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PROSTAGLANDIN MODULATION OF AIRWAY HYPERRESPONSIVENESS AND INFLAMMATION IN MURINE MODELS OF ASTHMA

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ABSTRACT

Allergic asthma is an inflammatory disease of the airways and is characterised by chronic inflammation, reversible bronchoconstriction and airway hyperresponsiveness (AHR). The cyclooxygenases (COXs) are key enzymes in the biosynthesis of prostaglandins, which have diverse physiological and pathological functions, and are also important modulators of inflammatory responses in the lung. However, the role of COX-derived prostanoids in AHR and airway inflammation is still not fully understood. The overall aim of this Thesis was to define the role of COX-derived products in allergic airway responses in experimental mouse models of asthma.

In the present work, lung physiology was measured using the flexiVent® system for direct measurements of respiratory system mechanics. Inflammation was assessed by measuring numbers of inflammatory cells in bronchoalveolar lavage (BAL) fluid as well as levels of inflammatory mediators in BAL fluid and lung tissue. Histological analysis of the lung tissue was also performed to assess possible structural changes. The aim of the first part of this Thesis was to develop an improved protocol for experimental studies of AHR and airway inflammation in sensitised and challenged mice, in order to optimise the delivery route and doses of allergen for antigen challenge. The result established that intranasal challenge of BALB/c mice caused a dose-dependent increase in AHR, whereas the BAL fluid cell response was augmented to the same extent by all doses of allergen. Intranasal challenge causes a more pronounced induction of both AHR and inflammation in sensitised BALB/c mice, compared to aerosol delivery of the same antigen.

The second part of this Thesis aimed to define the function of the prostaglandins in the mouse models of allergic airway inflammation. The role of the prostaglandins was investigated both by using intervention with non-steroidal anti-inflammatory drugs (NSAIDs), as well as genetically modified mice where the synthesis of prostaglandin (PG) E_2 had been disrupted by removal of one of the prostaglandin E synthases (mPGES-1). The findings support the concept that in general, COX products have a bronchoprotective function in the airways, i.e. local release of PGE_2 modulates the airway smooth muscle reactivity and protects against excessive airway narrowing. Furthermore, the bronchoprotective effects in allergic airway inflammation are mainly due to PGE_2, primarily generated by mPGES-1. The two isoenzymes, COX-1 and COX-2, also appeared to have separate functions, whereby COX-1 predominantly generated the prostaglandins recovered in BAL fluid and COX-2 activity was associated with the accumulation of inflammatory cells in BAL fluid. It is not clear, which prostaglandin mediated the COX-2 dependent effects on cellular inflammation. However, the observations suggest that neither mPGES-1 nor PGE_2 are involved in the inhibitory function of COX-2 on cell recruitment in the allergic airway reaction.

Taken together, the present Thesis supports the concept that AHR and the inflammatory response are distinct, and at least in part, uncoupled events. Furthermore, the findings raise awareness that BAL fluid inflammation is not a predictive surrogate marker of AHR. Finally, inhibition of PGE_2 has been suggested as a target for anti-inflammatory therapies in humans, as PGE_2 is a pro-inflammatory mediator and is involved in many inflammatory diseases. However, it is possible that inhibition of mPGES-1-derived PGE_2 may have negative consequences in the airways.

LIST OF PUBLICATIONS

This Thesis is based on the following publications:

I. Linda Swedin, Russ Ellis, Cecilia Kemi, Åke Ryrfeldt, Mark Inman, Sven-Erik Dahlén and Mikael Adner
Comparison of aerosol and intranasal challenge in a mouse model of allergic airway inflammation and hyperresponsiveness
International Archives of Allergy and Immunology 2010; 153(3):249-58

II. Linda Swedin, Theresa Neimert-Andersson, Josephine Hjoberg, Sofia Jonasson, Marianne van Hage, Mikael Adner, Åke Ryrfeldt, Sven-Erik Dahlén
Dissociation of airway inflammation and hyperresponsiveness by cyclooxygenase inhibition in allergen challenged mice
European Respiratory Journal 2009(1);34: 200–8

III. Linda Swedin, Russ Ellis, Theresa Neimert-Andersson, Åke Ryrfeldt, Gunnar Nilsson, Mark Inman, Sven-Erik Dahlén and Mikael Adner
Prostaglandin modulation of airway inflammation and hyperresponsiveness in mice sensitized without adjuvant
Prostaglandins & Other Lipid Mediators, 2010 Mar, in press

IV. Linda Swedin, Cecilia Kemi, Susanna Lundström, Silvana Lindgren, Craig Wheelock, Åke Ryrfeldt, Mikael Adner and Sven-Erik Dahlén
Deletion of microsomal PGE synthase-1 enhances airway hyperresponsiveness in a mouse model of chronic airway inflammation
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

AHR	Airway hyperresponsiveness
AIA	Aspirin-intolerant asthma
ASM	Airway smooth muscle
BAL	Bronchoalveolar lavage
BHR	Bronchial hyperresponsiveness
C_L	Dynamic lung compliance
COX	Cyclooxygenase
cPGES	Cytosolic prostaglandin E synthase
CysLTs	Cysteinyl-leukotrienes
EAR	Early allergic reaction
ED_{50}	Effective dose for half maximal response
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
FcεRI	High-affinity receptors for IgE
FEV_1	Forced expiratory volume in one second
FLAP	5-LO-activating protein
FOT	Forced oscillation technique
G	Tissue damping, reflects energy dissipation in the tissue
GPCR	G-protein coupled receptor
H	Tissue elastance, reflecting energy storage in the tissue
I.p.	Intraperitoneal
I.v.	Intravenous
IgE	Immunoglobulin E
IL	Interleukin
LAR	Late-phase allergic reaction
5-LO	5-lipoxygenase
LT	Leukotrienes
MCh	Methacholine
MC_T	Mucosal mast cells
MC_{TC}	Connective mast cells
mPGES-1	Microsomal Prostaglandin E synthase-1
mPGES-2	Microsomal Prostaglandin E synthase-2
NSAIDs	Non-steroidal anti-inflammatory drugs
OPLS	Orthogonal partial least squares regression of latent structure
OVA	Ovalbumin
PBS	Phosphate-buffered saline
PCA	Principal component analysis
PG	Prostaglandin
PLA_2	Phospholipase A_2
QP-3	Quick Prime-3
R_L	Dynamic lung resistance
R_N	Newtonian resistance
TX	Thromboxane
Zrs	Respiratory system input impedance

1 GENERAL INTRODUCTION

This Thesis summarises investigations into the role of cyclooxygenase (COX) products in airway hyperresponsiveness (AHR) and airway inflammation in mouse models of allergic asthma. The COXs are key enzymes in the biosynthesis of prostaglandins (PGs) which have diverse physiological and pathological functions, and are key modulators of inflammatory responses (1, 2). Although the bronchoconstrictive effects of primary PGs were described in the early 1970's (3-5), the role of COX-derived eicosanoids in AHR and inflammation is still unclear. The discovery that the two COX isoenzymes, COX-1 and COX-2, are amenable to selective pharmacologic inhibition (6) has created new opportunities for investigating the role of prostanoids in asthma and airway inflammation.

Certain asthmatics display aspirin-intolerant asthma (AIA), and drug regulatory authorities in many countries, including Sweden, advise all asthmatics to not use aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs). Recent studies have shown that the intolerance reaction to NSAIDs in AIA is mainly due to inhibition of COX-1, since selective COX-2 inhibitors are tolerated by patients with AIA (7). Furthermore, the withdrawal of the COX-2 inhibitor rofecoxib (Vioxx®), because of unacceptable cardiovascular side effects, highlights the importance of defining the role of the two COX isoenzymes in inflammation (6), but also the subsequent enzymes involved in the synthesis of the prostaglandins.

The overall aim of this Thesis was to define the role of the COX isoenzymes during allergic airway inflammation and how COX activity affects AHR and airway inflammation. This was achieved by intervention with NSAIDs as well as the use of genetically modified mice where the synthesis of prostaglandin (PG) E_2 had been disrupted by removal of one of the prostaglandin E synthases in *in vivo* mouse models of asthma. For studying the pathophysiology of asthma and the role of the two COX enzymes, it is important to use *in vivo* models relevant to human asthma. Therefore, a secondary aim was to develop improved protocols for experimental studies of AHR and airway inflammation in mice.

2 BACKGROUND

2.1 ASTHMA

Asthma is a complex, multifactorial disease of the airways. The prevalence has been increasing worldwide for the past 30 years, however, in some countries the prevalence appears to have reached a plateau. Approximately 300 million people have asthma (8) and in Sweden about 8-10% of the population are asthmatics (9). The disease may develop either early in childhood or later on in life (10, 11) and there is an ongoing debate regarding definition of the different asthma phenotypes (12). A number of studies have shown gender differences in the prevalence of asthma where during childhood, the prevalence is higher in boys than girls, yet after puberty females predominate. These gender differences have been suggested to be directly related to hormonal changes during development with current hormonal status, airway size, inflammatory conditions as well as smooth muscle and vascular functions all being of importance (13).

The cardinal features of asthma typically include episodes of airflow obstruction, chest tightness, wheezing and cough (10). The airflow obstruction is caused by smooth muscle contraction, bronchial wall oedema and mucus plugging leading to a closure of the airways, either partial or complete, that is in part reversible following the administration of bronchodilator (14, 15). Factors that can provoke asthma exacerbations include allergy, viral infections, exercise, and airborne irritants such as cigarette smoke, strong odours, dusts and other inhaled irritants (10). Asthma is pathophysiologically characterised by epithelial disruption, airway smooth muscle hypertrophy and hyperplasia, increased mucus secretion, basement membrane thickening, increased cytokine production and chronic infiltration of inflammatory cells (10). These structural changes are often referred to as remodelling, a term used to define complex morphological changes involving all structures within the bronchial wall (10). AHR is associated with asthma, however, this objective measurement is assessed with bronchial provocation tests.

The guidelines for treatment of asthma are based on stepwise increases in medication, usually inhaled glucocorticoids and β_2 adrenoceptor agonists are the main therapies. If asthma is not controlled using current therapy, the treatment should be stepped up until control is achieved. At each step, bronchodilators such as short-acting β_2 adrenoceptor agonists are recommended for short-term relief of symptoms in all patients. For further treatment of mild asthma, low-dose inhaled glucocorticoids or an anti-leukotriene are recommended. In moderate asthma, a treatment with a low-dose of inhaled glucocorticoid in combination with a β2 adrenoceptor agonist is often suitable. For more severe asthma, a higher dose of glucocorticoid in combination with a long-acting inhaled β_2 adrenoceptor agonist is often recommended (16). Regular use of long-acting β_2 adrenoceptor agonists alone may be harmful. Therefore, the long-acting β_2 adrenoceptor agonist is not recommended as a monotherapy in asthma and should always be used together with an inhaled glucocorticoid (16).

2.2 AIRWAY HYPERRESPONSIVENESS

AHR is one of the cardinal features of asthma. Our understanding of the allergic, inflammatory and immunological mechanisms of asthma has markedly increased, however, the mechanisms of AHR are still not fully understood (17). AHR is usually measured using bronchial provocation tests where the subject inhales a bronchoconstrictive agent. The provoking agents can be divided into direct and indirect stimuli. Direct bronchconstrictive agents are methacholine (MCh) or histamine which act directly on airway smooth muscle receptors. MCh stimulates contraction by acting on the muscarinic cholinergic receptors that are expressed both in the central airways and in the lung parenchyma (18). Indirect stimuli, such as allergens, adenosine and mannitol, cause stimulation of inflammatory cells to release mediators or stimulation of neural pathways that induce airway smooth muscle contraction (19). The airway response to the bronchoconstrictive agent is usually measured as forced expiratory volume in one second (FEV_1; 17).

In the present work, the lung function in mouse models of allergic asthma was investigated using the flexiVent® for direct measurements of respiratory system mechanics. However, making valid assessments of lung function in mice has been challenging, particularly due to the small size of the animal. The ability to measure lung function in mice has been greatly improved by the development of measurement techniques. The use of mice for studying lung functions has increased dramatically over the years and different techniques have been used for this purpose. Since most of the techniques used for studying lung functions are highly invasive and suffer from many sampling limitations, a need arose to develop methods for measuring overall lung function and in particular, the mechanical properties of the peripheral lung (20). Technical development has resulted in a method for the analysis of pressure and flow signals measured at the airway opening, enabling investigation of all lung regions (20). These measurements of lung functions can be assessed by the use of the flexiVent®, and by applying mathematical modelling.

2.3 AIRWAY SMOOTH MUSCLE

An important role for the airway smooth muscle (ASM) in asthma has long been accepted (21). There is remarkable hyperplasia/hypertrophy of the smooth muscle in the walls of bronchi and bronchioles in asthmatics (10). As the basic features of asthma are airway inflammation and remodelling, it has been believed in the past decade, that the smooth muscle was primarily a passive responder to bronchoconstrictive agonists, whereas airway inflammation was thought to be the mechanism underlying AHR (21). It has recently become clear that the ASM cells not only have contractile and proliferative functions, but also appear to be very active in producing pro-inflammatory cytokines and growth factors (21-23). Rather than directly influencing ASM cells, the released pro-inflammatory mediators may upregulate adhesion molecules that can attract inflammatory cells, which in turn can stimulate ASM cells to proliferate (22). This implicates that ASM may be, at least in part, a regulator of the inflammatory

events leading to the structural changes in asthmatic airways. Although the ASM is involved in the pathophysiology of asthma, this does not necessarily mean that the muscle itself is abnormal (21). There is evidence demonstrating that asthmatic smooth muscle shows a normal sensitivity to bronchoconstricting agents, but there are also studies suggesting that a greater smooth muscle mass might generate greater constrictive forces and with a greater maximal response (24). In contrast, studies in animal models of asthma and in *in vitro* preparations obtained from asthmatic patients have suggested that it is the relaxant response instead that may be impaired (23).

2.4 INFLAMMATORY CELLS IN AIRWAY INFLAMMATION

One function of the immune system is to protect multicellular organisms from pathogens and form a dynamic network of a variety of cells and molecules that act together for the recognition and elimination of foreign invaders. Although the immune system is developed to protect the host from infections, inappropriate responses of this system can lead to allergy and asthma.

Asthma is associated with pronounced inflammatory changes in the airways and it is now accepted that asthma is a chronic inflammatory disease. Evidence of inflammation can be observed in mild, moderate and severe conditions of this disease. Many cell types are involved in allergic airway inflammation, such as lymphocytes, macrophages, eosinophils, neutrophils and mast cells (25). However, the pattern of inflammation in the airways, i.e. the type of inflammatory cells and the site of inflammation may differ between asthma phenotypes (26).

The main function of the lymphocyte is to respond to invading antigens after activation by antigen-presenting dendritic cells. T-lymphocytes (T-cells) can be divided into different subpopulations depending on their receptors and functions, T-helper cells and cytotoxic T-cells (27). The T-helper cells can be further divided into T_H1 and T_H2 cells and they differ in the repertoire of cytokines produced. The T_H1 response results in a cytokine profile that activates mainly cytotoxic T-cells (Interleukin-2; IL-2) and macrophages (INF-γ) whereas the T_H2 response activates mainly mast cells, eosinophils, and B-cells (IL-4, IL-5 and IL-13). Several T_H2 secreted cytokines, such as IL-13, have the potential to initiate and regulate the inflammatory process, including tissue eosinophilia. An imbalance between T_H1 and T_H2 responses is thought to play an important role in several immune disorders, which in the case of allergic disease is dominated by T_H2 cells (27).

Monocytes/macrophages have important functions in the processing and presentation of antigens to T-lymphocytes, as well as in the regulation of T-lymphocyte responses. They are capable of secreting a variety of enzymes and mediators involved in inflammatory reactions, including eicosanoids, lysosomal enzymes and interleukins (28). Approximately 80% of the cells in bronchoalveolar lavage (BAL) fluid are macrophages. Yet, in most studies the number of macrophages in BAL fluid is not increased in asthmatics compared to healthy individuals. However, it has been

suggested that activated macrophages contribute to airway inflammation in asthma by their capacity to secret inflammatory mediators, though, the precise function of monocytes/macrophages in asthma and allergic inflammation is not clear (28).

Eosinophils have for some time been considered the major cell type involved in the pathophysiology of asthma, but more recently this view has been questioned (29, 30). Once at the site of inflammation, eosinophils release toxic granule proteins and pro-inflammatory mediators that contribute to the features of asthma (31). In subjects with asthma, increased numbers of eosinophils have been described in lung tissue, BAL fluid, bone marrow and blood (32). However, less than 50% of asthma subjects display evidence of eosinophilia (33). In most cases of non-eosinophilic asthma, the inflammation is associated with increased numbers of neutrophils, although a phenotype without either eosinophils or neutrophils has also been described (33).

Following allergen challenge, neutrophils are the first of the inflammatory cells to respond and enter the lung but these cells also disappear again very rapidly. It is clear that the neutrophil is present in the asthmatic airways and tissues, however, its involvement in asthma and allergic inflammation is not fully understood. It is possible that the neutrophil is involved in promoting airway remodelling by the release of mediators and this cell type does seem to have an important function in the inflammatory response (34).

The mast cell plays an important role in allergic disorders and the quantity of mast cells has been reported to be increased in the ASM of asthmatics. Furthermore, there is also a correlation of mast cell numbers with disease severity (35, 36). Mast cells are found in almost all normal human tissues and are especially abundant in tissues forming interfaces with the external environment, where they are believed to be important in tissue homeostasis, wound healing and host defence (35, 37, 38). They are usually divided into two subpopulations, where the granule content of tryptase and chymase is used to identify so-called mucosal mast cells (MC_T) and connective tissue mast cells (MC_{TC}), a common means of classification of human mast cell subtypes (39). In healthy individuals, both MC_T and MC_{TC} populations are present in all anatomical compartments of the lung with a gradual increase in density towards the alveolar parenchyma. However, MC_T is the most abundant subtype (40). Site-specific size differences among MC_T and MC_{TC} populations have also been reported, where small and large airways, pulmonary vessels and the alveolar parenchyma each possess site-specific subpopulations of mast cells beyond the classically determined MC_T and MC_{TC} populations (40).

2.4.1 Mast cell activation and mediator release

Allergen specific immunoglobulin E (IgE) binds to the high-affinity receptors (FcεRI) for IgE on mast cells and thereby sensitises them to respond when re-exposed to the allergen. The assumed dominating signal for mast cell activation in asthma is cross-linking of FcεRI-bound IgE with allergen which thereby induces the aggregation of two or more FcεRI molecules. IgE dependent activation of mast cells results in

degranulation and release of both preformed mediators, which are stored in the cytoplasmic granules of the cells, and *de novo* synthesised eicosanoids (41).

Inhalation of an antigen in allergic asthma patients leads to an early allergic reaction (EAR), usually within 15-30 min after inhalation of the specific allergen. The EAR is initiated after activation of airway mast cells and macrophages by allergen specific IgE. The activated cells release mediators such as histamine, prostanoids and cysteinyl leukotrienes, which cause bronchoconstriction, vasodilatation, increased airway permeability and increased mucus production. In addition, the mediators contribute to the development of airway inflammation by promoting an influx of inflammatory cells (42-45).

A second wave of airway constriction, the late-phase allergic reaction (LAR), may occur within 2-6 h after allergen provocation and is fully developed after 12 h. The LAR follows the EAR in 25-50% of subjects and has many features in common with the early allergic reaction (46). During the LAR, the airway constriction is more sustained and the inflammatory cells are matured, released from the bone marrow into the circulation and recruited into the airways (42).

