 594 after 1, 2, 3, 4 hours.

### 6.3. Naive mice, kidney stained for DAPI, mice received OVA 594 AF, i. n.

Fig.3 shows staining of kidney from naive mice stained with DAPI antibody. We can clearly see the difference between the time points. After one hour there are not so many cells picked up OVA 594 AF (red cells). But during the time there are more OVA positive cells.

The cells seem to be big. Some of them also seem to form clusters. Also it is difficult to say if all OVA positive cells are also cells stained for DAPI. Mostly cells are double positive for OVA and DAPI.

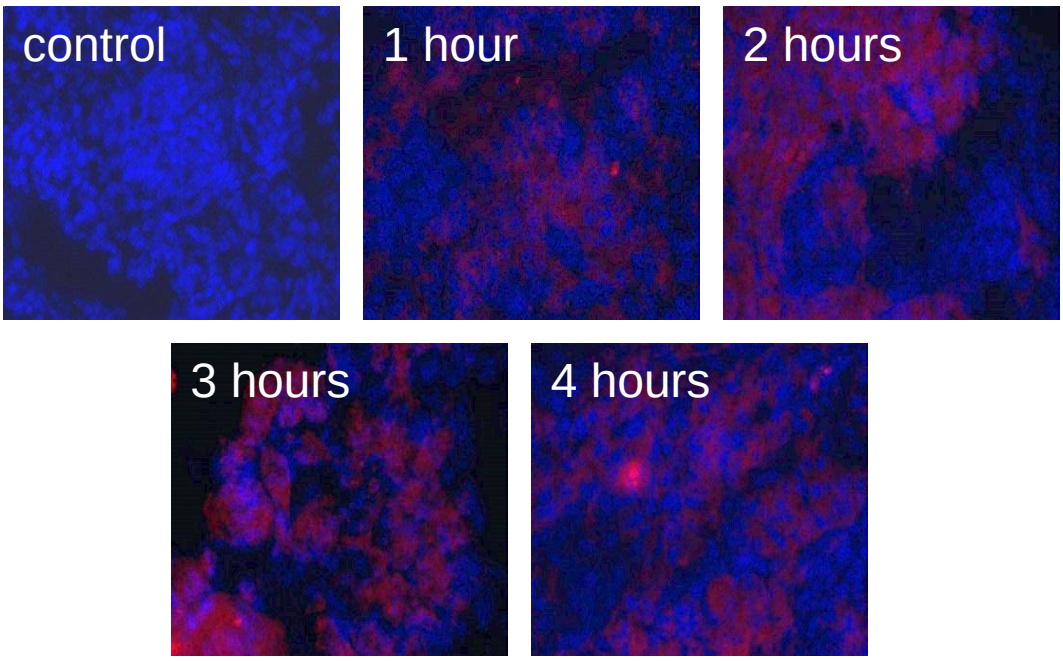



Figure 3. Kidney sections of naive mice treated with OVA 594.

The sections were stained with DAPI. The cells that picked up OVA 594 are red and cells with nucleus are blue because of DAPI staining. Frozen lung sections of naive mice (without treatment) and with i.n. administration of OVA 594 after 1, 2, 3, 4 hours. Shown are tissue section stained with DAPI at magnification of 40x.

### *6.4. Lung tissue, naive control, triple filter,without OVA 594 AF, i. n.,double staining, biotin – blue, secondary antibody green*

Mice did not receive OVA, there are no red cells. Antibodies conjugated with biotin were very bad visible. It is difficult to recognize if there is a double staining. In the tissue stained with CD 11c CD11b bio are few CD11b biotin positive cells and a lot of CD11c positive cells in the clusters. Mostly single staining.

F4/80bio, MHCII

There are a lot of MHC II positive cells, hardly any F4/80 biotin positive cells.

No double stained cells. There some F4/80 biotin cells around the airway.

F4/80 biotin, CD11b

Hardly any F4/80 biotin positive cells but a lot of CD11b+ cells, distributed over the lung tissue, do not build the clusters.

CD11c bio, MHCII

Very weak CD11c staining, only MHCII+ cells are visible, localized over the all tissue.

CD11b+F4/80-

MHCII+CD11c-

CD11b+CD11c

CD11b+F4/80-

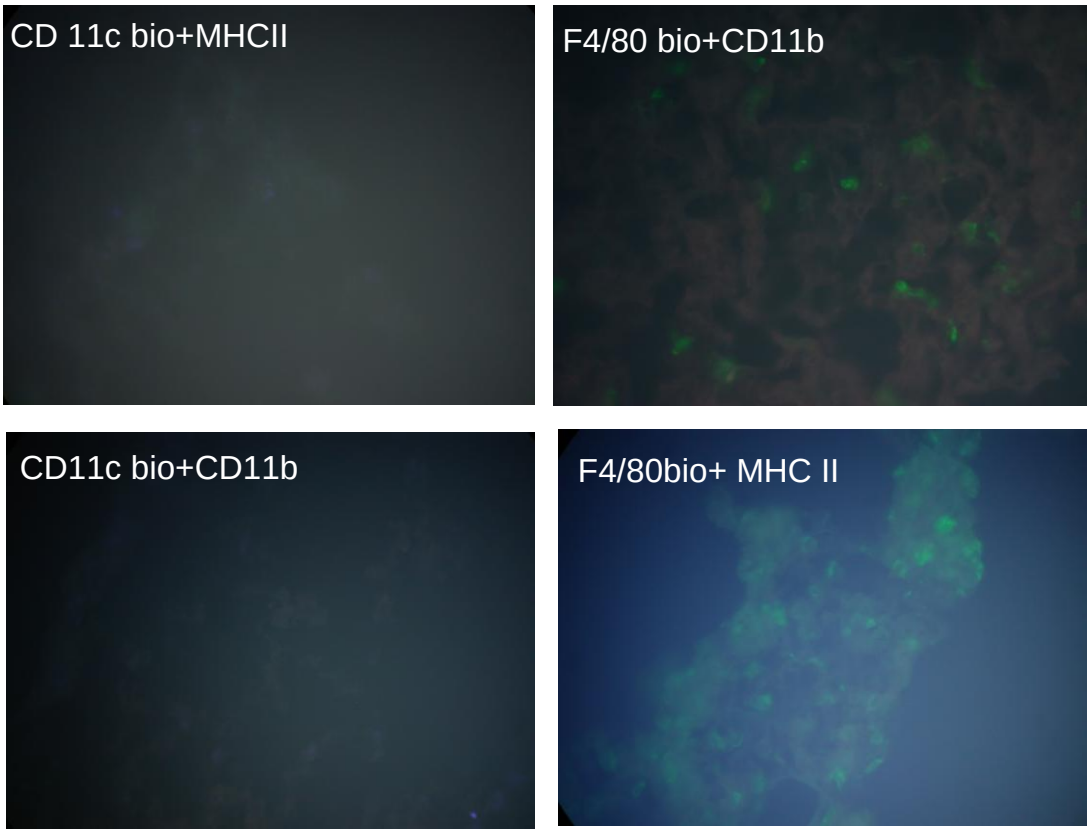



Figure 4. Lungs of naïve control mice stained with CD11c, F4/80, MHCII, CD11b.

Frozen lung sections from naïve control mice without treatment and with i.n. instillation of OVA 594. The lung sections were stained with biotin, cells are visible as blue cells and antibody not conjugated with biotin are green.

### *6.5. Lung tissue naive 1 hour, triple filter, OVA 594 AF, i. n., double staining, biotin – blue, secondary antibody green, red – OVA*

CD11cbiotin, MHC II

There are CD11c+ and MHCII bio+ cells but it looks like there is no double staining, also mostly red cells are not double stained. Who picked up the OVA? Only few cells are OVA MHC II positive cells.

Because of weak CD11c the CD11c+ cells are bad detectable. There are some cells OVA and CD11c positive cells, but is seems to be that CD11b biotin cells did not pick up OVA, but they are spread over the lung tissue.

F4/80 biotin, CD11b

There are hardly any F4/80 positive cells. CD11b are good visible, localized over the all lung tissue, cells which picked up OVA are also distributed everywhere, but mostly they are single stained, again there is the question which cells picked up the OVA?

But we can find there some cells OVA CD11b positive cells.

F4/80bioMHCII

The MHCII staining is very strong and so F4/80 stained cells are not visible. There are a lot MHCII positive but there are not cells which picked up OVA. MHCII are located over the all tissue. If we overlay triple filter and green filter there are some MHC II OVA positive cells.