2.5 EICOSANOIDS

Eicosanoids are bioactive twenty carbon polyunsaturated fatty acid derivatives that are synthesized *de novo* from membrane-bound arachidonic acid in response to various stimuli (47). The eicosanoid family consists of three major groups, the prostanoids (prostaglandins and thromboxanes), the leukotrienes and a number of other compounds biosynthesised in many different reactions. Individual members of each group possess distinct profiles of biological activity (48). Arachidonic acid is the major precursor for the generation of eicosanoids in mammalian cells and it is liberated from phospholipid cell membranes by phospholipase A_2 (PLA_2; 2). There are several forms of PLA_2 but the type IV cytosolic PLA_2 is the key enzyme for eicosanoid production, since cells lacking this enzyme are not able to produce eicosanoids (47). The release of arachidonic acid is usually receptor mediated and is activated by many types of stimuli, such as hormones, inflammatory mediators, growth factors, antigens and immune complexes (1, 2).

2.5.1 Cyclooxygenase pathway of arachidonic acid metabolism

Prostanoids are synthesised from arachidonic acid and related polyunsaturated fatty acid in reactions catalysed by the COX enzymes (47). COXs are key enzymes in the biosynthesis of prostanoids. In a two step reaction, COX converts arachidonic acid to the prostaglandin endoperoxides PGG_2 and PGH_2 (2, 47). These two reactions occur at distinct but structurally and functionally interrelated sites (49). Finally, PGH_2 is converted to the various bioactive PGs of the series of D_2, E_2, I_2, $F_{2\alpha}$ and tromboxane (TX) A_2 (2) by specific tissue isomerases or synthases (50), each with different structures and specific distributions (Figure 1).

There are two isoforms of COX referred to as COX-1 and COX-2, which both catalyse conversion of arachidonic acid to PGH_2 (6). The genes for COX-1 and COX-2 are located on two different chromosomes but share 60-65% protein homology (49, 51). The reason for the existence of two isoenzymes is unknown. Generally, COX-1 is constitutively expressed in most tissues and has been considered the "housekeeping" isoform which produces prostanoids required for the maintenance of physiological responses and homeostasis (1, 2, 6), whereas COX-2 is constitutively expressed to some extent but may also be induced upon inflammation. It is suggested that COX-2 is responsible for the production of prostanoids that are involved in inflammatory diseases and cancer (2, 6).

Both COX-1 and COX-2 are membrane-bound proteins localised in the endoplasmatic reticulum (51) but also associated with the nuclear envelope (49, 52). COX-2 appears to be more concentrated at the nuclear envelope and it has been suggested that the products formed via this enzyme affect nuclear events (1, 49). COX-1 and COX-2 are often co-expressed in the same cell and the different requirements of arachidonic acid concentrations may be one factor allowing the two COX isoenzymes to function in the same cell. Low concentrations may favour prostanoid generation via COX-2, whereas high concentrations favour COX-1 (49). The COX enzymes are an important pharmacological target. Structural differences between the two isoenzymes, that have important pharmacological and biological consequences, have made it possible to develop selective COX inhibitors towards the different COX isoenzymes (49).

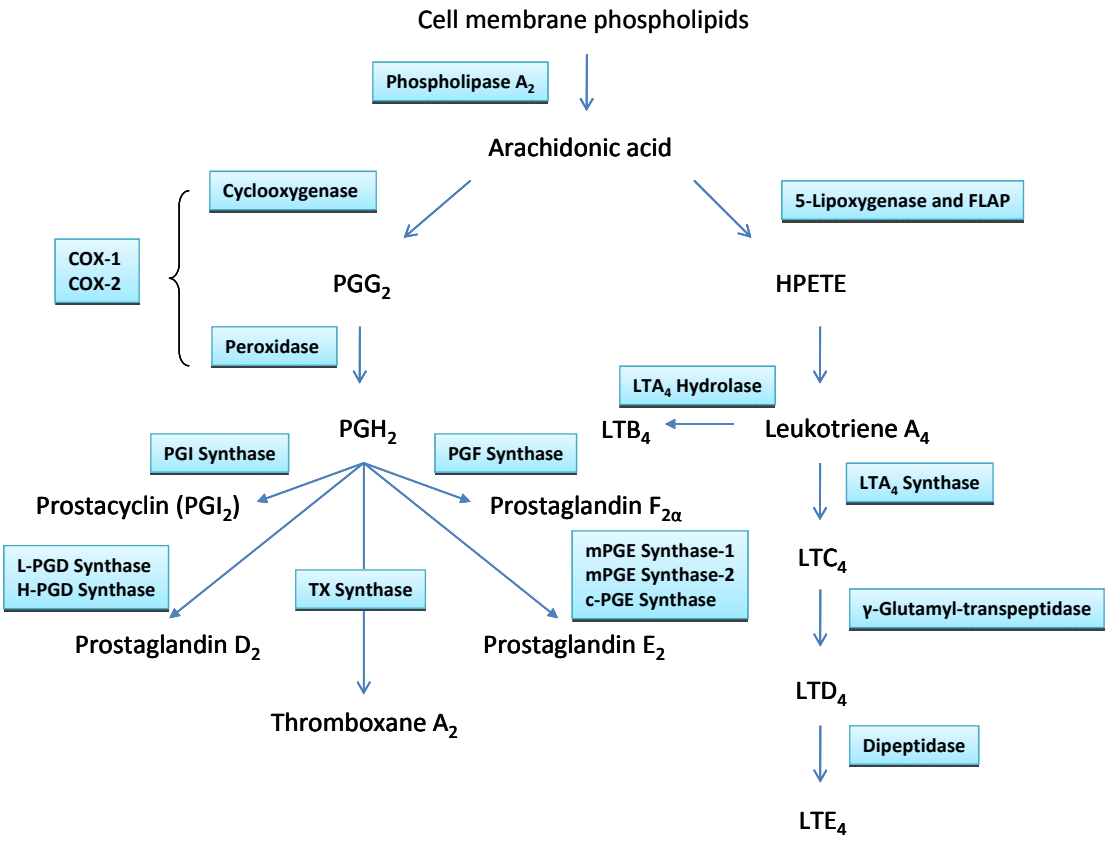


Figure 1. Biosynthesis of the eicosanoids through the COX- and 5-LO pathways.

2.5.2 Prostaglandin E synthase and synthesis of PGE_2

The most abundant prostaglandin in the body is PGE_2, which is formed by PGE synthases (PGES). Three PGES enzymes have been cloned and characterised, cytosolic PGE synthase (cPGES), microsomal PGE synthase 1 and 2 (mPGES-1 and mPGES-2, respectively; 53, 54). They all act downstream in the COX pathway and catalyse the isomerisation of PGE_2 from the unstable cyclic peroxide PGH_2. mPGES-1 is membrane associated (55) and is functionally coupled with COX-2 (55). Colocalisation of the two enzymes at the perinuclear membrane is thought to explain the functional coupling (53). This allows an effective transfer of the unstable PGH_2 between the two enzymes. Both COX-2 and mPGES-1 are upregulated after various inflammatory stimuli (55, 56). In order to understand the specific effect of PGE_2 synthesised via mPGES-1, murine models of genetic deletion of mPGES-1 have been generated. Data in these models supports the notion that this pathway has a key function in several pro-inflammatory conditions such as arthritis, fever and pain (54). However, the contribution made by mPGES-1 generated PGE_2 to airway physiology and AHR is not known.

In contrast, the two other PGES enzymes, mPGES-2 and cPGES, are constitutively expressed in most cells (53) and are thought to function as homeostatic regulators of prostaglandin production (55). It is believed that cPGES is coupled to COX-1, whereas mPGES-2 appears to act upon both COX-1- and COX-2-derived PGH_2 (53, 56). Microsomal PGES-2 is, however, upregulated in human colorectal cancer indicating a functional role in physiological responses other than inflammation (53). It is unclear to what extent cPGES contributes to the physiological production of PGE_2 and the regulation of basal homeostasis (56).

2.5.3 Biological effects of the prostanoids

Prostanoids exert their biological effects via specific prostanoid receptors, seven transmembrane spanning proteins known as G-protein coupled receptors (GPCRs), almost all of which are located in the plasma membrane, but sometimes also in the nuclear envelope. There are nine known prostanoid receptors in humans and several additional splice variants (47, 52). Three of these receptors bind PGD_2 (DP_1, DP_2/CRTH2 and TP), four bind PGE_2 (EP_1-EP_4) and three bind to $PGF_{2\alpha}$, PGI_2 and TXA_2 (FP, IP and TP, respectively; (47; Figure 2). The prostanoid receptors are divided into three clusters with distinct effects based on their coupling to different G_α-subunits. The three clusters include G_s-coupled receptors (IP, DP_1, EP_2 and EP_4) that mediate increases in intracellular cAMP, the G_q-coupled receptors (EP_1 and FP and TP) that mediate increases in intracellular Ca^{2+} and the G_i-coupled receptors (EP_3 and CRTH2) that mediate decreases in intracellular cAMP formation (47).

Prostaglandin D_2 is a pro-inflammatory mediator in human airways mainly released by mast cells during an allergic reaction. PGD_2 has been shown to be increased in BAL fluid from asthmatic patients (52). PGD_2 activates three receptor subtypes. Acting at the DP_1 receptor, PGD_2 mediates a weak relaxation in bronchial and pulmonary vein tissue, whereas binding to the CRTH2 receptor mediates effects such as T-lymphocyte

and eosinophil chemotaxis as well as degranulation (52). It is also known that PGD_2 can cause airway smooth muscle contraction via the TP receptor (57; Figure 2). However, there are species differences of the effects mediated by PGD_2, which in contrast induce a strong and potent airway smooth muscle relaxation in mouse airways (58).

Prostaglandin E_2 is able to mediate different biological actions depending on which receptor subtype is activated. In addition to the pro-inflammatory actions of PGE_2, there may also be beneficial bronchoprotective and anti-inflammatory properties within the airways of asthmatics (59-62). PGE_2 has been proven to inhibit both the early and late allergic response to inhaled allergen challenge. All four EP receptors are expressed in the airways (63), where the EP_2 receptor mediates mainly smooth muscle relaxation and the EP_1 receptor mediates smooth muscle constriction (47, 63, 64; Figure 2).

The role of prostacyclin, (PGI_2) in the airways is less defined, but it is known to be a potent vasodilator. In experimental models, PGI_2 has been associated with an inhibition of eosinophil recruitment and mediator release suggesting an important function in inhibiting the inflammatory response as well as reducing AHR (65, 66). In addition, the IP receptor has been shown to be involved in airway remodelling since mice lacking the receptor have increased airway goblet cell hyperplasia and subepithelial fibrosis (67; Figure 2).

Prostaglandin F_{2a} has been proven to cause ASM contraction in both healthy people and asthmatics (68, 69) via activation of the TP receptor (70). The relevance of the effect of FP receptor activation in human airways is ambiguous, yet PGF_{2a} has been reported to cause ASM contraction in cats and dogs (64), and similar effects have also been reported in the airways of mice (71; Figure 2).

Thromboxane A_2 is rapidly metabolised to the stable inactive metabolite TXB_2 (47). TX acts at the TP receptor and causes effects such as smooth muscle contraction, vasoconstriction and platelet aggregation (47; Figure 2).

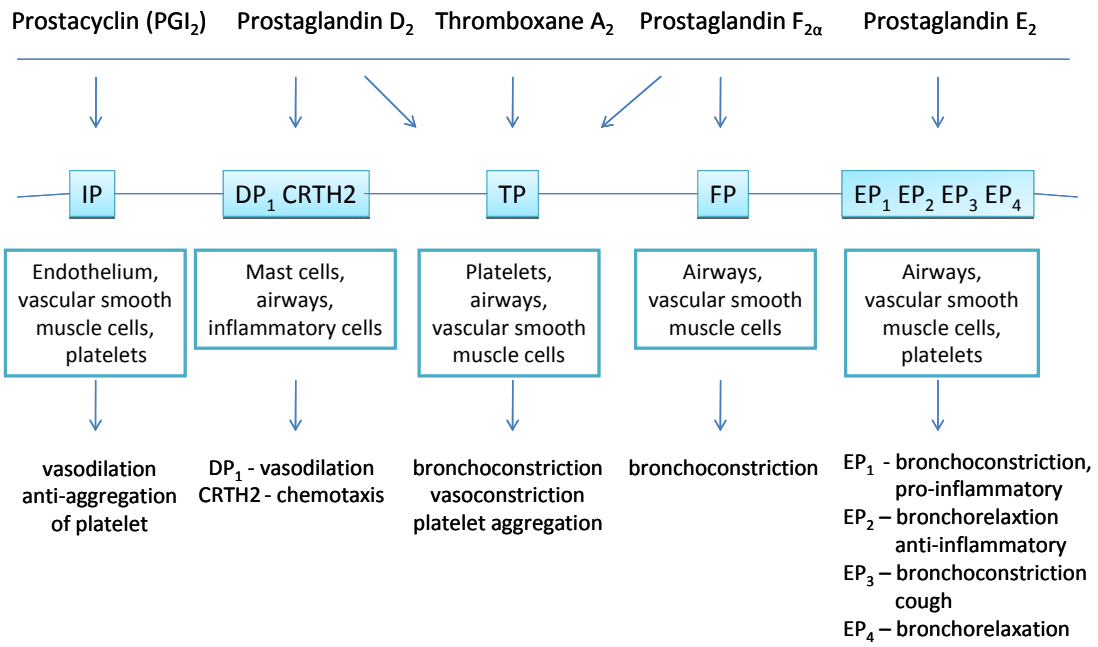


Figure 2. Prostanoid receptors and their potential actions in the airways. (Figure drawn by Daniel Andersson).

2.5.4 Non-steroidal anti-inflammatory drugs

The COX enzymes are important pharmacological targets. NSAIDs are known to inhibit COX enzymes with varying degrees of selectivity towards COX-1 and COX-2 (50). NSAIDs possess analgesic, antipyretic and anti-inflammatory effects. Aspirin was the first NSAID introduced in 1889. Later other NSAIDs have been introduced, namely, indomethacin (1963), diclofenac (1965) and ibuprofen (1969).

The therapeutic actions of the NSAIDs are believed to be due to inhibition of the enzyme that leads to the generation of prostaglandins. COX inhibition is not the only, but the main mechanism of action of the NSAIDs. Aspirin causes irreversible inactivation of the COX enzyme by acetylation of a serine residue in the active site of the COX enzyme (72). Other NSAIDs cause irreversible COX inhibition without covalent modification of the enzyme or are competitive inhibitors of COX (73). However, other effects of NSAIDs such as modulation of signalling through NF-κB has also been suggested (74, 75). NF-κB is located in the cytosol complexed with the inhibitory proteins IκB. One possible explanation for the anti-inflammatory effects of these NSAIDs is that due to their ability to inhibit the kinase that phosphorylates IκB, and thereby prevent degradation of IκB and the activation of NF-κB, that in turn inhibits the upregulation of genes involved in inflammatory responses (76). In addition, it has been shown that indomethacin exerts CRTH2 agonism (77, 78) and thereby promotes accumulation of cells into the airways.

NSAIDs are also associated with severe side effects. Non-selective inhibition of both COX isoenzymes has been related to gastrointestinal damage, platelet dysfunction, and impaired renal function (50, 79). After the discovery of the second inducible COX isoform, COX-2, it was suggested that the anti-inflammatory actions of the NSAIDs was due to inhibition of COX-2, while the unwanted side effects were caused by inhibition of the constitutively expressed COX-1. The inducible COX-2 has been identified as a distinct isoform of COX responsible for the generation of prostaglandins during acute and chronic inflammation (80). This has led to attempts to identify selective inhibitors of COX-2, to avoid the severe side effects caused by ordinary NSAIDs (50). The selective COX-2 inhibitors appeared to have the same anti-inflammatory efficacy as traditional unselective NSAIDs but with less gastrointestinal bleeding (49, 81-84). However, the selective COX-2 inhibitors were found to be associated with unacceptable cardiovascular side effects. The physiological balance between the pro-thrombotic and vasoconstrictory actions of COX-1-derived TXA_2 and the anti-aggregatory and vasorelaxant actions of COX-2-derived PGI_2 are likely to be the major underlying mechanisms involved in the increased cardiovascular risk (6, 50, 85). This has consequently limited their use, although selective COX-2 inhibitors are still valuable drugs as they have shown to be well tolerated in aspirin-intolerant asthmatics (86).

However, over the years it has become clear that the theory of COX-2 inhibition accounting for the generation of pro-inflammatory prostaglandins is more complex than it may seem. It has been demonstrated that COX-2 is constitutively expressed in certain cells (87, 88) and that COX-2 also plays a role in the biosynthesis of prostaglandins under physiological conditions. A third isofom of COX (COX-3) has also been proposed (89), the relevance and physiological functions of which are unclear.

To study the role of COX-activity in the present thesis, COX inhibitors with different selectivity towards the two COX isoenzymes were used (Table 1).

	IC_{50} towards the COX isoenzymes		
	COX-1 $\mu g \cdot mL^{-1}$	COX-2 $\mu g \cdot mL^{-1}$	COX-1/COX-2 ratio
Diclofenac	0.5 (1.6 µM)	0.35 (1.1 µM)	1.43
Indomethacin	0.01 (0.03 µM)	0.6 (1.7 µM)	0.02
FR122047	0.028 (0.06 µM)	65 (135 µM)	$0.43 \cdot 10^{-3}$
Lumiracoxib	67 (228 µM)	0.13 (0.44 µM)	515.38

Table 1. The COX inhibitors and their selectivity towards COX-1 and COX-2 in murine macrophages (diclofenac and indomethacin) and from human recombinant or whole blood assays (FR122047 and lumiracoxib; 90-92).

2.5.5 5-lipoxygenase pathway of arachidonic acid metabolism

Leukotrienes (LTs) are potent mediators of inflammation and bronchoconstriction in asthma but also participate in the modulation of homeostasis. The LTs are synthesised from arachidonic acid through the 5-lipoxygenase (5-LO) pathway, a process that occurs predominantly in inflammatory cells of myeloid origin such as mast cells, macrophages, and leukocytes (93, 94). The 5-LO enzyme is located in the cytosol of certain cells (peritoneal macrophages, monocytes, neutrophils and eosinophils), whereas in mast cells and alveolar macrophages 5-LO is localised both in the cytosol as well as in the nuclear envelope (94). Upon Ca^{2+}-dependent activation, 5-LO is translocated to the nuclear envelop and transformation of arachidonic acid to LTA_4 is initiated by activation of 5-LO-activation protein (FLAP). LTA_4 in turn is converted to LTB_4 by LTA_4 hydrolase, or can be converted to LTC_4 by LTC_4 synthase. LTB_4 and LTC_4 are transported from the cell by different transport proteins. Extracellularly, the released LTC_4 is converted into LTD_4, which in turn undergoes conversion to LTE_4 (95). Furthermore, LTC_4, LTD_4 and LTE_4 are together referred to as cysteinyl leukotrienes (CysLTs; Figure 1).

2.5.6 Biological effects of the leukotrienes

Leukotrienes bind to specific GPCR receptors on the outer plasma membrane of structural and inflammatory cells (95). LTB_4 mediates most, if not all, of its chemoattractant and pro-inflammatory actions through the high affinity receptor, BLT_1. However, LTB_4 can also act via the lower-affinity receptor, BLT_2, but the physiological functions of this receptor are less well known (95). The CysLTs can mediate sustained bronchoconstriction, mucus production and cause oedema in the airways through the cysteinyl leukotriene receptor 1 ($CysLT_1$). Less is known about the effects of leukotrienes acting at the $CysLT_2$ receptor. However, murine studies have revealed that $CysLT_2$ is involved in inflammation, vascular permeability and tissue fibrosis through this receptor (95).

To inhibit the action of CysLTs in asthma an anti-leukotriene therapy has been evaluated and studies in children and adults with asthma have shown improved pulmonary function, fewer exacerbations and increased quality of life. However, the response to anti-leukotriene therapy varies greatly amongst patients with asthma and the discordance in symptom improvement might be due to differences among patients regarding their CysLT biology, genotype or clinical characteristics (95).

2.6 CYTOKINES, CHEMOKINES AND GROWTH FACTORS IN INFLAMMATION

Cytokines

Cytokines are a group of low-molecular-weight regulatory proteins that serve as messengers in the immune system. Cytokines generally act locally and bind to specific high affinity receptors on the membranes of target cells, triggering alterations in gene expressions that evoke specific biological activities. Cytokines can be produced by

almost all cell types and may activate an entire network of interacting cells (27). In the present work, a panel of cytokines were measured that are important mediators of allergic airway inflammation. Table 2 summarises the source, receptors and actions of the cytokines that have been investigated in this Thesis (adapted from ref nr 96).

Cytokines	Source	Receptor	Action
IL-1α	Mo, Epi	IL-1R1, IL-1R2	T-cell and Mo activation (type-1, mediates inflammatory effects, type-2, suppressor of this activity)
IL-1β	Mo, Epi	IL-1R1, IL-1R2	T-cell and Mo activation (type-1, mediates inflammatory effects, type-2 suppressor of this activity)
IL-2	T_H1, Eos	IL-2R	T-cell growth factor, differentiation and survival (lymphocytes)
IL-4	T_H2, Eos, MC, Bas	IL-4R	B-cell switch to IgE synthesis, MC development, Eos and Bas activation and recruitment, AHR, mucus secretion, favours T_H2 production
IL-5	T_H2, Eos, MC, Bas	IL-5Rα	Eos, Bas differentiation, maturation and activation
IL-6	T_H1/T_H2, Mo, Endo	IL-6R, sIL-6R	T- and B-cell growth factor, cofactor for IgE synthesis, inhibitory effects on TNF-α and IL-1
IL-10	T reg T (T_H1), Mo	IL-10Rα	Suppresses T_H1/T_H2 function, activation of Eos, MC, Bas, favours Treg production
IL-12	Mo, B	IL-12R (IL-12Rβ1, IL-12Rβ2)	Favours T_H1 production, inhibits IgE synthesis, IL-12Rβ2 is considered to play a key role in IL-12 function
IL-13	T_H2, Eos, MC, Bas	IL-4Rα, IL-13R	MC develop, B-cell switch to IgE production, eosinophilia, AHR, mucus production
IL-17	T memory cells, T_H17	IL-17R, IL-17RA, IL-17RB, IL-17RC	Induces cytokine production by Fib, Mo, Epi, Endo, provokes neutrophilia. IL-17R is a heteromeric complex consisting of at least IL-17RA and IL-17RC but there are existing variants including IL-17RB
IFN-γ,	T_H1, T_H2, NK	IFNGR1, IFNGR2	Inhibits IgE synthesis, inhibits T_H2 induction, activates Eos, Mo
TNF-α	Mo, NK, T	TNFR1, TNFR2	Activation of Epi, Endo, involved in systemic inflammation and stimulates acute phase reaction. The receptor type 1 is expressed in most tissue and type 2 in cells in the immune system
GM-CSF	T_H1/T_H2, Mo, Eos, MC, Bas, Fib, Epi, Endo	GM-CSFR	Differentiation and activation of Eos, Neut, Bas, MC, Mo

Table 2. Bas, basophil; B, B-cell; DC, dendritic cell; Endo, endothelial cell; Epi, epithelial cell; Eos, eosinophil; Fib, fibroblast; L, lymphocyte; MC, mast cell; Mo, monocyte/macrophage; Neut, neutrophil; NK, natural killer cell; T, T-cell, T_H, T helper cell; Treg, regulatory T-cell.

Chemokines

The primary function of chemokines is to direct cellular trafficking along a concentration gradient (chemotaxis), either to sites of lymphoid activation for production of cellular immunity (antibody secretion) or to direct cell migration. Chemokines can also activate many cells, such as T-cells, monocytes, eosinophils and basophils, to secret a wide variety of mediators, for example leukotrienes, histamine, cytokines and chemokines (27). In the present work, a panel of chemokines were measured that are important for cell activation, degranulation and chemotaxis, and which are of particular importance in allergic airway inflammation. Table 3 summarises the source, responding cells and actions of the chemokines that have been investigated in this Thesis (adapted from ref nr 97).

Chemokines	Former designation	Source	Receptor	Responding cell
CCL2	MCP-1	Mo, L, Fib, Endo, Epi, Neut, MC, DC	CCR2	Mo, DC, T, Neut
CCL3	MIP-1α	Mo, L, Neut, Eos, Fib, MC	CCR1, CCR5	Mo, DC, Eos, T, NK
CCL5	RANTES	Mo, T, Fib, MC	CCR5, CCR1, CCR3	Mo, Eos, T, MC, NK, DC
CCL11	EOTAXIN	Epi, Endo, Eos, MC	CCR3, CCR5	Eos, T, MC
CXCL1	KC/GRO-α	Mo, Neut, Endo, Fib, MC	CXCR2	Neut, Mo
CXCL9	MIG	Mo, Neut	CXCR3	T, MC
CXCL10	IP-10	Mo, Neut, Fib, Endo	CXCR3	T, MC

Table 3. DC, dendritic cell; Endo, endothelial cell; Epi, epithelial cell; Eos, eosinophil; Fib, fibroblast; L, lymphocyte; MC, mast cell; Mo, monocyte/macrophage; Neut, neutrophil; KK, natural killer cell; T, T-cell.

Growth factors

Growth factors are hormone-like proteins capable of regulating a variety of cellular processes, such as cell growth, proliferation and cellular differentiation (98). Growth factors usually act as signalling molecules between cells and bind to specific receptors on the surface on their target cells. In this Thesis two different growth factors, fibroblast growth factor 2 (FGF2; previously basic FGF) and vascular endothelial growth factor (VEGF) have been measured.

FGF proteins are one of the largest families of growth factors and their biology is unique because they often function together as intracellular signalling molecules mediating communication between adjacent tissues. FGF is named after its capacity to stimulate the proliferation of fibroblasts. FGF2 is the most studied member of the FGF family although its role in the pathogenesis of asthma is not yet known. It has been demonstrated that FGF2 is overexpressed in asthmatics and is believed to induce ASM cell hyperplasia in asthmatic airways (99).

VEGF is a central growth factor for endothelial cells and it also has an extensive impact on endothelial cell function, such as triggering of mitosis, survival and differentiation. VEGF has been suggested to play a role in chronic inflammation and remodelling, causing hypervascularity of the airways, mucosal oedema and stimulating the recruitment of eosinophils. However, further studies are required to establish a function for VEGF in asthma and inflammatory disease (100).

2.7 MOUSE MODELS AS A TOOL IN ALLERGIC AIRWAY DISEASES

Since asthma is a complex disease, there is a need for *in vivo* models to study features relevant to human asthma (101, 102). The mouse is currently the most widely used species in asthma research due to the availability of transgenic animals and because of the wide range of tools available for the analysis of cell and mediator responses (101, 102).

There are, however, limitations with the use of murine models in asthma research. Laboratory rodents do not spontaneously develop characteristics that can be considered to represent human asthma, and the allergic airway response has to be induced (101). A typical sensitisation protocol of airway inflammation generally involves two main phases. First, the animals are sensitised to produce antibodies towards a foreign antigen, most often ovalbumin (OVA). Intraperitoneal (i.p.) injections with antigen and adjuvant once a week for two weeks, is the most simple and effective method of inducing antigen sensitisation in mice (103-105). The animals are thereafter repeatedly challenged with inhalation of the same antigen to induce allergic airway inflammation. This challenge induces an inflammatory process through an allergic reaction that leads to increased numbers of inflammatory cells in the lungs or BAL fluid (106). Several experimental protocols have been developed for the induction of allergic responses in mice to closely resemble the clinical pathology of human asthma (105). They all include different allergens in varying doses, administration routes and time points, with sensitisation carried out with or without adjuvant. Animal models of airway inflammation can offer valuable information and are useful for understanding the pathophysiological mechanisms of human asthma and for studying potential new drug therapies (101). However, the interpretation of results obtained from animal models and extrapolation of the results to human asthmatics is dependent on the outcome studied and the species used (101).

It is known that there is strain-specific phenotype variation of lung function parameters among common inbred mouse strains. This significant variability may be attributed to differences in genetic background. Hence, the genetic background of the mice used is important when inducing inflammatory disease. Different strains might therefore be more suitable for modelling particular asthma characteristics than others, i.e. AHR and airway inflammation (101, 107), and this needs to be considered when trying to extrapolate findings to human asthma.

Histamine and leukotrienes are known to be important inflammatory mediators in human asthma. In contrast, mice do not respond, or respond only weakly to these mediators. However, the actions of prostaglandins in the airways of mice are reasonably similar to known effects of the prostaglandins in the human lung. Thus, the use of mouse models for studying prostaglandin-mediated mechanisms can be of relevance to human disease.

3 AIMS OF THE THESIS

The overall aim of this Thesis was to understand the role of COX products on AHR and airway inflammation in mouse models of allergic asthma. In addition, a secondary aim was to develop improved models for experimental studies of AHR and airway inflammation in sensitised and challenged mice.

Specific aims:

- The primary objective of paper I was to optimise the delivery route and doses of allergen for antigen challenge in mice with respect to the induction of AHR and airway inflammation, but also to compare two commonly used strains of mice (BALB/c and C57BL/6).

- The aim of paper II was to study the effects of NSAIDs on AHR and airway inflammation induced by OVA challenge in allergic mouse models of asthma, and to define time points of action of COX inhibition on AHR and airway inflammation.

- The aim of paper III was to study the effects of NSAIDs on AHR and airway inflammation induced by OVA challenge in an adjuvant-free allergic mouse model of asthma.

- The aim of paper IV was to define the role of PGE_2 synthesised via mPGES-1 in a model of chronic allergic airway inflammation using genetically modified mice where mPGES-1 has been disrupted.

4 MATERIAL AND METHODS

Detailed description of the material and methods used in the present studies can be found in the publications and the manuscript included in this Thesis. The following sections provide an overview of methods used.

4.1 ANIMALS

Female BALB/c (papers I-III) and C57BL/6 (paper I) mice (8-13 weeks of age) were purchased from Charles River (Sulzfeld, Germany). For paper IV, mPGES-1 gene-disrupted female and male mice (*ptges*$^{-/-}$ mice) and C57BL/6 wild-type littermates (6 weeks of age) were kindly provided by AstraZeneca (Mölndal, Sweden). The animals were housed in plastic cages with absorbent bedding material and were maintained on a 12 h daylight cycle. Food and water were provided *ad libitum*. All animal experiments were approved by the regional committee of animal experimentation ethics (Dnr: 168/03, 201/04, 348/05, 152/06, 343/06; Stockholm North ethical committee for animal welfare, Stockholm, Sweden).

4.1.1 Targeted disruption of the mPGES-1 (*ptges*) gene

Generation of mPGES-1 null and conditional mice

In order to build up a mPGES-1 gene targeting vector, 4.0 kb, 0.9 kb and 1.8 kb genomic DNA fragments, for 5' homology, deletion and 3' homology regions respectively, were prepared by means of bacteria homologous recombination from a BAC clone which contains about 150 kb of 129/Sv genomic sequence including the mPGES-1 gene (ResGen, Invitrogen Corporation). The fragments were cloned into a modified loxP floxed PGKneo plasmid applying a standard cloning technique. To generate the mPGES-1 targeting construct, a 0.9 kb fragment containing exon 2 of mPGES-1 was floxed by an isolated loxP site and loxP-flanked neo cassette (Figure 3A). The targeting vector was linearized and electroporated into R1 mouse embryonic stem (ES) cells and ES cell clones were selected using G418 (300 μg/ml). The candidate ES cell clones underwent homologous recombination, were then screened by PCR and the result was confirmed by Southern blotting (Figure 3B). The targeted ES cells were injected into C57BL/6 blastocysts and the injected embryos were implanted into the uterus of pseudopregnant B6CBA female mice. Chimeric animals were mated with C57BL/6 mice to produce agouti heterozygous animals (F1). For reproduction of mPGES-1 null mice, the heterozygous F1 males were bred with Rosa26Cre transgenic mice that had a C57BL/6 background to delete the loxP floxed region including exon 2 and the neo cassette as well. The mPGES-1 null mice were obtained by intercrossing. Offspring were genotyped by PCR using primers 822, 823 and 824 that detected the wild type allele (0.279 kb fragment) and null allele (0.470 kb fragment), respectively (822, forward: 5'- AAGGTTGATGGTGCACGTCTA-3'; 823, reverse: 5'-TTTGTTTGGCCTCACTCTACA-3'; 824, forward: 5'-CTCAGGCAGGGCTCATCTACC-3'). For generating mPGES-1 conditional (floxed) mice, the heterozygous F1 mice were bred with EIIaCre transgenic mice that had a C57BL/6 background in order to delete the loxP flanked PGKneo cassette.

The genotyping for conditional mice was achieved by PCR using primers 822 and 823 to identify the wild-type allele (0.279 kb fragment) and floxed allele (0.399 kb fragment), respectively.

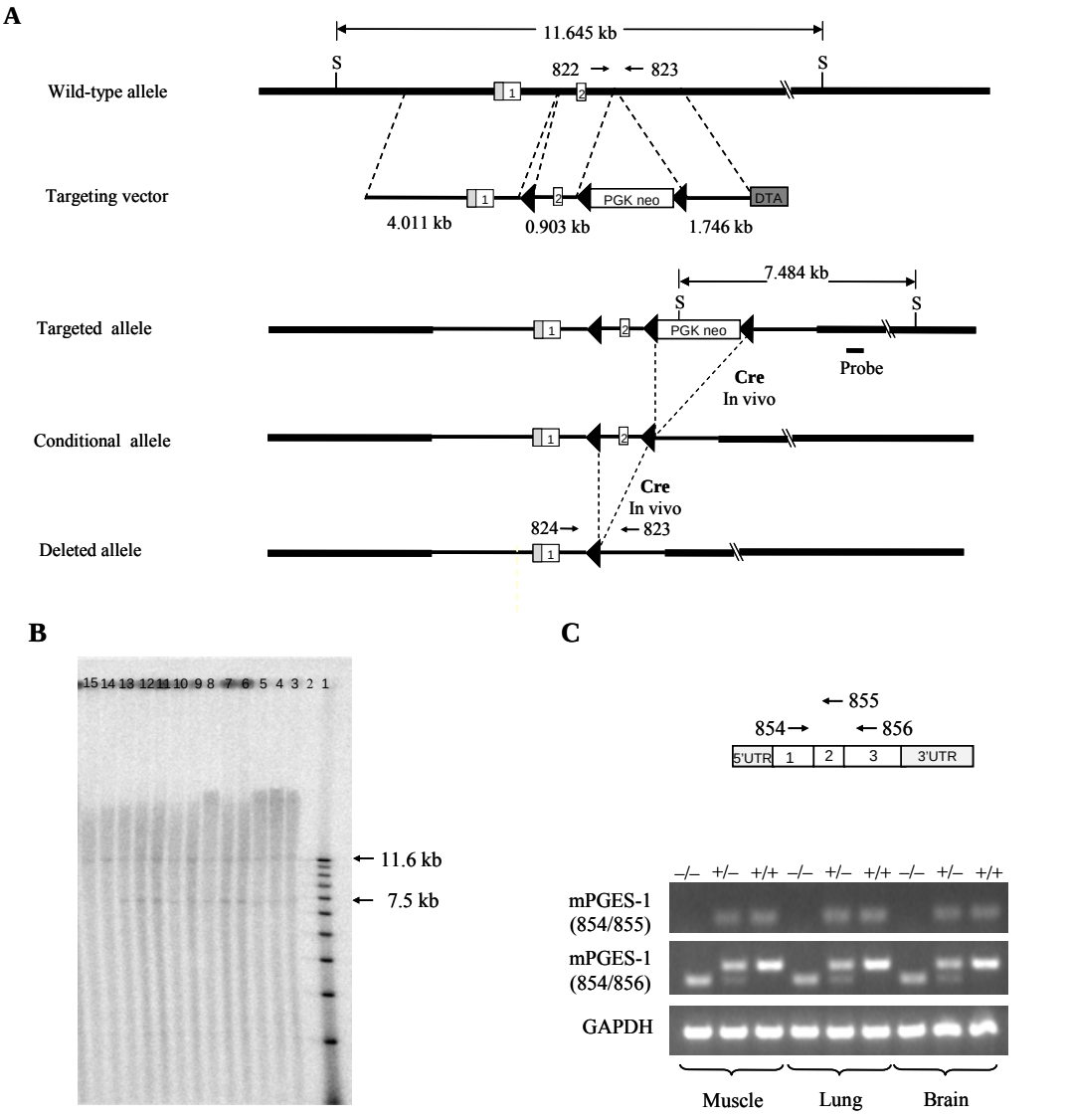


Figure 3. Targeted disruption of the mPGES-1 gene. A, mPGES-1 gene targeting strategy. Native 5' region of mPGES-1 gene and targeting vector (top), targeted and conditional allele (middle) and the disrupted mPGES-1 gene (bottom) were shown. A region containing exon 2 was deleted. The open rectangles with number indicate the exons. The filled rectangle shows untranslated region (UTR). Horizontal bars indicate probe used for Southern blotting. DTA, diphtheria toxin. A fragment gene cassette. S, SpeI. Triangle indicates loxP site. Arrow indicates the oligos used for genotyping. B; Southern blot analysis of the targeted ES clones. Genomic DNA was digested with SpeI and probed with a 3' flanking probe shown in (A). Expected sizes of DNA fragments of the wild-type and mutant alleles are indicated in (A). Lane 1 and 2, marker; Lane 3-14, targeted clones. Lane 15, wild type ES clone. C; mPGES-1 transcript (top) and RT-PCR analysis (bottom). Tissues from littermates of *ptges*$^{+/+}$ *ptges*$^{+/-}$ and *ptges*$^{-/-}$. Arrows indicate the oligos used for RT-PCR. (Figure kindly provided by Xiufeng Xu at AstraZeneca).