MHCII+CD11c-

CD11b+CD11c-

CD11b+F4/80-

MHCII+F4/80-

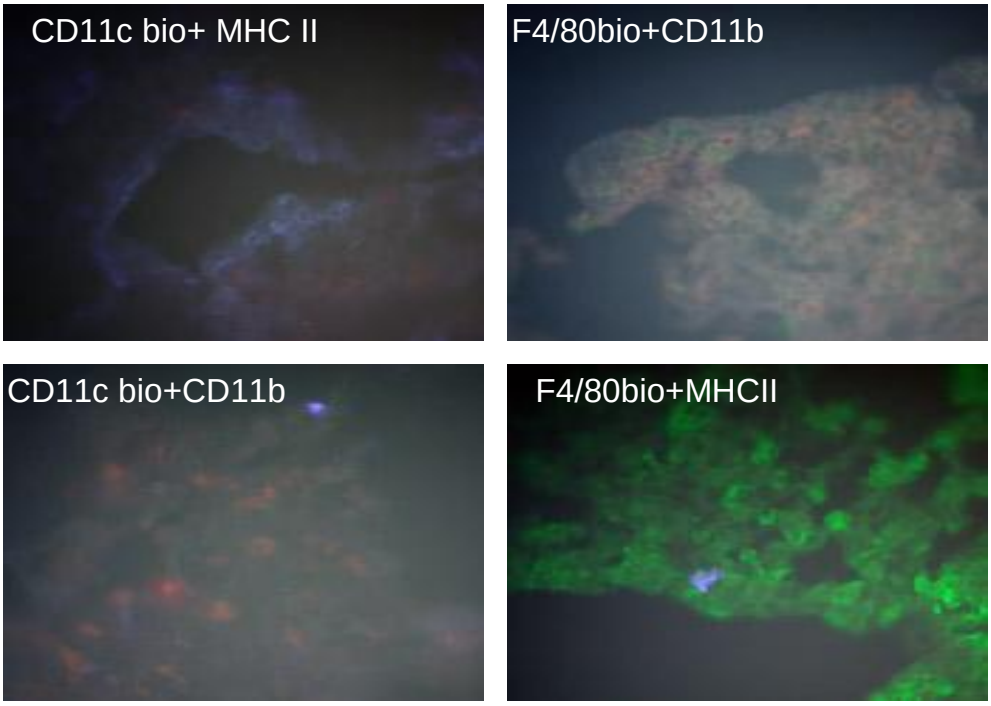



Figure 5. Lungs of naïve mice stained with CD11c, F4/80, MHCII, and CD11b.

Frozen lung sections from naïve mice without treated with OVA 594 AF i.n. The lung sections were stained with biotin, cells are visible as blue cells and antibody not conjugated with bio are green. Lung was taken out after 1 hour.

### 6.6. *Lung tissue, <u>naive 4 hours</u>, triple filter, OVA 594 AF, i. n., double staining, biotin – blue, secondary antibody green, red – OVA*

There are many MHC II positive cells, around the airways; few of them are double positive for CD11c biotin and MHCII. The anti-CD11c antibody does not lead to a signal as strong as the anti-MHCII antibody. However, it can be clearly seen that there are $CD11c^+$ cells.

Some cells are MHC $II^+CD11c^+$ but a big majority is single positive.

F4/80: antibody is very weak → hard to see $F4/80^+$ cells, but there are a lot of them which are also CD11b positive, some are also single positive; they are more located around the airways.

→ Some are CD11b $F4/80^+$, some cells are single positive for either CD 11b or F4/80

Hardly any double positive cells, only MHC II positive cells.

Conclusion: double positive CD11cMHCII, not double MHCII F4/80, only few F4/80CD11b positive

MHCII+CD11c+

CD11b+F4/80+

MHCII+F4/80-

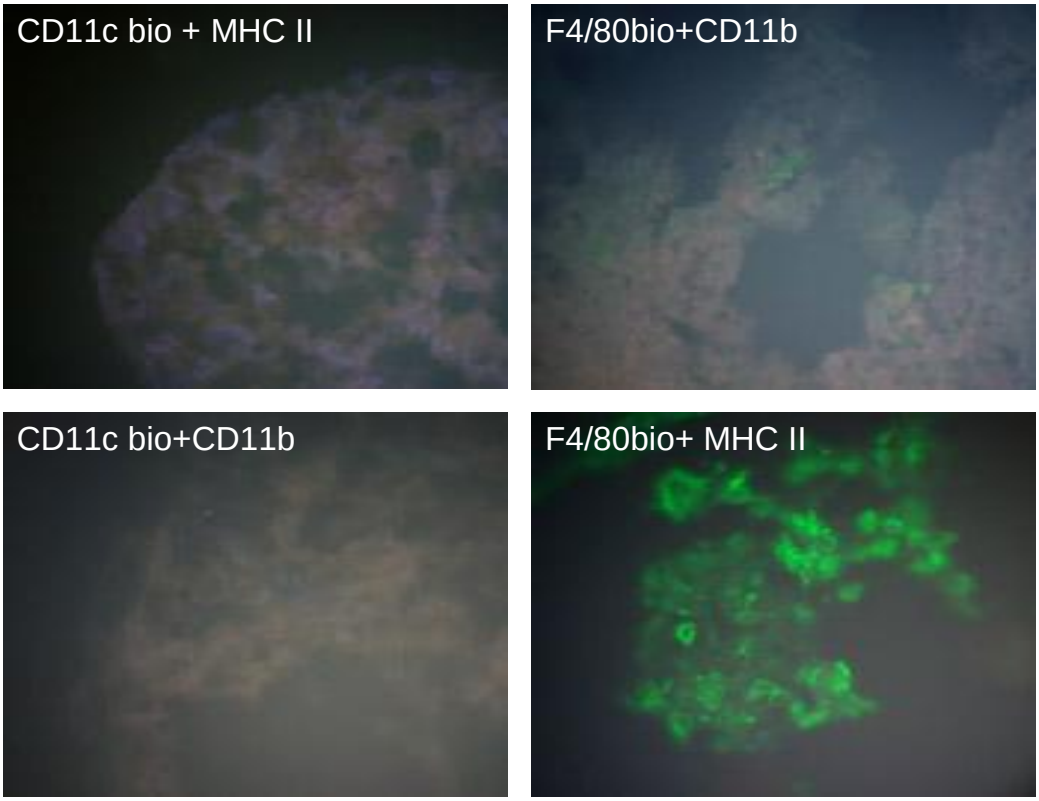



Figure 6. Lungs of naive mice stained with CD11c, F4/80, MHCII, and CD11b.

Frozen lung sections from naive mice without treatment. Mice received i.n. of OVA 594 and were euthanized after 4 hours. The lung sections were stained with biotin, cells are visible as blue cells and cells with antibody not conjugated with bio are green.

## *6.7. Fig. 7*

*Lung tissue, **recovered**, triple filter, without OVA 594 AF, i. n., double staining, biotin – blue, secondary antibody green*

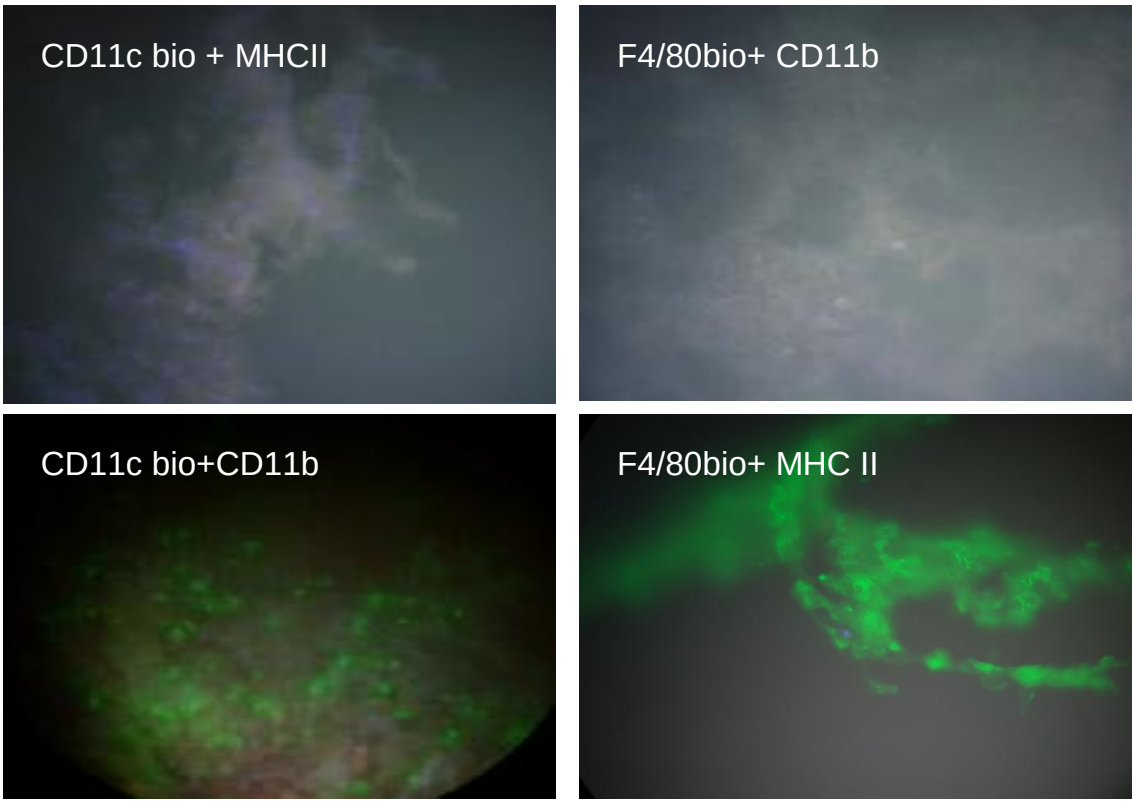



Figure 7. Lungs of recovered mice stained with CD11c, F4/80, MHCII, and CD11b.

Frozen lung sections from recovered mice without treatment and with i.n. instillation of OVA 594. The lung sections were stained with biotin, cells are visible as blue cells and antibody not conjugated with bio are green.

***Recovered mice*** - without OVA 594 AF i.n.

On the picture there are not red cells (OVA positive cells). We stained the lung tissue for CD11c bio with MHC II. CD11c bio positive cells are blue and MHC II is green. The problem was with CD11c (N 418) antibody, because it is not a good antibody. There is a lot of MHC II biotin positive cells, some of them

are also CD11c positive cells. MHC II positive cells are mostly around the airways.

Conclusion: a lot of MHC II positive cells, hardly any CD11c and MHC II double positive cells, because of not good quality CD 11c antibody.

In recovered tissue are a lot of CD 11b (green) positive cells, spread over the tissue, some cells are in the clusters. The tissue is not positive for CD11c biotin.

There are hardly any double positive cells for F4/80 biotin and CD11b, mostly single F4/80 positive cells. But the in other case the are lot MHC II positive cells but not double F4/80 biotin and MHC II double positive cells.

MHC II+CD11c-

CD11b+CD11c-

CD11b+F4/80-

MHCII+F4/80-

## *6.8. Fig. 8*

*Lung tissue **rechallenged 1 hours**, triple filter, OVA 594 AF, i. n., double staining, biotin – blue, secondary antibody green, red – OVA*