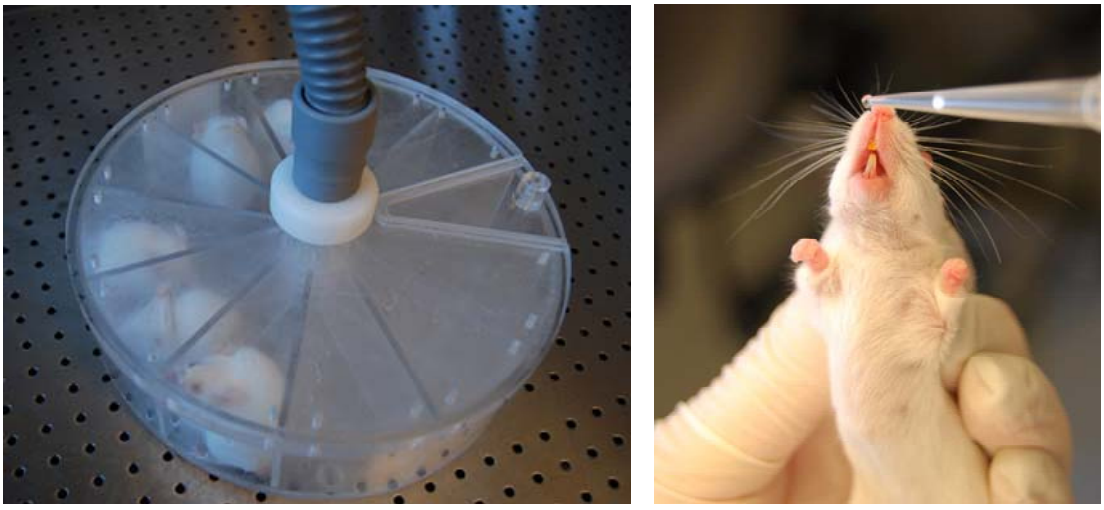
RT-PCR analysis

The brain, lung and muscle were dissected from 8-week littermates of mPGES-1 null, heterozygous and wild-type mice, respectively. Total RNA was extracted with a standard method. Reverse transcription was run with SuperScript First-Strand (Invitrogen) followed by PCR using primers located in a deleted exon and up- and down-stream intact exons (855, reverse: 5'- CGCTCCACATCTGGGTCACTC-3'; 854, forware: 5'- CCAGGTGCTCCCGGCCTTTCT-3' and 856, reverse: 5'- GTAGGCCACGGTGTGTACCAC-3' (Figure 3C).

4.2 ALLERGEN PROVOCATIONS

Sensitisation and challenge of animals

In the present studies, three different protocols for OVA-induced airway inflammation have been used with respect to the routes of delivery, dose and time intervals for the sensitisation and challenge phases. The protocols range from an acute 18-day protocol to a chronic 91-day protocol. Mice were sensitised by i.p. injection of 10 µg OVA (grade II, Sigma-Aldrich, St. Louis, MO, USA) with or without 1 mg $Al(OH)_3$ (Sigma-Aldrich) in a total volume of 200 µL phosphate-buffered saline (PBS; Sigma-Aldrich). The allergic airway inflammation was induced by challenge (Figure 4), with either aerosolised OVA (1 or 6%) for 30 min, or with different doses of OVA (20, 50, 100 and 200 µg) given intranasally following light anaesthesia (3.5% isoflurane, Forene, Abbot Scandinavia, Solna, Sweden). Control mice were immunised by i.p. injection of 10 µg OVA with or without 1 mg $Al(OH)_3$ and challenged with PBS.



A; Aerosol challenge B; Intranasal challenge

Figure 4. Mice challenged with OVA either as an aerosol (A) delivered with a nebulizer (UltraNeb® De Vilbiss) or intranasally (B) following light anaesthesia (3.5% isoflurane). (Photos taken by Jesper Säfholm).

Acute airway inflammatory protocol (18 days)

Mice were sensitised by i.p. injections of 10 μg OVA together with 1 mg $Al(OH)_3$ in PBS on days 1 and 8. The allergic airway inflammation was induced on days 15, 16 and 17 by challenge with OVA either as an aerosol or intranasally (Figure 5A). The outcome was assessed 24 or 48 h after the last challenge (Papers I-II).

Intermediate adjuvant-free airway inflammatory protocol (47 days)

Mice were sensitised by i.p. injections of 10 μg OVA in PBS on days 1, 3, 5, 7, 9, 11 and 13. The allergic airway inflammation was induced by intranasal challenge of 100 μg OVA in a total volume of 20 μL PBS on day 40, 43 and 46 (Figure 5B). The outcome was assessed 24 h after the last challenge (Paper III).

Chronic airway inflammatory protocol (91 days)

Mice were sensitised by a i.p. injection of 10 μg OVA together with 1 mg $Al(OH)_3$ in PBS on day 1 followed by both i.p. injection and intranasal instillation on day 11. The allergic airway inflammation was induced with 50 μg OVA given intranasally on two constitutively days every second week for 12 weeks (Figure 5C). The outcome was assessed 24 h after the last challenge (Paper IV).

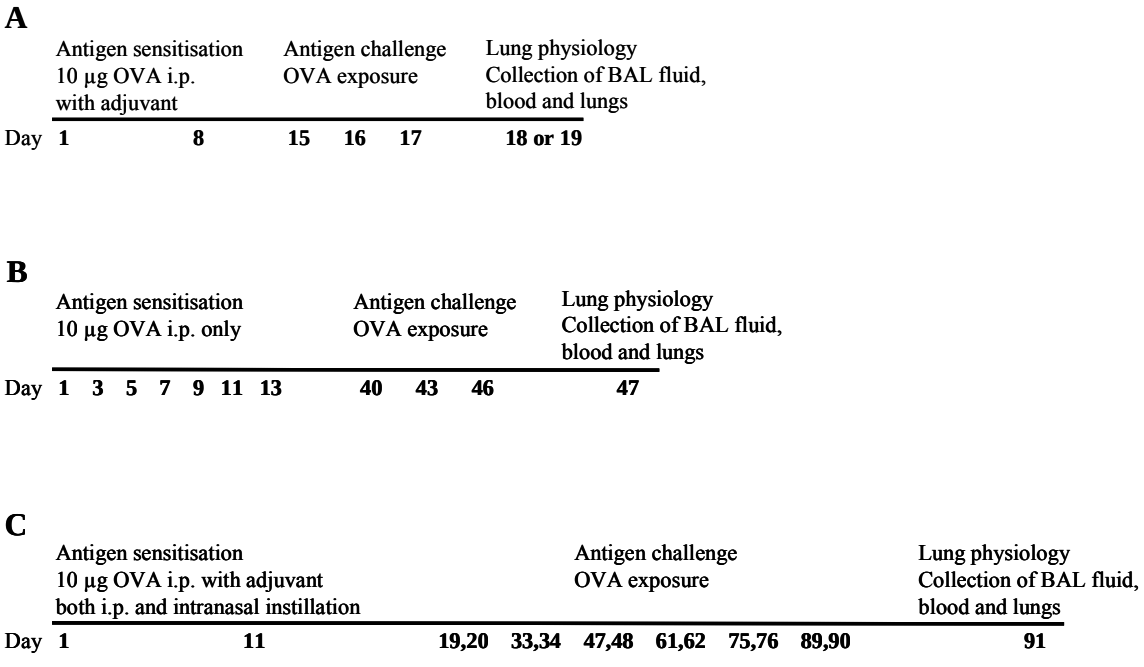


Figure 5. The three allergic airway inflammatory protocols used in the thesis; (A) the acute, (B) the adjuvant-free intermediate and (C) the chronic protocols.

4.3 TREATMENT

Intervention with COX inhibitors (papers II-III)

Animals were treated with injections of either diclofenac sodium (1 $mg \cdot kg^{-1}$ body weight; non selective COX inhibitor; Cayman Chemicals, Ann Arbor, Michigan, USA), indomethacin (4 $mg \cdot kg^{-1}$ body weight; non selective COX inhibitor; Sigma-Aldrich), FR122047 (5 $mg \cdot kg^{-1}$ body weight; selective COX-1 inhibitor; Cayman Chemicals), lumiracoxib (1 $mg \cdot kg^{-1}$ body weight; selective COX-2 inhibitor; SynphaBase, Muttenz, Switzerland) or solvent control i.p. 1 h before each OVA challenge, 1 h before the MCh challenge and as an intravenous injection (i.v.) at the start of the anesthesia. The doses of the COX inhibitors have previously been described to be both effective and selective (90-92). The doses of diclofenac and indomethacin used were selected from the IC_{50} described in mice (90) and the doses of FR122047 and lumiracoxib used were selected from *in vitro* studies in mice and rats (Table 1; 91, 92).

4.4 *IN VIVO* LUNG FUNCTION MEASUREMENTS

4.4.1 Lung mechanics

In this Thesis direct measurements of the respiratory system mechanics have been assessed using the flexiVent® system (Scireq, Montreal, PQ, Canada) and evaluated assuming two different models, a single compartment model (overall lung resistance and compliance) and a constant phase model (forced oscillation, separation of central and peripheral airways).

Single compartment model

A classic approach to assess lung mechanics in animals is the measurement of dynamic resistance (R_L) and compliance (C_L) assuming the single compartment model (108). This is the simplest model used for modelling pulmonary mechanics and is often described as a single homogenously ventilated tube connected to an elastic compartment (20, 108-110). R_L reflects both narrowing of the conducting airways and alterations in the lung periphery (108). Decreases in C_L reflect mainly events in the lung periphery, in particular, airway closure and lung unit derecruitment (108). This simple model is suitable if the lungs behave in a linear fashion. However, heterogeneous ventilation can occur, i.e. different distributions of ventilation throughout the lung (109). Consequently, the contribution of airway and tissue resistance is highly dependent on breathing frequency as well as lung volume, and measuring these parameters at a given frequency may not provide the most suitable information about the lung mechanics (111, 112). This insight led to the development of a more complex model, the constant phase model, and the use of the forced oscillation technique (FOT) for measuring lung function.

Constant phase model

FOT is applied to anaesthetised and tracheostomised animals to measure the impedance (Zrs, Figure 6) of the lungs. Measurement of the pressure and volume displacement inside the cylinder can be used to calculate the Zrs (111). The respiratory mechanics are calculated with a computer program, fitting a more complex model of the lung, the constant phase model, which allows partitioning of the lung mechanics into central and peripheral events in the lung (108-110, 112). During the forced oscillation, the ventilator piston interrupts the mechanical ventilation and oscillates (ranging from 0.25-20.5 Hz) the airflow into the airway for 2-16 seconds depending on the perturbation. The obtained parameters (Figure 6) are the Newtonian resistance (R_N), a close approximation of resistance in the central airways, tissue damping (G) reflecting energy dissipation in the lung tissue, tissue elastance (H) characterising tissue stiffness (reflects energy storage in the tissue) and inertance (I) representing the inertive properties of the gases in the airways (108-110, 113). However, the inertance in mouse airways is so small, it can be considered insignificant, although this is not the case in humans.

$$\mathrm{Zrs}(f) = \mathrm{R_N} + i\,2\pi f\,\mathrm{I} + \frac{\mathrm{G} - i\,\mathrm{H}}{(2\pi f)^{\alpha}} \qquad \alpha = \frac{2}{\pi}\tan^{-1}\frac{\mathrm{H}}{\mathrm{G}}$$

Zrs	Respiratory system input impedance
f	Frequency
R_N	Newtonian resistance
i	Positive square root of -1
I	Inertance
G	Tissue damping, related to tissue resistance, reflects energy dissipation in the tissue
H	Tissue elastance, reflecting energy storage in the tissue
α	Parameter that links G and H

Figure 6. Zrs was determined from each frequency contained in the flow signal applied by the flexiVent® and analysed using the equation above (the constant-phase model).

Preparation of animals for measurement of lung mechanics

Before the assessment of lung mechanics, mice were anaesthetised with pentobarbital sodium i.p. (70-90 mg·kg^{-1} body weight; Apoteket Produktion & Laboratorier AB, Stockholm, Sweden), to achieve an optimal sedation and placed on a 37 °C-heating pad to maintain body temperature during the anaesthesia. Mice were tracheostomised with a metal 18-gauge cannula and mechanically ventilated in a quasi-sinusoidal fashion (114) using the flexiVent® system at a sinusoidal frequency of 2.5 Hz and a tidal volume of 12 mL·kg^{-1} body weight. Once the ventilation was started, bilateral thoracotomies were performed to equalise the pleural pressure to atmospheric pressure and to exclude any chest wall contribution to pulmonary mechanics. The positive end-expiratory pressure

was set to 3 cmH_2O. To stabilise respiratory lung baseline readings and ensure similar volume history, four sigh manoeuvres at three times the tidal volume were performed at the beginning of the experiment defined as incremental increases and decreases of lung volume during a period of 16 seconds (papers I-III), or four total capacity measurements during a 6 seconds period were performed (paper IV). The animals were allowed a five-minute resting period before the measurements of lung mechanics began.

Measurement of airway responsiveness

The small animal ventilator has a piston pump that ventilates the animals mechanically. The computer controls the movement of the piston and the timing of the inspiratory and expiratory valves. During a perturbation the piston interrupts and sends a predetermined volume perturbation into the airways.

Airway responsiveness was used as a measurement of AHR to MCh (acetyl-β-methylcholine chloride; Sigma-Aldrich) according two different approaches. In papers I-III, R_L and C_L were measured continuously using a standardised script and the piston delivered the predetermined volume perturbation every eighth breath for approximately 3 min (50 measurements). Changes in reactivity and sensitivity were assessed using non-linear regression analysis to calculate the maximum responses to each MCh dose and the effective dose for half maximal response (ED_{50}). C_L is expressed as the maximal decrease to each MCh dose.

In papers I and IV, the airway responsiveness was assessed by the use of FOT. During a forced oscillation manoeuvre the ventilator piston interrupts the mechanical ventilation and delivers 13 superimposed sinusoidal frequencies, ranging from 1 to 20.5 Hz, as an input signal during a period of 3 seconds (referred to Quick Prime-3; QP-3). The maximum response of each MCh dose for R_N, G and H was assessed by altered measurements of R_L/C_L and QP-3 in similar standardised scripts as described previously for approximately 3 min (20 measurements of each perturbation). Since the animals are prepared in an open chest system, R_N gives a good approximation of the resistance of the conducting airways, while G and H characterise the peripheral airways and lung tissue.

To assess airway responsiveness, the animals were given injections of increasing doses (0.03, 0.1, 0.3, 1 and 3 $mg \cdot kg^{-1}$ body weight; in paper IV up to 10 $mg \cdot kg^{-1}$ body weight) of MCh through the lateral tail-vein at four-minute intervals. Heparin (10 $U \cdot mL^{-1}$PBS) was added to prevent clotting of the i.v. catheters. As a dose of 10 $mg \cdot kg^{-1}$ body weight of MCh sometimes causes such bradycardia that it results in cardiac arrest with no further increases in AHR, the maximal resistance was reached at 3 $mg \cdot kg^{-1}$ or, in some experiments, even earlier due to the same phenomenon.

4.5 SAMPLE COLLECTION, PROCESSING AND ANALYSIS

Bronchoalveolar lavage fluid

Directly after AHR measurements, collection of BAL fluid was performed to monitor inflammatory processes of the airway lumen. A total volume of 1 mL PBS containing 0.6 mM EDTA was used to lavage the lungs three times. When required, red blood cells were removed by resuspending the BAL fluid cells in 100 µL lysis buffer for 2 min at room temperature (RT) followed by washing in 1 mL PBS. The total number of cells was then counted and adjusted back to cells·mL^{-1} BAL fluid. For differential cell counts, a minimum of 300 cells were counted per BAL fluid sample.

IgE and IgG1 analysis

Following collection of BAL fluid, blood was sampled by cardiac puncture and sera were stored at -20 °C for measurement of antibody titres using enzyme-linked immunosorbent assay (ELISA) as described previously (115). Plates were coated with 5 µg·mL^{-1} OVA or 2 µg rat anti-mouse IgE (BD Biosciences, San Diego, CA, USA). Values were expressed in units of optical density (OD).

Lung histology and morphometry

Following the collection of blood, lungs were fixed by inflation with 4% formalin (Sigma-Aldrich) under a pressure of 20 cmH_2O for 24 h. Lung histology and morphometry were performed as previously described (116-121). The left lobe was separated transversely into two segments, just below the first-generation of airways, and the upper segment was embedded with the trimmed side down (116). Three µm thick lung sections were cut and assessed using the following stains: hematoxylin (Sigma-Aldrich) and eosin (Sigma-Aldrich) to quantify tissue eosinophils, α-smooth muscle actin (α-SMA, DAKO, Glostrup, Denmark) to evaluate changes in smooth muscle thickness, picro-sirius red stain (PSR, Sigma-Aldrich) to assess the presence of collagen, periodic acid-Schiff (PAS, Sigma-Aldrich) to assess mucin-containing goblet cells and toluidine blue (Sigma-Aldrich) to quantify mast cells. Morphometric analyses of the stained sections were performed using a digital image analysis system (Northern Eclipse, Version 7.0; Empix Imaging Inc., Mississauga, Ontario, Canada).

Immunohistochemical staining for COX-1 and COX-2

Initially, the paraffin-embedded lung sections were hydrated to tap water. Slides were then treated with proteinase K for 8 min to digest tissue samples prior to pre-treatment with 3% hydrogen peroxide for 5 min to inhibit endogenous peroxidases and protein block (Dako, X0909) for 10 min to prevent nonspecific reactivity. The sections were then incubated with COX-1 (1:400, RT for 1 h) or COX-2 (1:100, 4 °C over night) primary antibody (Cayman Chemicals). Following incubation with COX antibodies,

sections were treated with secondary antibody (1:100, AbCam, 6720) for 2 h at RT. After washing, sections were incubated with streptavidin peroxidase for 45 min. Colour was developed by adding AEC chromogen for 20 min (Sigma-Aldrich). The slides were counterstained with hematoxylin and mounted with faramount (DAKO). Negative control sections were treated in exactly the same way but primary antibodies were omitted.

Measurements of eicosanoid levels in BAL fluid

To define whether the two COX isoenzymes are responsible for the formation of different eicosanoids, the levels of PGD_2, 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2), PGE_2, TXB_2 (a stable metabolite of TXA_2) and CysLTs (measured as LTE_4, the end metabolite of LTC_4 and LTD_4) were measured in BAL fluid by Enzyme Immunoassay (EIA; Cayman Chemicals). The assay is based on competition between free eicosanoid in the sample and an added eicosanoid-acetylcholinesterace (AchE)-linked tracer. The complexes formed, subsequently bind to an antibody that has previously been attached to each well on a 96-well plate. After washing, Ellerman's reagent containing acetylthiocholine, a substrate for AchE was added. This reaction yields a coloured product that can be measured spectrophotometrically at 412 nm. The assay detection limits for the different mediators were 3.9 $pg{\cdot}mL^{-1}$ for PGD_2 and 6-keto-$PGF_{1\alpha}$, 7.8 $pg{\cdot}mL^{-1}$ for PGE_2, TXB_2, and LTE_4. Results below the detection limit were set at the detection limit for statistical evaluation. The EIA specificity for cross-reactivity between the different mediators was less than 0.01% with a few exceptions; TXB_2 EIA cross-reacted with PGD_2 (0.53%), PGE_2 (0.09%) and 6-keto-$PGF_{1\alpha}$ (0.08%), PGE_2 EIA cross-reacted with 8-iso-PGE_2 (37.4%) and 6-keto-$PGF_{1\alpha}$ (1%), 6-keto-$PGF_{1\alpha}$ EIA cross-reacted with PGE_2 (1.5%) and TXB_2 (0.5%) and CysLT EIA cross-reacted with LTC_4 (50%) and LTD_4 (100%). PGD_2 was measured with the PGD_2-MOX assay where methoxime treatment of all samples creates a PGD_2 derivative that does not cross-react with $PGF_{2\alpha}$ or F-type isoprostanes.

Measurement of inflammatory mediators in BAL fluid and lung homogenate

To examine whether prostaglandins are able to modulate the release of cytokines, chemokines and growth factors, which also may contribute to manifestations of allergic reactions, these mediators were measured in BAL fluid or homogenised lung using cytometric bead arrays or Luminex® methodology.

In paper II, the levels of certain cytokines in BAL fluid were analysed in all control and treated animals by the flow cytometric analysis of fluorescence-labelled beads (Cytometric Bead Array, BD Biosciences BD™), following the manufacturer's protocol, and compared with known standards (detection levels were 5 $pg{\cdot}mL^{-1}$).

In paper III, a mouse 20-plex bead set (Invitrogen, Carlsbad, CA, USA) was used for the assay and measured using Luminex® methodology. Labelled antibodies against various cytokines/chemokines and growth factors were used to analyse multiple

cytokine responses in BAL fluid according to manufacturer's instructions. Values below the detection limit were set at the detection limit for statistical evaluation.