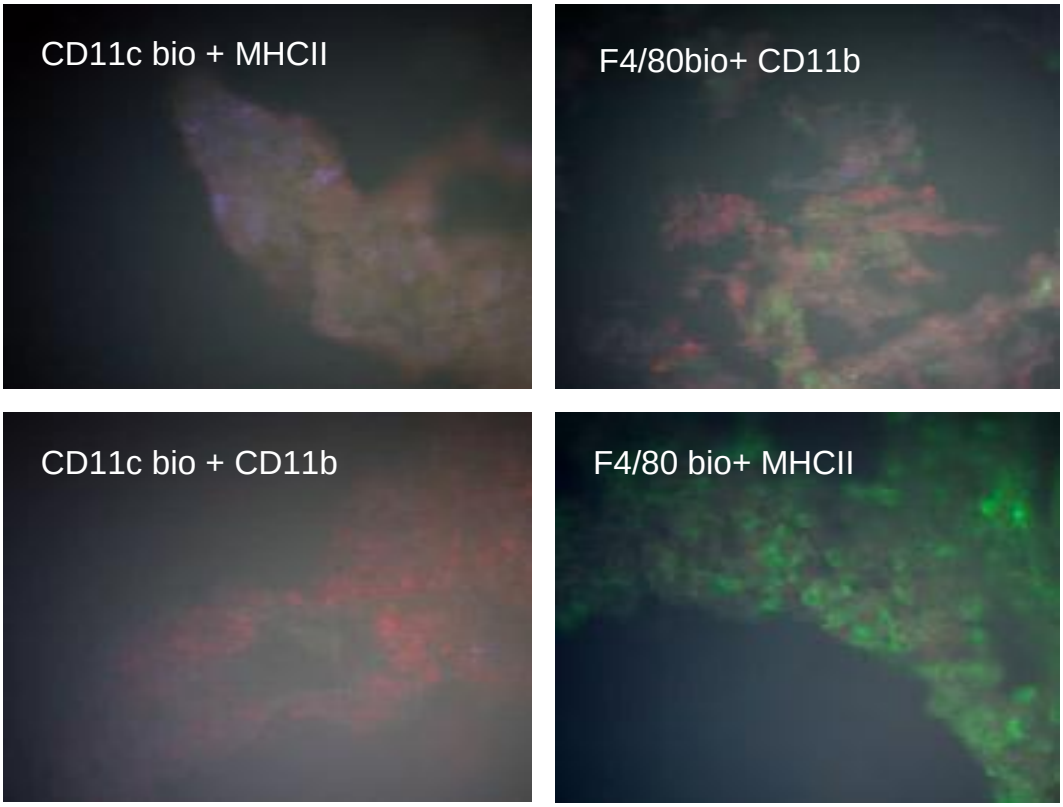



Figure 8. Lung sections of rechallenged mice after one hour.

Frozen lung sections of rechallenged mice euthanized one hour after the OVA 594 treatment. The sections were stained for MHCII, CD11b, CD11c, and F4/80 which are all seen in green. Cells that picked up antibody with conjugated with bio are seen as blue.

### *Rechallenged 1 hour*

Many single positive F4/8O cells and CD11b

Only MHC II positive cells, hardly any F4/80

Hard to see double positive CD11c and CD11b, but there are some

Few double positive CD11c and MHCII

CD11b+F4/80-

MHCII+F4/80-

CD11b+CD11c-

MHCII+CD11c-

## *6.9. Fig. 9*

*Lung tissue **rechallenged 4 hours**, triple filter, OVA 594 AF, i. n., double staining, biotin – blue, secondary antibody green, red – OVA*

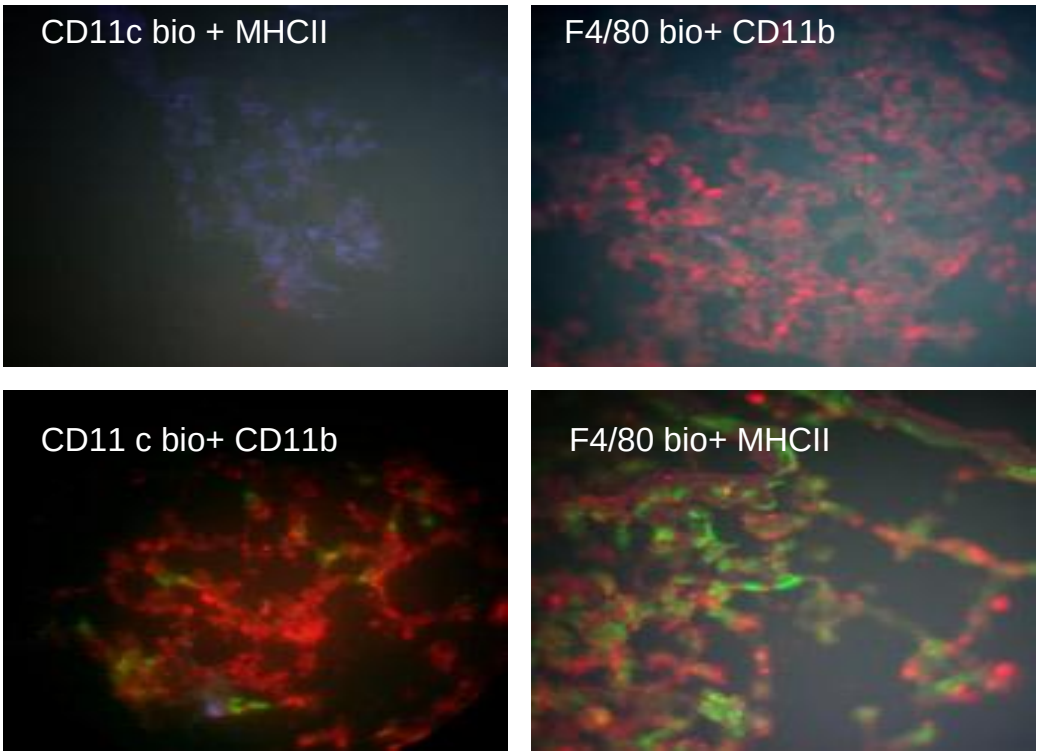



Figure 9. Lung sections of rechallenged mice after four hours.

Frozen lung sections of rechallenged mice euthanized after four hours of OVA 594 treatment. The sections were stained for MHCII, CD11b, CD11c, F4/80 which are all seen in green. Cells that picked up antibody with conjugated with bio are seen as blue.

### *Rechallenged 4 hours*

There are many MHCII single positive cells, not CD11c bio positive cells.

Double positive F4/80 and CD11b

Single MHCII but not F4/80

Few CD11c bio and CD11b double positive

MHCII+CD11c-

CD11b+F4/80-

MHCII+F4/80-

CD11b+CD11c-

## *6.10. Fig. 10*

***Lung tissue, single staining***

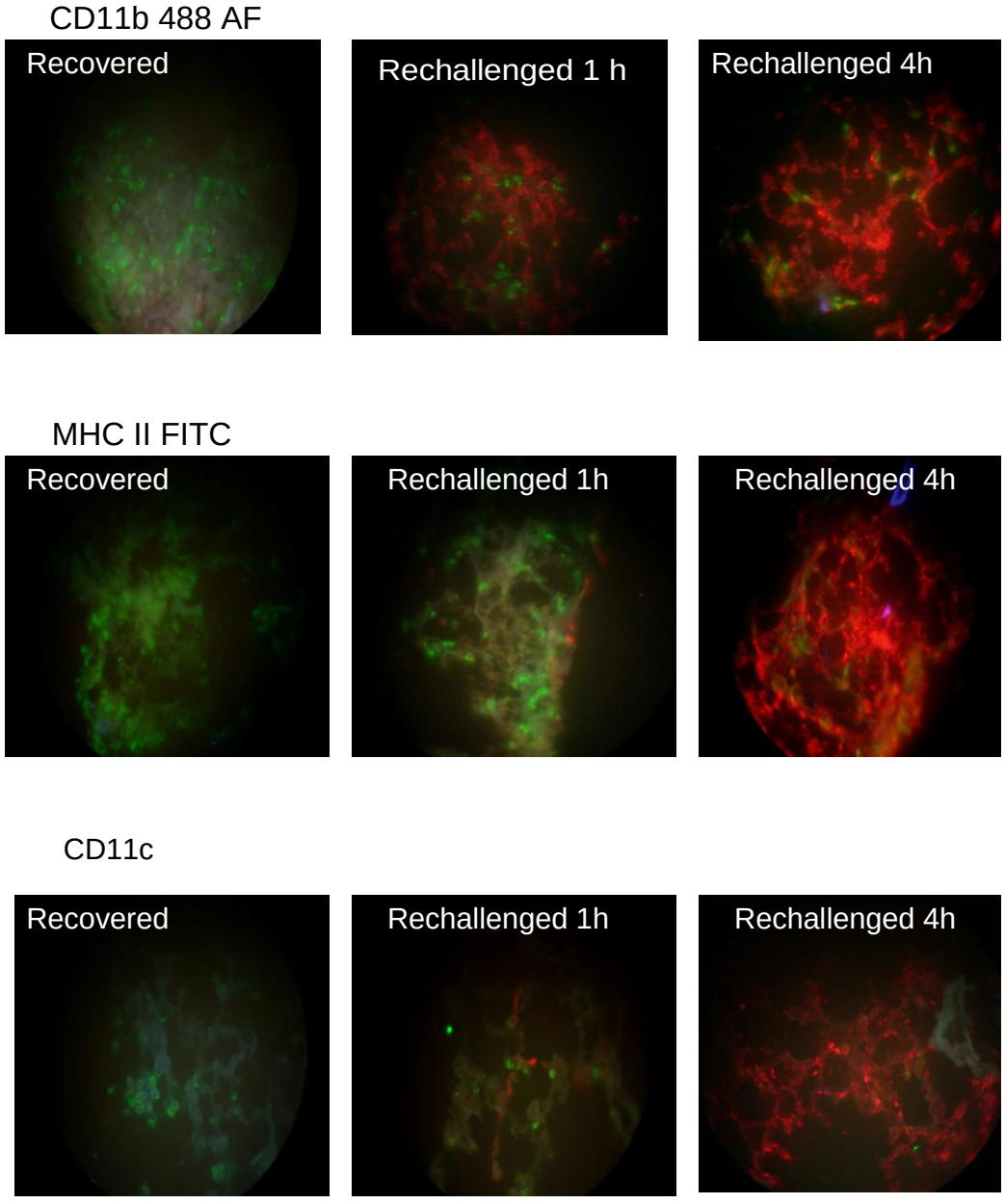



**Fig. 10** Lungs of recovered and rechallenged mice.

Frozen lung sections of recovered mice without treatment and rechallenged mice with i.n. administration of OVA 594 after 1 and 4 hours. The lung sections were stained with anti-MHCII monoclonal antibody, anti-CD11b monoclonal antibody or anti-CD11c monoclonal antibody, all of them being green fluorescent labelled. OVA positive cells are red.