In paper IV, the levels of 19 cytokines/chemokines and growth factors were measured in BAL fluid and homogenised lung using a milliplex^{TM} map kit (Millipore Corporation, MA, USA) and assayed by Luminex$^{®}$ methodology. Lung tissue was homogenised with tissue extraction reagents according to the manufacturer's protocol (Invitrogen). Results below the detection limit were set at the detection limit for statistical evaluation. In addition to evaluating the influence of each mediator individually, a multivariate statistical analysis of the cytokine data was performed in order to explore correlations between the cytokine levels in BAL fluid and lung homogenate, as well as to examine how levels of mediators in lung homogenate correlate between treatments (PBS/OVA), between the different genotypes (wildtype/*ptges*$^{-/-}$) and between the genders.

Statistical Analysis

All data are presented as mean ± S.E.M. Differences among the treatment groups were assessed by unpaired *t*-test, one-way or two-way analysis of variance (ANOVA). Significant ANOVA was analysed further using the Bonferroni post hoc test. The two-way ANOVA test was used to analyse the dose-response curves and unpaired *t*-test or one-way ANOVA was used in all other statistical analyses, except for the analysis of cytokines, chemokines and growth factors in paper IV where multivariate modelling also was used. Changes in reactivity and sensitivity were assessed using non-linear regression analysis to calculate the maximum responses (E_{max}) and ED_{50} values. Log-values of ED_{50} were expressed in order to fit a normal distribution. A p-value of less than 0.05 was considered significant. Statistical analysis and graphics were all performed using Graph Pad Prism (version 5.0 GraphPad software Inc., San Diego, CA, USA).

For multivariate modelling, the principal component analysis (PCA) and orthogonal partial least squares regression of latent structure (OPLS) analyses (SIMCA-P+12, Umetrics, Lund, Sweden), were performed on the cytokine data. PCA analysis is a useful unsupervised approach for studying general trends in sample sets containing multiple variables. Mathematically it is a multivariate projection method designed to extract and display the systematic variation in a data matrix X. OPLS is a regression extension of PCA, which is used to connect the information of two blocks of variables X and Y to each other. OPLS is a supervised approach for studying the relationship between a single Y variable, such as gender or treatment and a range of measured variables (X) in a biological system. All variables used during PCA and OPLS analysis were subjected to mean centering and univariate scaling prior to modelling. Model performance was reported as cumulative correlation coefficient for the model (R^2) and predictive performance based on cross validations (Q^2).

5 RESULTS AND DISCUSSION

5.1 RATIONALE AND MAIN FINDINGS

Summary of the main results obtained in this Thesis:

Paper I:
The primary aim of this paper was to optimise the delivery route and dose of allergen used for antigen challenge in mice, with respect to the induction of AHR and airway inflammation.

Aerosol challenges using different concentrations of OVA and at different time points caused similar increase in both AHR and the infiltration of cells into the BAL fluid. Intranasal challenge of BALB/c mice caused a dose-dependent increase in AHR, whereas the BAL fluid cell response was augmented to a similar extent by all doses tested. Intranasal challenge caused a more pronounced induction of both AHR and inflammation in sensitised BALB/c mice, compared to aerosol delivery of the same antigen.

The intranasal dose of OVA that resulted in the greatest increase in AHR and infiltration of cells into the BAL fluid in BALB/c mice was applied to C57BL/6 mice. Intranasal OVA challenge caused strong inflammatory cell responses in BAL fluid to a similar extent in both C57BL/6 and BALB/c mice, but no change in AHR was observed in the C57BL/6 mice, indicating that this strain may not be optimal for the study of AHR.

Paper II:
The aim of this paper was to study the effects of NSAIDs on AHR and airway inflammation in allergic mouse models of asthma and to define time points of action of COX inhibition on AHR and airway inflammation.

Administration of FR122047 (COX-1 inhibitor) during OVA challenge and prior to MCh challenge enhanced AHR without affecting the inflammatory cell response. In contrast, administration of lumiracoxib (COX-2 inhibitor) during the same time period had no effect on AHR but reduced the inflammatory cells in BAL fluid. Non-selective COX inhibition with diclofenac both enhanced the AHR and reduced the inflammatory cells. Furthermore, investigation of the time point of action of the COX inhibition revealed that administration of diclofenac only during OVA challenge reduced the cells in BAL fluid without any changes in AHR, whereas administration of diclofenac only, prior to MCh-challenge enhanced AHR but did not affect the cells in BAL fluid.

It was concluded that COX-1 activity predominantly generated prostanoids that are bronchoprotective and serve to protect against increases in AHR. The bronchoprotective effect of COX-1 activity could presumably be explained in terms of the removal of PGE_2 and PGD_2. COX-2 activity was associated with an infiltration of inflammatory cells into the lung, lending support to a pro-inflammatory function.

It was not clear which prostaglandin mediated the COX-2 dependent effects on cellular inflammation. These findings demonstrate that AHR and the inflammatory response are distinct and at least in part, uncoupled events.

Paper III:
It is not known whether the distinct roles of prostanoids generated by the COX-1 and COX-2 pathways in paper II were dependent upon specific experimental conditions in that study, or whether the findings may be generalised. The aim of this paper was to study the effects of NSAIDs on AHR and airway inflammation in an adjuvant-free allergic mouse model of asthma.

Administration of either diclofenac, indomethacin, FR122047 or lumiracoxib enhanced AHR. Only diclofenac and lumiracoxib reduced the inflammatory cell content of BAL fluid. Moreover, levels of prostaglandins in BAL fluid were reduced by diclofenac, indomethacin and FR122047 but were unaffected by lumiracoxib. However, none of the COX inhibitors displayed major effects on the production of cytokines, mast cells, smooth muscle mass, number of goblet cells, or collagen deposition in the airways.

It was concluded that, in a prolonged adjuvant-free protocol, both COX-1 and COX-2 generated prostanoids were able to protect against increases in AHR. The bronchoprotective action of COX activity would seem to be explained by the removal of PGE_2, PGD_2 and PGI_2. However, the two COX isoenzymes also appear to have separate functions whereby COX-1 activity predominantly generates prostaglandins found in BAL fluid, whereas products of the COX-2 pathway regulate the inflammatory response in BAL fluid. Paper III provides further evidence that AHR on the one hand, and the inflammatory response and generation of prostanoids on the other, are dissociated uncoupled events.

Paper IV:
The aim of this paper was to define the role of mPGES-1 in a mouse model of chronic allergic airway inflammation. Our hypothesis was that, during inflammation, the production of PGE_2 is increased via upregulation of mPGES-1 expression. Hence, removal of mPGES-1 activity in genetically modified mice where this particular enzyme has been disrupted, would result in a loss of PGE_2-mediated bronchoprotection.

Absence of mPGES-1 resulted in a further increase in AHR after antigen challenge without affecting the infiltration of inflammatory cells in BAL fluid, or cytokine, chemokine and growth factor profiles in the lung. PGE_2 generated by mPGES-1 has a bronchoprotective function. The experiments with mPGES-1 deleted animals provided evidence to support the involvement of PGE_2 in protection against further increases in AHR, and also suggests it may be the main COX product to mediate the bronchoprotective function. This paper also indicates that the inflammatory response observed during allergic airway inflammation may not be regulated by the activity of mPGES-1.

5.2 MODEL CHARACTERISATION (PAPER I)

5.2.1 Optimisation of allergen dose and delivery route for the antigen challenge protocol

Although a variety of different sensitisation and challenge protocols have been used, one basic model for acute allergic airway inflammation is consistently applied. This type of sensitisation protocol usually requires multiple systemic administration of the allergen together with an adjuvant. OVA derived from chicken egg is a frequently used allergen that induces a robust allergic reaction in the airways of laboratory animals (122). Thus, a standard short protocol using OVA as an allergen was used to evaluate the optimal dose and delivery route of allergen for antigen challenge with respect to the outcomes of AHR and inflammation.

Initially, the effects of aerosol delivery were investigated using two different concentrations of OVA at two different time points. Aerosol challenge with both OVA concentrations tested (1 or 6%), as well as the two different time points (24 or 48 h), resulted in similar development of AHR and eosinophil-driven inflammation in the airways. The protocol using 1% OVA, and assessment 24 h after challenge was considered more suitable for studying AHR and airway inflammation, since the magnitude of the BAL fluid cell responses were similar and these conditions were associated with a lower variability in reactivity and cell responses, as well as an increased sensitivity to MCh at 24 h.

The aerosol delivery technique was then compared to different doses of OVA given intranasally. The main findings were that intranasal challenge caused a dose-dependent increase in AHR, whereas the BAL cell response was augmented to a similar extent by all doses of allergen delivered intranasally. Consistent with previous studies in mice, eosinophils were the dominating inflammatory cell type in allergic airway reactions induced by OVA (105). Furthermore, intranasal challenge caused a more pronounced induction of both AHR and inflammation compared to aerosol delivery. It was presumed that the AHR and BAL fluid cell responses were lower following aerosol challenge compared to the intranasal procedure, due to the aerosol exposure causing a less efficient distribution of aerosolised antigen within the lung. The findings suggest that separate pathways cause AHR and inflammation since the thresholds for reaching maximal allergen-induced AHR and inflammatory responses appear to be dissociated. Thus, intranasal challenge was more effective than aerosol challenge in inducing both AHR and airway inflammation in BALB/c mice. One potential limitation with the intranasal challenge is that it requires light isoflurane anaesthesia. It has been suggested that isoflurane may have an anti-inflammatory effect (123, 124). The substantial inflammatory responses obtained, would suggest that any possible inhibitory effects of isoflurane were insignificant under these particular experimental conditions.

Histological analysis of the pulmonary tissue demonstrated an increased infiltration of eosinophils, as well as elevated numbers of goblet cells in OVA treated mice compared to controls, regardless of whether OVA was administrated intranasally or as an aerosol. Intranasal OVA induced a greater influx of eosinophils into the tissue compared to

aerosol challenge, but there were no differences in goblet cell numbers between the two procedures. Neither aerosol nor intranasal challenge induced changes in the smooth muscle area or collagen deposition. These findings suggest that structural changes in the airways are not necessary for the development of AHR.

5.2.2 Comparison between BALB/c and C57BL/6 mice using the intranasal challenge protocol

The intranasal challenge procedure that resulted in the greatest increase in AHR and BAL fluid cells in BALB/c mice was selected for further comparisons between the two most commonly used strains of mice, BALB/c and C57BL/6 mice using the forced oscillation technique. As expected, BALB/c mice displayed an increase in AHR and a clear effect of MCh on both central resistance (R_N) and peripheral tissue dampening (G). In contrast, the same procedure did not change these parameters in C57BL/6 mice, but did result in a similar accumulation of cells in BAL fluid. Furthermore, the baseline physiological measurement of tissue elastance (H) was enhanced in both BALB/c and C57BL/6 mice. These alterations in both strains may be caused by infiltration of cells into the parenchyma which in turn may lead to airway closure due to mucus production, leakage of plasma proteins onto the airway surface and an increase in airway surface tension (125). The study confirms the difficulties C57BL/6 mice have in developing AHR after antigen challenge (107, 126-130) and this strain is not optimal for studying AHR in a short standard eosinophil-driven murine model of allergic airway inflammation. The findings also demonstrated that forced oscillation is a valuable method for evaluating mechanical parameters of both the central airways and peripheral lung.

5.3 EFFECTS OF INHIBITION OF PROSTAGLANDIN SYNTHESIS ON AIRWAY RESPONSES (PAPER II-IV)

In this Thesis, three different protocols for OVA-induced airway inflammation have been used to gain a more detailed understanding of the role of the different COX isoenzymes in mouse models. This was achieved by the application of COX inhibitors with differing selectivities towards the COX isoenzymes, or by using mPGES-1 gene-disrupted mice. The protocols have induced different types of airway inflammation that can be classified as acute eosinophil-associated, adjuvant-free model as well as a more chronic airway inflammation in which several types of inflammatory cells are involved, particularly neutrophils.

5.3.1 COX products as modulators of airway responsiveness

Regardless of the protocol used for antigen sensitisation and challenge, OVA challenge consistently increased the AHR to MCh in mice. When examining the total lung resistance, the maximal response to MCh differed, both between protocols and between different strains of mice, although the extent of the antigen-induced increases

in total lung resistance are similar when compared to PBS challenged control mice (Figure 7).

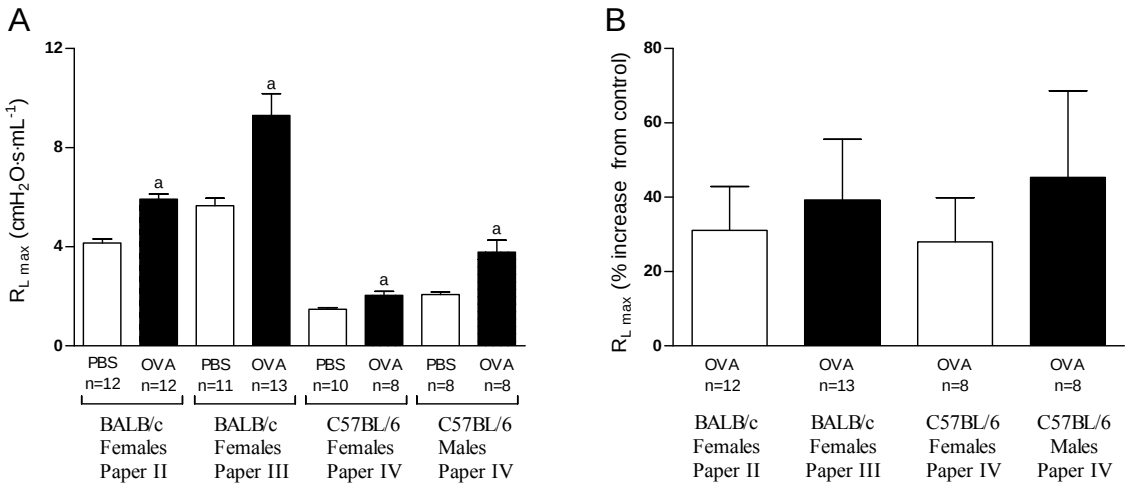


Figure 7. A; Maximal lung resistance ($R_{L\ max}$) to MCh in three mouse models of allergic airway inflammation and two different strains of mice, B; Antigen-induced maximal lung resistance, percentage of increase from PBS controls. The results represent mean±SEM. a, $p<0.05$ compared to PBS controls.

In paper II, treatment with the non-selective COX inhibitor diclofenac, as well as the selective COX-1 inhibitor FR122047, caused further increases in airway reactivity to MCh, whereas selective COX-2 inhibition by lumiracoxib did not change the AHR induced by OVA alone (Figure 8). Thus, the findings from paper II identified that COX-1 activity is critical for modulation of AHR.

In contrast, in paper III, the administration of diclofenac, indomethacin, FR122047 and lumiracoxib during OVA challenge resulted in significantly enhanced airway reactivity compared to OVA control mice (Figure 8). Although paper II indicates that COX-1 mainly regulates the production of prostaglandins that in turn could have a direct effect on the airway smooth muscle, paper III indicates that in addition to COX-1, COX-2 activity may also contribute to this effect when tested in an adjuvant-free model.

The discrepancy in AHR after COX-2 inhibition in papers II and III may be partly related to the use of different challenge procedures. Another possible explanation might be the differences in pharmacokinetics amongst the COX inhibitors used, which presumably attained significance as the COX inhibitors were given every day before each antigen challenge in paper II, and every third day before each antigen challenge in paper III. The findings implicate that the effect of COX activity on AHR can be generalised and is not dependent on specific experimental conditions since consistent results were obtained in two separate independent experiments. Thus, prostaglandins generated by both COX isoenzymes have a bronchoprotective role. From these

experiments it was assumed that PGD_2 and PGE_2 were presumably responsible for the effect as both induce a strong and potent relaxation of mouse airway smooth muscle (58, 61). This also highlights that the protocol selected for sensitisation and challenge has a clear impact on COX activity and its regulation of AHR.

In paper IV, the role of the mPGES-1 enzyme was investigated in a chronic mouse model of airway inflammation. It was discovered that removal of PGE_2 synthesised via mPGES-1 in OVA challenged *ptges*$^{-/-}$ mice caused an increase in AHR mainly in the central airways, along with a reduction of BAL fluid PGE_2 regardless of gender. The data established that the total lung resistance in *ptges*$^{-/-}$ mice and AHR induced by the COX inhibitors in papers II and III were increased to a similar extent (Figure 8). These findings strengthen the importance of COX enzymes in regulating the reactivity of airway smooth muscle by protecting against increases in AHR and PGE_2 may be the main COX product to mediate the bronchoprotective function.

The experiments with mPGES-1 deleted animals also showed that *ptges*$^{-/-}$ mice displayed normal baseline physiology and respiratory mechanics in both females and males, which supports the notion that mPGES-1 is involved in the production of prostaglandins during inflammation but is not necessary for the regulation of basal airway tone. Paper IV reveals for the first time that the major bronchoprotective function of PGE_2 is generated by mPGES-1 in allergic airway inflammation.

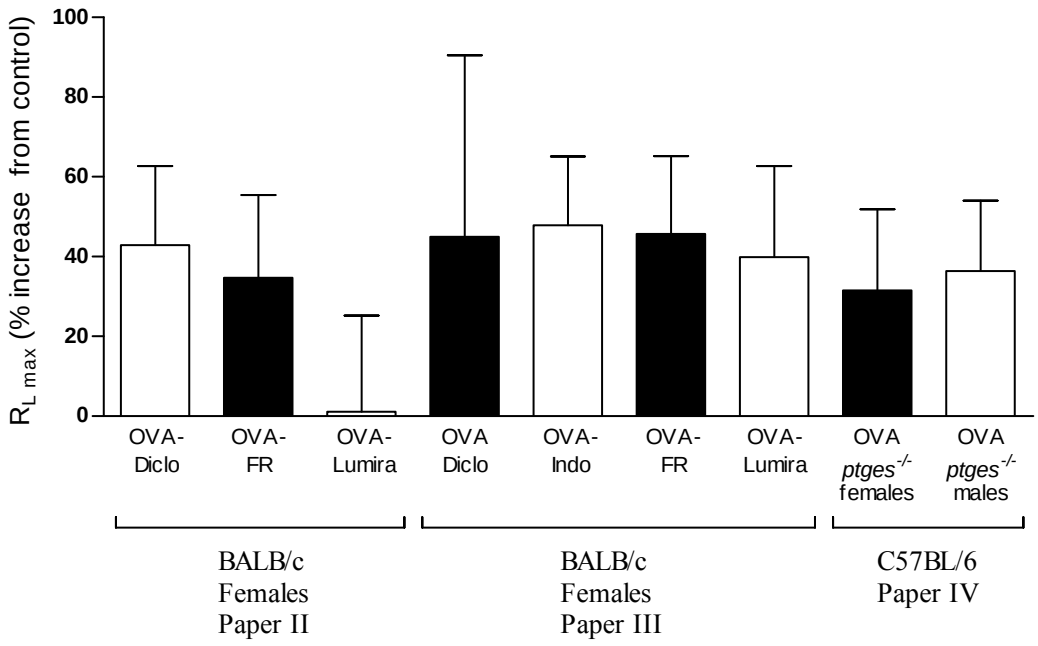


Figure 8. Maximal lung resistance ($R_{L\ max}$) to MCh induced by either COX inhibition or by genetically deleted mPGES-1 in three different mouse models of allergic airway inflammation and two different strains of mice. The results represent mean±SEM. Antigen-induced maximal lung resistance, percentage of increase from PBS controls. Diclo, diclofenac; Indo, indomethacin; FR, FR122047; Lumira, lumiracoxib.

The further decrease in compliance caused by COX inhibition that was observed in paper II could not be confirmed in paper III. A decrease in compliance is thought to be dominated by changes in airway closure, particularly in the peripheral small airways (108), however, C_L is influenced by increases in R_L. In addition, the change in C_L is also fairly small. Therefore, the COX-induced increase in AHR is probably located at the level of the proximal airways and the primary bronchi, as an effect on airway smooth muscle reactivity. This finding was also supported by the substantial response observed in the central airways after the removal of mPGES-1-derived PGE_2 in paper IV. Consistent with this observation, the increased AHR measured after COX inhibition was a result of modulation at the level of the central airways, which also suggests that the decreased C_L observed in paper II after COX inhibition is most likely a consequence of the great increase in R_L.

5.3.2 COX products as modulators of inflammatory cells in BAL fluid

Regardless of the protocol used for antigen sensitisation and challenge of mice, OVA induced an infiltration of cells into the airway lumen (Figure 9A). Although the cellular composition in BAL fluid differs between protocols as well as between strains of mice, the antigen induces a significant increase in the total lung resistance compared to PBS challenged controls.

As shown in Figure 9B, total numbers of BAL fluid cells were decreased by the different COX inhibitors with various degrees of efficacy relative to OVA controls, however, only diclofenac and lumiracoxib were able to significantly reduce BAL fluid cell numbers. Eosinophils were the predominating inflammatory cell type and COX-2 inhibition mainly decreased this eosinophilic inflammation. This indicates that COX-2 generates prostaglandins that mediate the accumulation of cells in the airways.

In paper III, indomethacin showed a similar lack of effect to that observed with the COX-1 inhibitor on the OVA induced cellular response, in contrast to the other non-selective COX inhibitor, diclofenac. The finding that diclofenac and indomethacin gave rise to contrasting results regarding the reduction of cells in BAL fluid is most likely due to the differing selectivities of the two drugs towards the COX isoenzymes, with diclofenac being more COX-2 selective and indomethacin more COX-1 selective (90). Another possible explanation might be that indomethacin exerts DP_2/CRTH2 agonism (77, 78) and thereby promotes accumulation of cells into the airways, which may have contributed.

In paper IV, the OVA induced infiltration of cells into BAL fluid was similar in mPGES-1 disrupted mice compared to wild-type mice, an effect therefore not dependent on mPGES-1 (Figure 10). During the chronic allergen exposure, the major inflammatory cell type found in the BAL fluid was the neutrophil (Figure 10). Furthermore, the findings from paper II and III support the fact that COX-2 inhibition reduced the infiltration of inflammatory cells into the BAL fluid, and together with the observations in paper IV allow for a more comprehensive interpretation of the findings.

As the deficiency of mPGES-1-derived PGE_2 did not reduce inflammatory cell numbers in BAL fluid, this suggests that mPGES-1 is not involved in the inhibitory function of COX-2 products on cell recruitment. The observations suggest that PGE_2 does not mediate the inflammatory cell response in the allergic airway reaction in this particular model. This is in line with data where inhalation of PGE_2 in mPGES-1 deleted mice actually suppressed the infiltration of cells into the airway lumen (131).

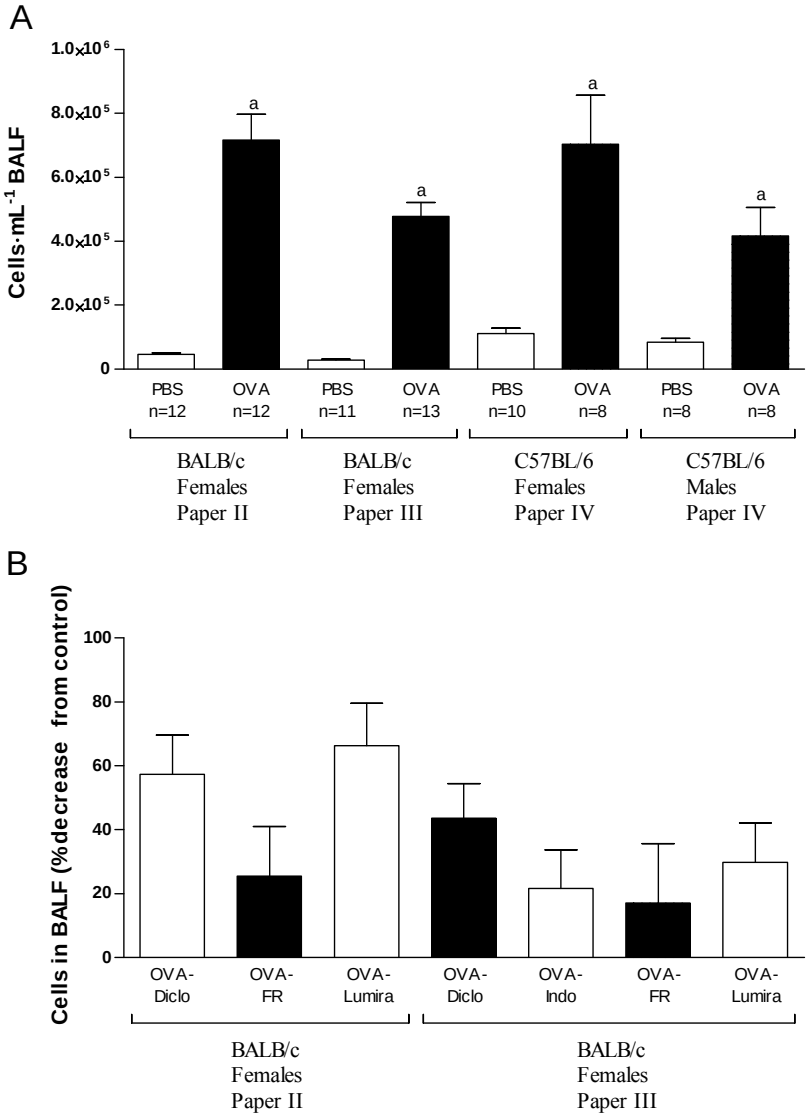


Figure 9. Antigen-induced accumulation of cells in BAL fluid. A; Total cells induced by PBS or antigen challenge in three mouse models and two different strains of mice. B; Antigen-induced total cells in BAL fluid, percentage of decrease from OVA controls by COX inhibition**.** The results represents mean±SEM. a, $p<0.05$ compared to PBS controls. Diclo, diclofenac; FR, FR122047; Indo, indomethacin, Lumira, lumiracoxib.

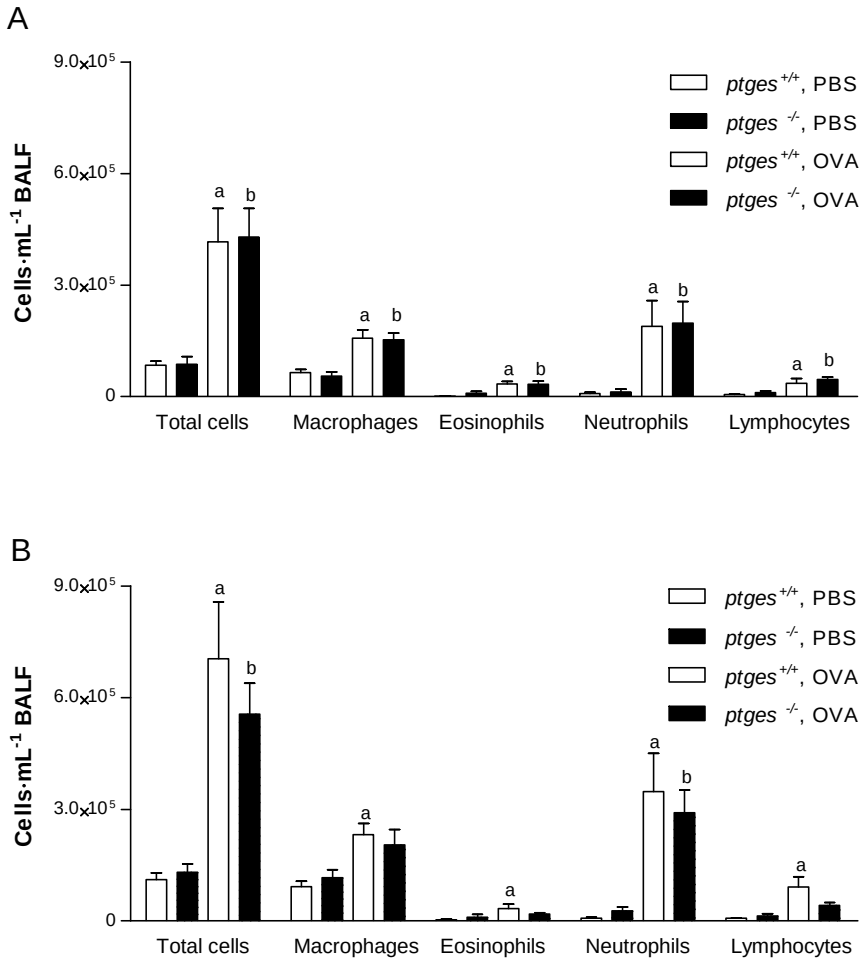


Figure 10. Total cells and composition of cells in BAL fluid in *ptges*$^{+/+}$ (wild-type mice) and *ptges*$^{-/-}$ mice challenged with PBS or OVA (n=8-12). The cellular response in BAL fluid in A; males and B; in females. Data are represented as mean ± SEM. a, $p<0.05$ OVA challenged *ptges*$^{+/+}$ (wild-type) mice compared to controls, b, $p<0.05$, OVA challenged *ptges*$^{-/-}$ mice compared to controls.

Taken together, these findings suggest that the two COX isoenzymes have separate functions with respect to regulation of the cellular inflammatory response where only COX-2 activity was found to be associated with the accumulation of cells in BAL fluid. However, the data do not define which prostaglandin mediated the COX-2 dependent effect on cellular inflammation. It is possible that locally released prostaglandins may act directly, by an as yet undiscovered mechanism, on cellular recruitment in a way that may not be reflected by the pattern of COX product formation recovered in BAL fluid. Further investigations using selective agonists and antagonists are required to identify which particular prostaglandins might be responsible for the cellular response implied by the COX inhibitors. Although a decrease in inflammation was observed following COX-2 inhibition in paper II and III, the allergen-induced AHR was further enhanced, indicating that these processes are distinct and not always coupled events.

5.3.3 Time point of action of COX inhibition

In paper II, the time point of action of COX inhibition was defined by administration of the non-selective COX inhibitor diclofenac, using two different protocols. Thus, the effects on AHR and BAL fluid cell responses were compared when diclofenac was given either only during the OVA challenge, or only prior to the MCh challenge. The findings showed that administration of diclofenac only during the OVA challenge reduced the cell response in BAL fluid without any effect on the increased AHR induced by the antigen itself (Figure 11A and B). In contrast, administration of diclofenac only prior to the MCh challenge enhanced the allergen-induced AHR to MCh but did not affect the cell response in the BAL fluid (Figure 11C and D). These findings indicate that there is a difference in the time taken for the two separate reactions to occur during antigen challenge.

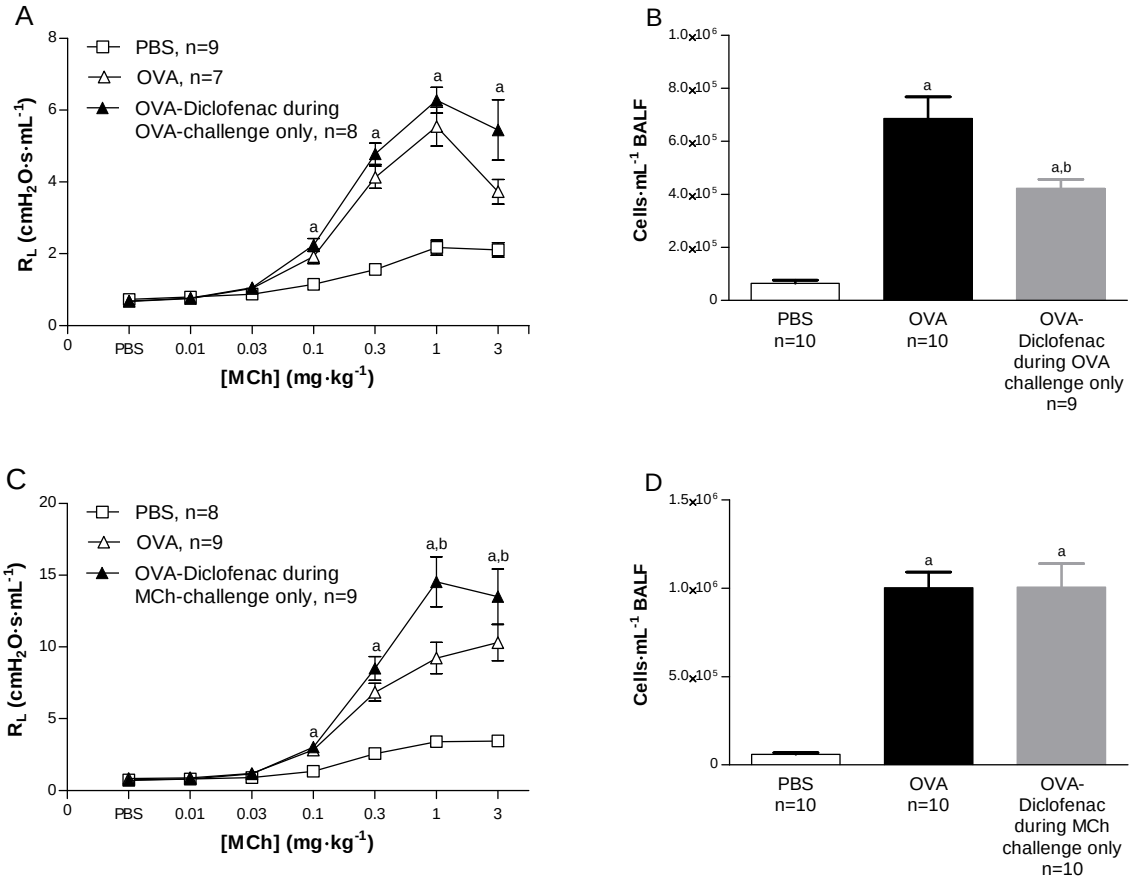


Figure 11. The time point of action of COX inhibition. OVA sensitisation and challenge in mice treated with diclofenac only during OVA challenge (A-B) or only during the MCh challenge (C-D). A; Lung resistance to MCh, B; total cell count in BAL fluid, C; lung resistance to MCh, D; total cell count in BAL fluid. Datapoints represented as mean ± SEM. a, $p<0.05$ compared to PBS challenged controls and b, $p<0.05$ diclofenac compared to OVA controls.

5.4 EFFECTS OF THE INHIBITION OF PROSTAGLANDIN SYNTHESIS ON MEDIATOR RELEASE (PAPER II-IV)

To define whether the distinct effects of the two COX isoenzymes on AHR and the accumulation of cells in BAL fluid were associated with differential formation of eicosanoids, prostanoids and CysLTs were measured in BAL fluid (paper II-IV). A possible modulatory effect of prostaglandins on the inflammatory mediator profile was also investigated and a panel of cytokines, chemokines and growth factors were measured in BAL fluid (paper II-IV) and lung homogenate (paper IV).

5.4.1 Prostanoid and leukotriene levels in BAL fluid

Measurements of prostanoids in BAL fluid showed that PGD_2, PGE_2, TXA_2 and 6-keto-$PGF_{1\alpha}$ (the metabolite of PGI_2) increased significantly above basal levels in OVA challenged mice. The relations of the levels of these mediators measured were 6-keto-$PGF_{1\alpha}$>>PGE_2 >PGD_2>TXA_2.

In consistency, both paper II and III, showed that COX-1 inhibition by diclofenac, indomethacin and FR122047 resulted in a reduction in the levels of PGD_2, PGE_2 and TXA_2, whereas inhibition of COX-2 by lumiracoxib had no or little effect on these mediators. The observation that the levels of the prostaglandins reduced by COX inhibition were different between the two studies may again be explained by the different challenge procedures and the different types of inflammation being induced in these particular studies. These data support a predominantly COX-1 mediated release of prostanoids in the airways and lends support to the concept that the two COX isoenzymes have separate roles regarding the generation of prostanoids in the airways during allergic airway inflammation.

As shown in paper IV, when using mPGES-1 gene-disrupted mice, it was concluded that approximately half of the antigen-induced PGE_2 production (50% in males and 26% in females) in BAL is catalysed by mPGES-1 in chronic airway inflammation (paper IV). Thus, the bronchoprotective action of COX products in allergic airway inflammation is presumably explained in terms of the removal of local PGE_2 that is mainly synthesised by mPGES-1. The effect of PGE_2 on airway responses to allergen may be due to smooth muscle relaxation via the EP_2 receptor (60, 61) and by inhibition of the release of mast cell mediators (132). However, in mice, PGD_2 also induces a potent relaxation of airway smooth muscle (58) and it is possible that this prostaglandin may have contributed as well. A novel finding in paper III was the high level of the PGI_2 metabolite 6-keto-$PGF_{1\alpha}$ present in BAL fluid after antigen challenge. It is not unlikely that PGI_2 may also be involved in the allergic inflammatory process. In fact, PGI_2 has been suggested to be one of the prostaglandins with an inhibitory role in allergic responses and large amounts of this mediator are produced during allergic reactions in the human lung (133, 134).

The levels of CysLTs were significantly increased above basal levels when mice were challenged according to the short standard eosinophil-driven protocol. Similarly, mice challenged with OVA according to the adjuvant-free protocol had higher levels of

CysLTs compared to PBS challenged controls, although these differences did not reach statistical significance. This variance may again be explained by the different challenge procedures used in these particular studies. However, consistent with these findings, COX inhibition did not alter or change the level of CysLTs.

5.4.2 Inflammatory mediators in BAL fluid and lung homogenate

The levels of cytokines, chemokines and growth factors were measured in BAL fluid in paper II-IV. Generally, an increase in IL-4 and/or IL-5 was observed in OVA challenged mice regardless of the protocol used for sensitisation and challenge. In the short standard protocol (paper II), an increase in IL-13 levels was also observed that could not be confirmed in models of adjuvant-free induced inflammation (paper III) or chronic airway inflammation (paper IV). Interestingly, indomethacin was the only COX inhibitor to cause increased levels of IL-4, IL-5, IL-13, MIG and MIP-1α compared to OVA controls. The finding that it was essentially only indomethacin and not the other non-selective COX inhibitor, diclofenac, that had significant effects on BAL fluid cytokine levels indicates that this effect may not be due to the inhibition of COX but result of another mechanism, possibly involving the by activation of CRTH2 (77, 78). Moreover, the enhanced cytokine levels measured following indomethacin treatment could not be correlated with the observed increase in AHR.

As OVA challenge and intervention with indomethacin had effects on cytokine release as shown in papers II and III, it was decided to investigate these inflammatory mediators as in both BAL fluid and lung homogenate in paper IV. Instead of only evaluating each mediator alone, a multivariate data analysis was performed. The data showed that antigen-induced mediators were more readily detectable in lung homogenate than those in BAL fluid. We speculated as to whether the inflammatory mediators found in BAL fluid were a consequence of "spill-over" of mediators released in the tissue or whether they might be released by the cells found in BAL. To address this query, we decided to perform an analysis to explore possible correlations between antigen-induced inflammatory mediators in BAL fluid and lung homogenate. The data revealed that MCP-1, MIG, IP-10, MIP-1α, TNF-α and IL-4 correlated closely between lung homogenate and BAL fluid and may be related to a "spill-over". However, this phenomena could not be applied to all mediators since the correlation between lung homogenate and BAL fluid was not a general event as certain mediators correlated well but others did not. Due to the fact that several BAL fluid mediators were below detection limits, we decided to focus the analysis of mediator profiles to the lung homogenate.