**Single staining**

***CD 11b***

Fig.10 shown the tissue section stained with anti CD11b at magnification of 40 x. Most cells demonstrate the typical morphology of immature DC. All the cells present large nuclei. In the single staining we tried to observe which cells picked up OVA and where these cells are located? Different groups of sensitised mice were exposed to OVA allergen. If we compare rechallenged and recovered group, it is shown that in rechallenged tissue cells picked up OVA. It is clearly seen that after four hours in lung tissue are more OVA positive cells than after OVA hours. The problem is to distinguish if the OVA are in the cells or in the all tissue. It seems to be OVA positive cells are located mostly around the airways, they built the clusters. Recovered didn't received OVA, so we can only see green cells positive for CD11b.

CD11b is a good antibody. In rechallenged 1 hour tissue is mostly single staining, hardly any double positive cells.

Conclusion:

1. Double staining in rechallenged 4 hours
2. Recovered look similar only single CD 11b positive cells
3. Double positive cells are around the airways
4. What are the cells who picked up the cells????? – DC or macrophages?!

***MHC II***

MHC II was a good antibody, same quality as CD11b.
Green MHCII is located over the tissue, in each tissue. In rechallenged 4h are a lot of red OVA positive cells that make the problem to detect green MHCII positive cells. MHC II positive cells do not build clusters. But red OVA positive cells are in the clusters around the airways. There are some double positive cells around the airways.

Conclusion:

1. Good staining, there are a lot of MHC II positive cells
2. Many OVA positive cells after 4h in rechallenged tissue
3. Double positive cells are mostly located around the airways

***CD11c***

The CD11c is very bad antibody; the cells are not good stained, in the comparing to others antibodies. There are hardly any green CD11c cells in all types of tissue. In rechallnged 4h tissue are occurred many OVA positive cells, cells are in clusters, mostly around the airways. In recovered tissue are CD11c positive cells in the clusters. In the all pictures it is seemed to be only single positive cells, no double positive cells. The question is what are these cells? CD11c is a surface marker for DC and for macrophages, if we can see only single positive cells, the question which cells picked up OVA is very important. Maybe it is because of not so good type of antibody. If we focus on the slides closely we can find only few double positive cells in rechallenge 4 h.

Conclusion:

1. CD11c is a bad antibody
2. Again a lot of red OVA positive cells in rechallenge 4h and n
3. Not so much double positive cells

If there are double cells, there are located around the airway

Findings: CD11c is very bad antibody and the staining is very weak, recovered tissue has only single positive cells because of not exposition to OVA.

1. after 4h there are a lot OVA positive cells, so that mean that number OVA positive cells depends on time, it increases with time
2. some CD11c, CD11b and MHCII are double positive cells, located around the clusters
3. number of OVA positive cells also depends on the type of tissue

# 7. DISCUSION

Dendritic cells play key role in response of allergic asthma. If we will be able to indicate different subsets, localization, migration and distribution of DC, then we are also able to modify bounding between DC and Th cells. This bounding is important for involving the cascade of inflammatory process. The first aim was to describe surface markers of DC and distinguish if it is DC or macrophage, second to detect the localization and distribution and third to describe different subpopulations.

Th2 responses, effector Th2 cells still depend on DCs for their activation in vivo (Lambrecht el al., 2000). We were able to show that conditional depletion of CD11c+ DCs from the airways of mice was able to completely suppress Th2-mediated effector responses (Epstein, 2005). The function of DCs might also directly suppress Th2 activation in already sensitized mice (Epstein 2004, Lambrecht el al., 2001).

For characterization of dendritic cells we were using immunohistochemistry staining. We detected DC in lung tissue, spleen, kidney and liver frozen section.

The main question in our experiment is which DC cells and their localization of DC play in the development of airway hyperresponsiveness and eosinophilic infiltration in the airways.

We examined the occurred changes in respiratory, during induced bronchoconstriction in BALB/c and B6 mice sensitized and challenged with ovalbumin in the different time. APC are recruited to the lungs during the active disease, persists within lung inflammatory infiltrates during remission. We hypothesize that CD11c+ cells which are important for maintaining Th2 memory cells within the lungs are long lived (Uphan et al., 2003).

To test this hypothesis we used different stages of sensitized mice, as well as naïve, recovered and rechallenged (memory). We tried to observe the

location and migration CD11c+ cells within the lungs and lymph nodes during acute disease, remission, relapses of allergic asthma. We suppose increased numbers of CD11c+ cells in the lungs during remission.

We are focusing on CD11+c cells, which include DCs and alveolar macrophages. To distinguish the populations, it is important to stain lung cells. It is necessary to distinguish CD11c+ macrophages and DC in the airway.

Also it is important to examine other surface markers as well as CD11b, MHCII, B220 (Lambrecht et al. 2000, Epstein 2005, van Rijt L.S. et al.,2005).