Further analysis of the lung homogenate established that the different mediator profiles found in the lung homogenate of OVA and PBS challenged mice were dependent on antigen treatment rather than genotype and gender. It was clear that the main contribution to the separation between the two treatments was related to MIG, MIP-1α, IL-17 and IP-10. Interestingly, three of those mediators were the same mediators that also correlated well with the mediators in BAL fluid. However, the contributions made by these inflammatory mediators to this particular model is not clear. MCP-1, MIG,

IP-10 and MIP-1α can be produced by many cells in the airways, including neutrophils (135), which were the major inflammatory cell type in BAL fluid induced in the present study of chronic airway inflammation. It is not unlikely that secretion of chemokines by the accumulated neutrophils found in BAL fluid may be responsible for the correlation found between lung homogenate and BAL fluid. These findings indicate that lack of mPGES-1-derived PGE_2 does not alter the inflammatory mediator profiles. Thus, the findings demonstrate that the cytokine levels in BAL fluid are not affected by COX inhibition in general, nor specifically by PGE_2.

5.5 EFFECTS OF INHIBITION OF PROSTAGLANDIN SYNTHESIS ON STRUCTURAL CHANGES (PAPER III)

In paper III, it was investigated whether AHR caused by COX inhibition could possibly be directly related to structural changes, i.e. remodelling in the airways.

5.5.1 Effects on tissue eosinophilia

Using the adjuvant-free mouse model, a substantial infiltration of eosinophils was observed in OVA sensitised and challenged mice compared to PBS controls. Although a significant decrease in BAL fluid eosinophils was observed after COX-2 inhibition, no decrease in tissue eosinophilia was found following treatments with any of the COX inhibitors tested. The findings implicate that the increased total number of cells in BAL fluid may not necessarily reflect the accumulation of cells in the lung tissue. Accordingly, other observations confirm that factors regulating the accumulation of cells in these two compartments do not always correlate (136, 137).

5.5.2 Effects on mast cells

The importance of mast cells in asthma has been highlighted by increased mast cell numbers found in the smooth muscle layer of asthmatics (36). Previous data obtained by Williams *et al* which showed that the number of mast cells in the lung was unaffected by antigen challenge was confirmed in the present work. However, this does not exclude the possibility that mast cells are key regulatory cells and may drive the response in this particular adjuvant-free model (138). Indeed, it is known that a relatively small number of activated mast cells can mediate AHR in mice (139) and also be important triggers of subsequent responses by virtue of mast cells being stationary rather than early migrating cells. Treatment with COX inhibitors had no effect on the number of mast cells in lung tissue. However, regardless of the influence of mast cells, the data suggest that COX products do not regulate the infiltration of mast cells into the lung.

5.5.3 Effects on smooth muscle

An increase in smooth muscle mass was observed in OVA sensitised and challenged mice compared to PBS controls. However, the increase in smooth muscle mass after COX administration was at the same high level as in that observed in OVA controls. These findings suggest that the enhanced AHR caused by COX inhibition is due to an effect on smooth muscle hyperreactivity. In line with the findings, there are observations in asthmatics that support a primary modulation of airway smooth muscle induced by drug treatments (140, 141). Thus, it is possible that COX inhibition modulates airway smooth muscle reactivity as a distinct effect of AHR.

5.5.4 Effects on goblet cells

Antigen challenge induced an increase in goblet cell numbers compared to PBS controls. However, COX inhibition did not affect the goblet cell hyperplasia which was at the same high level as that in OVA controls. As COX inhibition did not affect the allergen-induced goblet cell hyperplasia, it is concluded that this is not the mechanism underlying AHR in the current model.

5.5.5 Effects on collagen formation

Antigen challenge according to the adjuvant-free intermediate protocol did not affect collagen composition in the lung tissue nor did COX inhibition affect the amount of collagen deposition. This would suggest that collagen is not necessary for development of the AHR. Studying changes in the airways related to collagen deposition would require repeated exposures using OVA as an allergen for longer periods of time.

5.5.6 Summary of structural changes in the airways

In summary, it was concluded that the enhanced AHR observed after COX inhibition is rather due to altered functional properties of the airways than alterations in the structural changes, since the current findings show that COX inhibition did not affect the structural features examined. Accordingly, structural changes in the airways are not necessary for the development of AHR.

5.6 EXPRESSION OF COX-1 AND COX-2 (PAPER III)

In an attempt to explain the increased activity of COX after OVA challenge, the expression and location of the two COX isoenzymes was investigated in lung tissue. Expression of COX-1 was found to be located in epithelial cells, large inflammatory cells in the lung tissue and the smooth muscle layer. The most striking observation was that COX-1 expression decreased in the epithelium after OVA exposure, which may be due to the elevated numbers of goblet cells (Figure 12). Expression of COX-2 was concentrated in airway smooth muscle in both PBS and OVA challenged mice, the latter also displaying a greater smooth muscle mass (Figure 12). The discrepancy

between the effects of COX-2 on AHR in papers II and III may possibly be explained by the short standard protocol used in paper II not inducing an increase in smooth muscle (paper I). The expressions of the COX isoenzymes were not affected by antigen challenge, however, the enzymatic activity is not only determined by protein expression but also by factors that regulate the action of the enzymes. Although our data indicate that COX-1 is central in regulating the production of prostaglandins that affect the airway smooth muscle, the finding in an adjuvant-free mouse model indicates that COX-2 activity during inflammation also contributes to the smooth muscle response. Since COX-2 expression was concentrated in the airway smooth muscle it is possible that COX-2 generates a local autocrine release of prostaglandins that directly acts on the airway smooth muscle to regulate AHR.

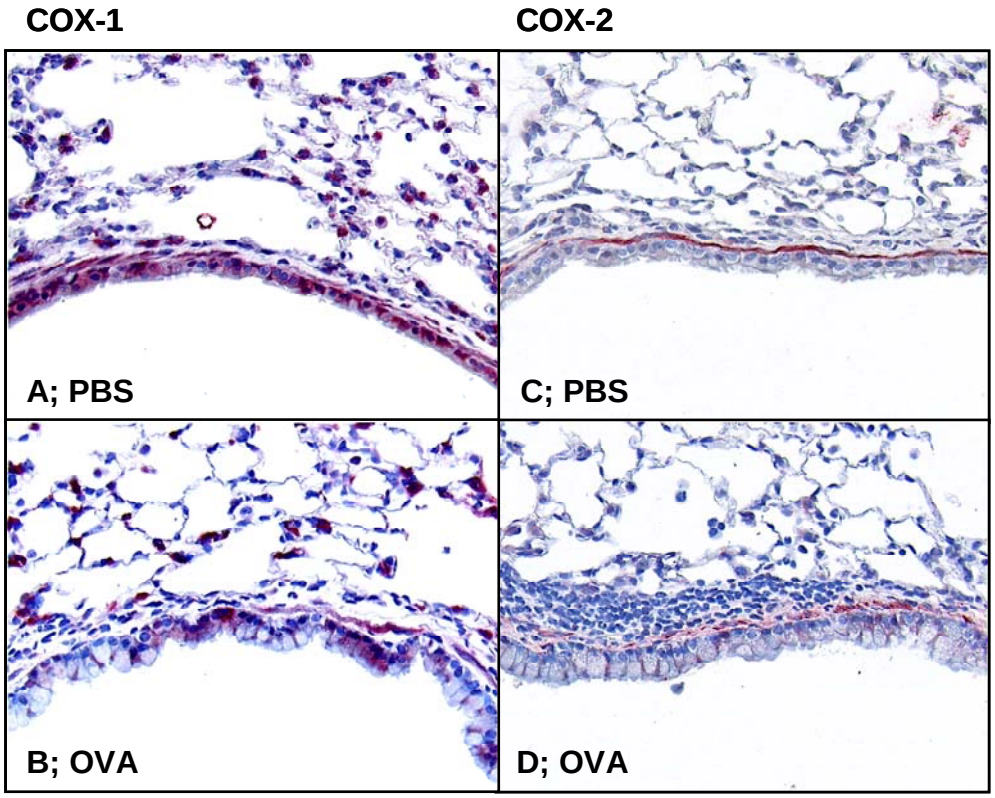


Figure 12. Immunohistological staining showing the expression of COX-1 and COX-2 in lung tissue from OVA sensitised mice. Representative pictures after challenge with PBS (A and C) or OVA (B and D). Expression of COX-1 was located in epithelial cells as well as large inflammatory cells in the lung tissue and in the smooth muscle layer whereas the expression of COX-2 was concentrated in the airway smooth muscle of both PBS and OVA challenged mice.

5.7 SUMMARY

On the basis of the studies in this Thesis, it is possible to assess the relative contribution of the two COX isoenzymes to the events produced in the airways by allergen challenge of sensitised mice (Figure 13). Thus, both COX-1 and COX-2 generated prostanoids that inhibited the reactivity of the airway smooth muscle. The experiments with mPGES-1 deleted animals provides, for the first time evidence to support PGE_2 being directly involved in regulating AHR, although release of PGD_2 and PGI_2 may contribute as well.

Furthermore, whereas COX-1 predominantly generated the prostaglandins recovered in BAL fluid, COX-2 activity is associated with the accumulation of inflammatory cells found in BAL fluid. This difference was not explained by the present experiments and it remains unclear which prostaglandin mediated the COX-2 dependent effects on cellular inflammation. The experiments in the mPGES-1 deficient animals, however, argue against PGE_2 being a mediator responsible for the inflammatory cell response in the allergic airway reaction observed in this particular model.

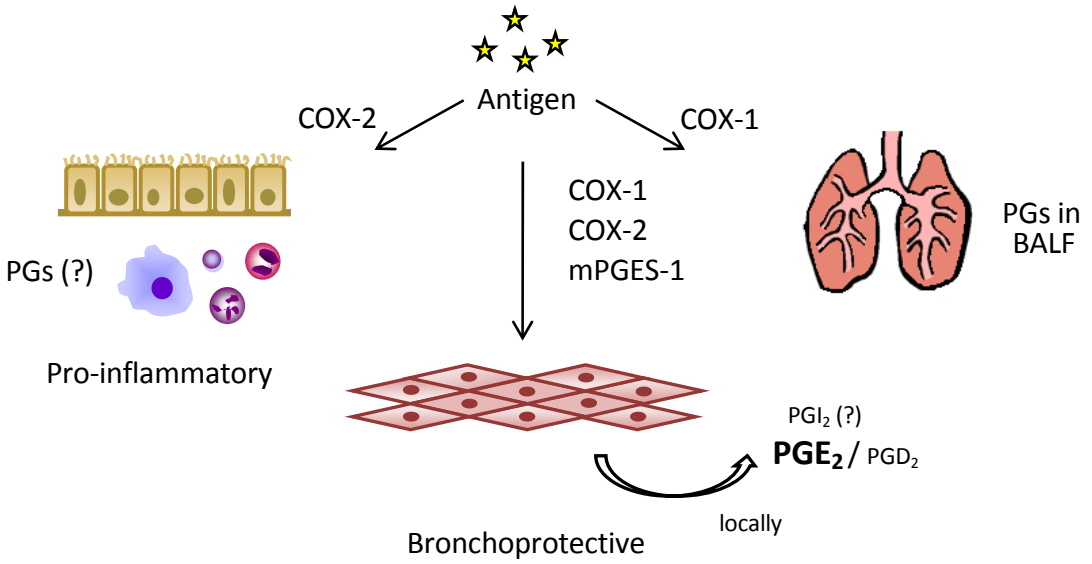


Figure 13. A simplified illustration on how the two COX isoenzymes might function in response to antigen challenge. (Figure drawn by Daniel Andersson).

6 GENERAL DISCUSSION

The findings in this Thesis support the concept that COX products have an overall bronchoprotective function in the airways of mice. The conclusion is based on a series of experiments where intervention with non-selective or isoenzyme selective COX inhibitors has caused enhanced AHR in animals with allergic inflammation. Treatment with COX inhibitors did not change baseline airway responsiveness to MCh in animals without allergic airway inflammation. In the study where the effect of interventions at different time points was studied (paper II), it was shown that enhanced AHR could only be produced when animals with induced airway inflammation were treated with a COX inhibitor on the day of the experiment. It is therefore possible to conclude that the allergic inflammation appears to cause increased biosynthesis of bronchoprotective prostanoids that serve to protect against excessive increases in AHR which was a consequence of allergen challenge.

The enhancement of AHR by COX inhibitors, or by deletion of mPGES-1, was observed in a short protocol with eosinophilic inflammation, an intermediate adjuvant-free protocol associate with mast cell activity, as well as in a chronic protocol involving the activation of several inflammatory cell types, which is also known to induce airway remodelling. This would suggest a common mechanism for AHR that is independent of the type of inflammation and structural changes involved. Further support for a general mechanism is provided by observations that the enhanced AHR after COX inhibition was independent of the different composition of cells within the airway lumen, as well as the cytokine, chemokine and growth factor profiles produced. However, the enhancement in AHR was related to changes in the formation of COX products, in particular PGE_2.

The specific roles of the different COX products on the various effects observed, remains to be conclusively established. The experiments with mPGES-1 deleted animals indicated that PGE_2 may be the main COX product to mediate the bronchoprotective function that is lost after treatment with NSAIDs and accordingly, the biosynthesis of PGE_2 was diminished to about half of the levels measured in wild-type littermates. Interestingly, the effect of the mPGES-1 deletion was very similar to the effect of the COX inhibitors, suggesting that PGE_2, with its airway relaxing properties, is indeed the main mediator of COX-dependent bronchoprotection. However, as PGD_2 also is a bronchodilator in mice (58) and PGI_2 has been shown to have an inhibitory role on inflammation in certain mouse models (65, 66), the role of these two prostaglandins remains to be defined in the future. In view of the findings that the level of PGI_2 in BAL fluid after allergen challenge was actually higher than those of PGE_2, one may speculate that there is a role for PGI_2. In the future, investigations using selective receptor agonists and antagonists are required to identify exactly which prostaglandins mediate the responses implied by the COX inhibitors.

Whereas intervention with different COX inhibitors generally caused an increased AHR in mice with allergic airway inflammation, COX-2 inhibitors often attenuated the accumulation of inflammatory cells in BAL fluid. This consistently observed dissociation between the effects of the COX inhibitors on inflammation and AHR questions the dogma that the degree of airway inflammation determines the extent of AHR.

Several other observations from the experiments included in this Thesis support the lack of correlation between BAL fluid cells and AHR. Firstly, intranasal allergen challenge caused dose-dependent increases in AHR, but constant stereotypical increases in BAL fluid cells (Paper I). Secondly, C57BL/6 mice displayed the same BAL fluid inflammatory cell response after allergen challenge as BALB/c mice, but only BALB/c mice developed AHR (Paper I). Thirdly, there were differences in time for development of AHR and BAL fluid cells after COX inhibition during the allergen challenge (Paper II). Fourthly, mPGES-1 deleted animals displayed enhanced AHR, but no change in inflammatory cell numbers in the BAL fluid (paper IV).

In this context, it is worth considering that several investigators make claims about mechanisms in AHR, based on the effects of drugs or other interventions on BAL fluid cell counts in experiments where airway physiology not is assessed. Perhaps some of the disappointing results associated with new molecular targets that have been identified in mice models relate to incorrect conclusions drawn from studies using less relevant end-points. It is important to note that eosinophils and neutrophils in BAL fluid are not the sole determinants of AHR and additional mechanisms seem to be involved in explaining this feature. Inflammatory cell infiltration into BAL fluid is not an appropriate surrogate marker of AHR.

The investigations also provide insight into the importance of evaluating lung morphology. The findings implicate that the increased total number of cells in BAL fluid may not necessarily reflect the accumulation of cells in the lung tissue, and that regulation of the inflammatory cell response in these two compartments does not always correlate. However, this phenomenon has to be further investigated. Again, there are studies in this field where the inflammatory cell response in lung tissue is assumed to be an indicator of AHR, whereas in actual fact, such a correlation may not exist. With regard to the ongoing debate on therapies directed towards different phenotypes of asthma (12) it should also be recognised that the type of airway inflammation may not predict the degree of AHR in subjects with asthma (142).

Several of the observations in this series of experiments support the hypothesis that the main target of the bronchoprotective COX products induced during allergic inflammation is in fact the airway smooth muscle. Furthermore, it is likely that these products are produced locally in the airways. For example, the biosynthesis of PGE_2 is known to be prominent in both the airway epithelium and the smooth muscle itself (143, 144), and it is possible that the induced allergic inflammation causes an increase in PGE_2 release that in turn dampens the AHR.

The studies attempted to assess the role of the two COX isoenzymes in allergic airway inflammation. In the short protocol, the COX-2 inhibitor lumiracoxib inhibited BAL fluid cell levels but did not increase AHR, whereas the COX-1 inhibitor FR122047 enhanced AHR without affecting cell accumulation found in BAL fluid, supporting the conventional interpretation of COX-2 as being a pro-inflammatory pathway. However, when the effects of the selective inhibitors were studied in an adjuvant-free model, the outcome was more complex, and lumiracoxib was also able to enhance AHR. This suggests that with a more prolonged inflammation, COX-2 activity was also induced in tissues that regulated AHR. Thus, both COX-1 and COX-2 generated products may regulate AHR.

Furthermore, COX-2 activity was associated with the accumulation of inflammatory cells in BAL fluid. It is acknowledged that the present experiments do not define which prostaglandins mediated the COX-2 dependent effects on cellular inflammation. It is possible that locally released prostaglandins may act directly on cellular recruitment, which is not reflected by the pattern of COX products found in BAL fluid. The experiments in the mPGES-1 deficient animals, further, suggested that PGE_2 is not the mediator responsible for the inflammatory cell response underlying the allergic airway reaction in this particular model. In support of these findings, inhalation of PGE_2 in mPGES-1 deleted mice actually suppressed the infiltration of cells into the airway lumen (131). Taken together, the present findings indicate that the effects of the COX inhibitors on AHR is due to primary modulation of airway smooth muscle reactivity, rather than a consequence of the effects of the inhibitors on the inflammatory response in the airways, as monitored by the numbers of inflammatory cells in BAL fluid.

In many inflammatory diseases, PGE_2 is a pro-inflammatory mediator and inhibition of PGE_2 has therefore been a target for the development of new anti-inflammatory therapies. Indeed, in several models of pain and inflammation, mPGES-1 deleted mice display anti-inflammatory phenotypes (145-147). Attention has therefore been drawn towards mPGES-1 as a novel target for selective treatment of several inflammatory disorders. The need for new therapies lacking side effects is apparent, as the recently developed anti-inflammatory COX-2 inhibitors were associated with unacceptable cardiovascular side effects (6, 54, 148, 149). The findings in this Thesis implicate that inhibition of mPGES-1 may have negative consequences in the airways. Further preclinical and clinical studies are required to resolve potential safety issues related to mPGES-1 inhibition in human airway diseases.

As asthma is a complex disease, it is unlikely that one animal model of asthma replicate all of the morphological and functional features of the chronic human disease will ever be developed. Therefore, it is of great importance to improve and validate *in vivo* models so that they closely resemble the clinical pathology of human asthma. For improvement of the methodology, studies in paper I evaluated the effects of the delivery route and the doses of allergen for antigen challenge with respect to the induction of AHR and inflammation. The BALB/c mice displayed substantial airway reactivity to MCh. This phenomenon was observed regardless of the route of administration, dose of antigen or protocol used for sensitisation and challenge.