Although CD11c+ cells may home to lung infiltrates, persists in the lung and may be involved in the maintenance of memory cells (Uphan 2003).

If we be able to describe DC then we could be able to disrupt the interaction between APC and Th2 memory cells and that could lead to a reduction memory cells in the lungs. We predict that elimination CD11c+ cells will reduce or eliminate memory responses and disease relapse (Epstain, 2005).

Localization of APC

The results of this study show that macrophages exhibiting a dendritic morphology and expression of the CD11c cell surface marker and they are distributed widely throughout the mouse lung tissue. Examination of tissue sections stained with an antibody to CD11c, CD11b, and MHCII indicates that these cells are presented in airway epithelia as well as within the interstitial spaces in alveolar regions (de Heer H.J. et al., 2005)

At various times later, the mice were sacrificed, their lung was harvested and frozen section of lung was analyzed by florescence microscopy to determine the locations of the beads.

The lung DCs were harvested after Ag challenged and retained the ability to present Ag in vitro up to 4 hours after initial administration of the Ag.

The markers CD11b, F4/80 were chosen because of their suggested differential expression among the myeloid and lymphoid DC subsets (Lambrecht et al., 2000., Epstein, 2001.,Wallet M.A. et al.,2004)
CD11b is a myeloid specific marker (Vermaelen K.Y. et al., 2001., Lambrecht et al. 2000)

Conclusions:
There are some questions which are important to be known because of our next focusing.
1. The first is which subpopulations of DC are in the lungs.
2. Time point of OVA positive cells, which cells picked up the OVA
3. Localization of APC in the lung tissue
4. Distribution of the cells with nuclei in liver, kidney, spleen depends on the time

Ad1.

To characterize APC by description surface markers was the most difficult aim. It is because of used antibodies, some of them were too weak.

In the single staining we noticed the localization of the cells and which cells picked up the OVA.

We identified dendritic cells in lung tissue from B6 and Balb/c. Lung tissue sections were stained with different antibodies. Dendritic cells were present in the airway epithelium (Uphan J.W. 2003), close to the alveolar walls, interstitial spaces, and alveoli of lungs. At higher magnification, lung dendritic cells show typical morphology of DC.

Ad2. & Ad3. Time points

Single staining – CD11c, CD11b, MHCII

If we compare rechallenged 1h & 4h, we can clearly see the differences between rechallenged tissue 1 hour and 4 hours. After one hour there are not

so much OVA positive cells in the lung tissue, but after 4 hours there are many OVA positive cells. It is because mice were exposing to the allergen for four hours. The conclusion is that with the longer exposition the tissue has more cells that picked up OVA and cells are distributed not only to the lung but also they are localized in the liver, spleen and kidney (de Heer et al., 2005, Lambrecht et al., 2001).

Ad4. Distribution of the cells with nuclei in liver, kidney, spleen depends on the time

In the kidney there are good visible cells that picked up OVA and the increasing number OVA positive cells which depend on the time. In the comparing number OVA positive cells after one and four hours, we can notice that after one hour there are not so many OVA cells, but after 3 or 4 hours there are lots of OVA+ cells. There is no difference between 3h and 4h. DAPI is a dye used for staining the nuclei and morphology of cells. OVA are surrounded around the nuclei.

Spleen

Same finding is also in the spleen, the number of OVA positive cells depends on time exposition to OVA allergen. After 4 hours there are many OVA positive cells distributed all over the spleen. OVA are located around the nuclei. Some of them are in the clusters.

Liver

OVA are occurred in the DAPI+ cells. The situation is same, time point play key role in the occurrence of DAPI OVA positive cells.

We predicted the higher number of OVA positive cells actually in the spleen and liver. It is surprised us that similar number of OVA+ cells is found in the kidney as well.

It could be because of high blood circulation in liver. DAPI cells can be captured in the kidney tissue. We are not able to say which type of cells is there. With DAPI staining we can see only cells with nuclei. For better descriptions of these cells would be good to use flow cytometry and antibody for the detecting of cells phenotypes (Epstein 2001).

For better characterization would be the best way to use another type of monoclonal antibody CD11c and F4/80 (Uphan J.W.2003, Kuipers et al.). We can not say if there are DCs or macrophages, we can only mention that the most cells are single positive for MHCII and CD11b.

DCs were identified by N418 labeling. We have investigated the characteristics of populations of DC in the mouse spleen, kidney, liver, and lung.

Further phenotypic analysis of lung DC has shown that isolated DC express significant levels of typical macrophage markers CD11c, CD11b (de Heer et at., 2005) They express markers thought to be characteristic of macrophages, such as F4/80, CD11b (Epstein, 2005).

Using less sensitive immunohistology, however, a clear distinction can be detected between macrophages, which express high levels of F4/80 and CD11b, and the DC, which express lower levels of these markers(Epstein, 2005, Uphan J.W.2003).

The capacity of DC to phagocyte is likely indicative of the relative immaturity of these cells. DC populations seem to play fundamentally roles in the regulation of T cell responses in allergic asthma ( Lambrecht et al., 2000).

# 8. LITERATURE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