However, intranasal challenge caused a more pronounced induction of both AHR and inflammation compared to aerosol delivery, and the airway responsiveness increased in a dose-dependent fashion. It may also be necessary to adjust the dose of antigen used depending upon the aim of the outcome of the study. If a pharmacological treatment is expected to cause an increase in AHR, it may be more suitable to use a lower dose making it possible to detect an enhancement in AHR, whereas for studies of inhibition of AHR it might be more suitable to use a higher dose of antigen instead. Moreover, when the intranasal dose of OVA that resulted in the greatest increase in airway responses in BALB/c mice was applied to C57BL/6 mice, there was a similar, strong inflammatory cell response in the BAL fluid, but no change in AHR, indicating that this strain may not be optimal for studying AHR.

Several different protocols were used to resemble features of human asthma that included OVA models of acute airway inflammation (papers I and II), an intermediate adjuvant-free model (paper III) or a more chronic model of airway inflammation (paper IV). Eosinophils are present in the model of acute airway inflammation, whereas neutrophils are the predominant cell type in the chronic model of airway inflammation. Acute models reproduce many key features of clinical asthma, however, these models lack chronic inflammatory markers, airway remodelling and long-term AHR. From a clinical point of view, the chronic models are considered to be better at resembling human asthma. However, the design of the protocol used in different studies needs to be developed, depending on the objective of the study. So far, potential new asthma therapies that have been successfully developed in acute murine models of asthma but these promising results have failed to be reproduced, both in more chronic models of asthma and in human subjects with asthma (150).

Nevertheless, the consequences of COX inhibition on AHR, cellular inflammation and levels of prostaglandins recovered in BAL fluid were independent of the mouse model employed, and thus, the findings support the concept that COX products are involved in several mechanisms, as well as the fact that COX-induced bronchoprotection is a general event in mouse models. As inhalation of PGE_2 can protect against bronchconstriction induced by allergen-, exercise- and aspirin in humans (62, 151-153), it seems that the bronchoprotective functions of the prostaglandins in the mouse are similar to those in humans. Therefore, despite well founded criticism of the uncritical use of mouse models of asthma (154, 155), the use of mouse models for studying the bronchoprotective mechanism of prostaglandins appears highly relevant to the human situation. It is therefore concluded that the mouse models used in this Thesis are relevant for the analysis of bronchoprotective mechanisms mediated by prostaglandins in humans.

7 CONCLUSIONS

The main conclusions obtained from this Thesis are:

- Intranasal challenge was more effective than aerosol exposure in inducing both AHR and airway inflammation in BALB/c mice (paper I)

- The protocol that induced the maximal AHR in BALB/c mice failed to cause AHR in C57BL/6 mice (paper I)

- COX-1 predominantly generated prostanoids found in BAL fluid (papers II and III)

- COX-1 and COX-2 mainly generated bronchoprotective prostanoids that protect against increases in AHR (papers II and III)

- COX-2 products were associated with the accumulation of cells in BAL fluid (papers II and III)

- The experiments in mPGES-1 deficient mice supported PGE_2 as the main COX product mediating the bronchoprotective function in the airways (paper IV)

- PGE_2 synthesised via mPGES-1 was not responsible for the inflammatory cell response in the allergic airway reaction (paper IV)

- Several observations supported BAL fluid cell inflammation as an inappropriate surrogate marker of AHR (papers I-IV)

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Astma är en folksjukdom vars förekomst ökar över hela världen. I Sverige och i ett flertal andra länder har 8-10% av befolkningen astma, med tendensen att hos vuxna drabba fler kvinnor än män. Astma kännetecknas av luftvägsinflammation och luftvägsobstruktion i varierade svårighetsgrader. Sjukdomen är ofta relaterad till specifika antikroppar (IgE) som bildas vid exponering för olika allergen. Dessa IgE antikroppar kan bindas till mastceller som finns i lungorna. Denna reaktion stimulerar frisättning av inflammatoriska mediatorer från mastcellen. En del av dessa mediatorer är leukotriener, tromboxan och prostaglandiner som tillsammmans kallas för eikosanoider. Det enzym som genererar prostaglandiner kallas för cyclooxygenase. Den biologiska effekten av dessa eikosanoider är utbredd och dessa är involverade i många inflammatoriska processer i kroppen. Det är dock inte klarlagt vilken relativ betydelse de olika eikosanoiderna har i astma och allergiska svar. Syftet med studierna i denna avhandling var att definiera betydelsen av produkter genererade av cyclooxygenase i allergiska luftvägssvar i experimentella musmodeller av astma. De metoder som vi har använt syftar till att studera lungfunktion och inflammatoriska reaktioner efter olika interventioner av anti-inflammatoriska läkemedel i möss. Lungfunktionen har i denna avhandling studerats med hjälp av ett flexiVent® system där man mäter direkta förändringar av lungmekanik som svar på ett luftvägssammandragande ämne. Med denna teknik kan man mäta vanlig lungfunktion som beskriver variabler för lungans luftvägsmotstånd och tänjbarhet, samt precisionsmätning av lungmekanik i både centrala och perifera delar av luftvägarna.

Olika djurmodellsystem är viktiga för att studera mekanismer som finns vid humana sjukdomar. Det är dock viktigt att ha en kontinuerlig utveckling och utvärdering av modellerna för att de ska vara så relevanta för den humana situationen som möjligt. Musluftvägar är på många sätt lika människans, men dagens djurexperimentella protokoll har vissa brister. Därför var första delen av avhandlingsarbetet en modellutveckling där syftet var att optimera dos och administreringssätt för att göra möss allergiska mot äggalbumin. Studien påvisade att administrering av allergenet via nosen var mer effektivt än luftburet äggalbumin i att framkalla både allergisk luftvägsinflammation samt ökad luftvägskänslighet efter tillförsel av ett luftrörssammandragande ämne.

Den andra delen av avhandlingen syftar till att utreda betydelsen av prostaglandiner, dels med hjälp av anti-inflammatoriska läkemedel som selektivt hämmar olika enzymsystem och dels genom att använda möss där prostaglandin E syntesen är delvis utslagen, för att kartlägga de funktioner olika eicosanoider har vid allergenutlöst bronkkonstriktion i olika musmodeller. Studierna visar att luftvägshyperreaktivitet nedregleras av endogena prostaglandiner, sannolikt främst av prostaglandin E_2, medan ännu ej definierade prostaglandiner ökar invandringen av inflammatoriska celler i lungan. Dessa resultat styrker att prostaglandin E_2 har en skyddande effekt på luftvägarna och förmodligen inte är den mediator som reglerar det inflammatoriska cellsvaret vid det allergiska svaret i luftvägarna.

Sammanfattningsvis, studierna visar att luftvägskänslighet och luftvägsinflammation är parallella men ibland oberoende fenomen, samt att tidpunkten för behandling med anti-inflammatoriska läkemedel avgör vilken prostaglandineffekt som blockeras. Resultaten visar att studier av luftvägsinflammation utan bestämning av luftvägsfysiologi har begränsat värde då luftvägsinflammation och känslighet i luftvägarna inte alltid följs åt och att dessa dessutom delvis regleras genom olika mekanismer. Detta fynd stämmer också överrens med vad som ses i studier av personer med astma där inflammationen inte alltid är korrelerad till ökad luftvägskänslighet.

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10 REFERENCES

1. Morita I. Distinct functions of COX-1 and COX-2. Prostaglandins Other Lipid Mediat. 2002;68-69:165-75.
2. Carey MA, Germolec DR, Langenbach R, Zeldin DC. Cyclooxygenase enzymes in allergic inflammation and asthma. Prostaglandins Leukot Essent Fatty Acids. 2003;69:157-62.
3. Anggard E, Bergstrom S. Biological effects of an unsaturated trihydroxy acid (PGF2alfa) from normal swine lung. Acta Physiol Scand. 1963;58:1-12.
4. Hedqvist P, Holmgren A, Mathe AA. Effect of prostaglandin F 2 on airway resistance in man. Acta Physiol Scand. 1971;82:29A.
5. Mathe AA, Strandberg K, Astrom A. Blockade by polyphloretin phosphate of the prostaglandin F2a action on isolated human bronchi. Nat New Biol. 1971;230:215-6.
6. FitzGerald GA. COX-2 and beyond: Approaches to prostaglandin inhibition in human disease. Nat Rev Drug Discov. 2003;2:879-90.
7. Dahlen B, Szczeklik A, Murray JJ. Celecoxib in patients with asthma and aspirin intolerance. The Celecoxib in Aspirin-Intolerant Asthma Study Group. N Engl J Med. 2001;344:142.
8. Eder W, Ege MJ, von Mutius E. The asthma epidemic. N Engl J Med. 2006;355:2226-35.
9. Lundback B. Epidemiology of rhinitis and asthma. Clin Exp Allergy. 1998;28 Suppl 2:3-10.
10. Fireman P. Understanding asthma pathophysiology. Allergy Asthma Proc. 2003;24:79-83.
11. Blake K. Review of guidelines and the literature in the treatment of acute bronchospasm in asthma. Pharmacotherapy. 2006;26:148S-55S.
12. Handoyo S, Rosenwasser LJ. Asthma phenotypes. Curr Allergy Asthma Rep. 2009;9:439-45.
13. Almqvist C, Worm M, Leynaert B. Impact of gender on asthma in childhood and adolescence: a GA2LEN review. Allergy. 2008;63:47-57.
14. Tulic MK, Hamid Q. New insights into the pathophysiology of the small airways in asthma. Clin Chest Med. 2006;27:41-52, vi.
15. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004;59:469-78.
16. GINA workshop Report. Global strategy for asthma management and prevention [database on the Internet]. National institutes of Helth, National Heart, Lung and Blood Institute
17. Townley RG, Horiba M. Airway hyperresponsiveness: a story of mice and men and cytokines. Clin Rev Allergy Immunol. 2003;24:85-110.
18. Barnes PJ. Muscarinic receptor subtypes in airways. Life Sci. 1993;52:521-7.
19. Berend N, Salome CM, King GG. Mechanisms of airway hyperresponsiveness in asthma. Respirology. 2008;13:624-31.
20. Bates JH, Suki B. Assessment of peripheral lung mechanics. Respir Physiol Neurobiol. 2008;163:54-63.
21. An SS, Bai TR, Bates JH, Black JL, Brown RH, Brusasco V, et al. Airway smooth muscle dynamics: a common pathway of airway obstruction in asthma. Eur Respir J. 2007;29:834-60.
22. Black JL, Johnson PR. Factors controlling smooth muscle proliferation and airway remodelling. Curr Opin Allergy Clin Immunol. 2002;2:47-51.
23. Knox AJ, Pang L, Johnson S, Hamad A. Airway smooth muscle function in asthma. Clin Exp Allergy. 2000;30:606-14.
24. Dulin NO, Fernandes DJ, Dowell M, Bellam S, McConville J, Lakser O, et al. What evidence implicates airway smooth muscle in the cause of BHR? Clin Rev Allergy Immunol. 2003;24:73-84.
25. Busse WW, Lemanske RF, Jr. Asthma. N Engl J Med. 2001;344:350-62.
26. Hamid Q, Tulic M. Immunobiology of asthma. Annu Rev Physiol. 2009;71:489-507.
27. Kindt TG, R. Osborne, B. Kuby Immunology 6th ed ed. New York: W.H. Freeman and Company; 2007.
28. Ying S, Zhang G, Gu S, Zhao J. How much do we know about atopic asthma: where are we now? Cell Mol Immunol. 2006;3:321-32.
29. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. N Engl J Med. 2009;360:985-93.
30. Wenzel SE. Eosinophils in asthma--closing the loop or opening the door? N Engl J Med. 2009;360:1026-8.
31. Radinger M, Lotvall J. Eosinophil progenitors in allergy and asthma - do they matter? Pharmacol Ther. 2009;121:174-84.

32. Gleich GJ. The eosinophil and bronchial asthma: current understanding. J Allergy Clin Immunol. 1990;85:422-36.
33. Macdowell AL, Peters SP. Neutrophils in asthma. Curr Allergy Asthma Rep. 2007;7:464-8.
34. Sampson AP. The role of eosinophils and neutrophils in inflammation. Clin Exp Allergy. 2000;30 Suppl 1:22-7.
35. Forsythe P, Ennis M. Pharmacological regulation of mediator release from human mast cells. Clin Exp Allergy. 1998;28:1171-3.
36. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. N Engl J Med. 2002;346:1699-705.
37. Buckley MG, McEuen AR, Walls AF. The detection of mast cell subpopulations in formalin-fixed human tissues using a new monoclonal antibody specific for chymase. J Pathol. 1999;189:138-43.
38. Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. J Allergy Clin Immunol. 2006;117:1277-84.
39. Irani AA, Schechter NM, Craig SS, DeBlois G, Schwartz LB. Two types of human mast cells that have distinct neutral protease compositions. Proc Natl Acad Sci U S A. 1986;83:4464-8.
40. Andersson CK, Mori M, Bjermer L, Lofdahl CG, Erjefalt JS. Novel site-specific mast cell subpopulations in the human lung. Thorax. 2009;64:297-305.
41. Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. Nature. 2008;454:445-54.
42. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med. 2000;161:1720-45.
43. Tunon-De-Lara JM, Redington AE, Bradding P, Church MK, Hartley JA, Semper AE, et al. Dendritic cells in normal and asthmatic airways: expression of the alpha subunit of the high affinity immunoglobulin E receptor (Fc epsilon RI -alpha). Clin Exp Allergy. 1996;26:648-55.
44. Stingl G, Maurer D. IgE-mediated allergen presentation via Fc epsilon RI on antigen-presenting cells. Int Arch Allergy Immunol. 1997;113:24-9.
45. Campbell AM, Vachier I, Chanez P, Vignola AM, Lebel B, Kochan J, et al. Expression of the high-affinity receptor for IgE on bronchial epithelial cells of asthmatics. Am J Respir Cell Mol Biol. 1998;19:92-7.
46. O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic responses. Am Rev Respir Dis. 1987;136:740-51.
47. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 2001;294:1871-5.
48. Serhan CN. Lipoxin biosynthesis and its impact in inflammatory and vascular events. Biochim Biophys Acta. 1994;1212:1-25.
49. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem. 2000;69:145-82.
50. Capone ML, Tacconelli S, Di Francesco L, Sacchetti A, Sciulli MG, Patrignani P. Pharmacodynamic of cyclooxygenase inhibitors in humans. Prostaglandins Other Lipid Mediat. 2007;82:85-94.
51. Warner TD, Mitchell JA. Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. Faseb J. 2004;18:790-804.
52. Park GY, Christman JW. Involvement of cyclooxygenase-2 and prostaglandins in the molecular pathogenesis of inflammatory lung diseases. Am J Physiol Lung Cell Mol Physiol. 2006;290:L797-805.
53. Kudo I, Murakami M. Prostaglandin e synthase, a terminal enzyme for prostaglandin E2 biosynthesis. J Biochem Mol Biol. 2005;38:633-8.
54. Friesen RW, Mancini JA. Microsomal prostaglandin E2 synthase-1 (mPGES-1): a novel anti-inflammatory therapeutic target. J Med Chem. 2008;51:4059-67.
55. Xing L, Kurumbail RG, Frazier RB, Davies MS, Fujiwara H, Weinberg RA, et al. Homo-timeric structural model of human microsomal prostaglandin E synthase-1 and characterization of its substrate/inhibitor binding interactions. J Comput Aided Mol Des. 2008.
56. Murakami M, Nakatani Y, Tanioka T, Kudo I. Prostaglandin E synthase. Prostaglandins Other Lipid Mediat. 2002;68-69:383-99.
57. Coleman RA, Sheldrick RL. Prostanoid-induced contraction of human bronchial smooth muscle is mediated by TP-receptors. Br J Pharmacol. 1989;96:688-92.

58. Richter M, Sirois P. Effects of eicosanoids, neuromediators and bioactive peptides on murine airways. Eur J Pharmacol. 2000;389:225-34.
59. Pavord ID, Tattersfield AE. Bronchoprotective role for endogenous prostaglandin E2. Lancet. 1995;345:436-8.
60. Sheller JR, Mitchell D, Meyrick B, Oates J, Breyer R. EP(2) receptor mediates bronchodilation by PGE(2) in mice. J Appl Physiol. 2000;88:2214-8.
61. Tilley SL, Hartney JM, Erikson CJ, Jania C, Nguyen M, Stock J, et al. Receptors and pathways mediating the effects of prostaglandin E2 on airway tone. Am J Physiol Lung Cell Mol Physiol. 2003;284:L599-606.
62. Gauvreau GM, Watson RM, O'Byrne PM. Protective effects of inhaled PGE2 on allergen-induced airway responses and airway inflammation. Am J Respir Crit Care Med. 1999;159:31-6.
63. Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties, and functions. Physiol Rev. 1999;79:1193-226.
64. Coleman RA, Smith WL, Narumiya S. International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. Pharmacol Rev. 1994;46:205-29.
65. Idzko M, Hammad H, van Nimwegen M, Kool M, Vos N, Hoogsteden HC, et al. Inhaled iloprost suppresses the cardinal features of asthma via inhibition of airway dendritic cell function. J Clin Invest. 2007;117:464-72.
66. Takahashi Y, Tokuoka S, Masuda T, Hirano Y, Nagao M, Tanaka H, et al. Augmentation of allergic inflammation in prostanoid IP receptor deficient mice. Br J Pharmacol. 2002;137:315-22.
67. Nagao K, Tanaka H, Komai M, Masuda T, Narumiya S, Nagai H. Role of prostaglandin I2 in airway remodeling induced by repeated allergen challenge in mice. Am J Respir Cell Mol Biol. 2003;29:314-20.
68. Mathe AA, Hedqvist P. Effect of prostaglandins F2 alpha and E2 on airway conductance in healthy subjects and asthmatic patients. Am Rev Respir Dis. 1975;111:313-20.
69. Mathe AA, Hedqvist P, Holmgren A, Svanborg N. Bronchial hyperreactivity to prostaglandin F 2 and histamine in patients with asthma. Br Med J. 1973;1:193-6.
70. Devillier P, Bessard G. Thromboxane A2 and related prostaglandins in airways. Fundam Clin Pharmacol. 1997;11:2-18.
71. Sugimoto Y, Hasumoto K, Namba T, Irie A, Katsuyama M, Negishi M, et al. Cloning and expression of a cDNA for mouse prostaglandin F receptor. J Biol Chem. 1994;269:1356-60.
72. DeWitt DL, Smith WL. Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. Proc Natl Acad Sci U S A. 1988;85:1412-6.
73. Rome LH, Lands WE. Structural requirements for time-dependent inhibition of prostaglandin biosynthesis by anti-inflammatory drugs. Proc Natl Acad Sci U S A. 1975;72:4863-5.
74. McCarty MF, Block KI. Preadministration of high-dose salicylates, suppressors of NF-kappaB activation, may increase the chemosensitivity of many cancers: an example of proapoptotic signal modulation therapy. Integr Cancer Ther. 2006;5:252-68.
75. Niederberger E, Manderscheid C, Geisslinger G. Different COX-independent effects of the COX-2 inhibitors etoricoxib and lumiracoxib. Biochem Biophys Res Commun. 2006;342:940-8.
76. Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. Nature. 1998;396:77-80.
77. Hata AN, Lybrand TP, Breyer RM. Identification of determinants of ligand binding affinity and selectivity in the prostaglandin D2 receptor CRTH2. J Biol Chem. 2005;280:32442-51.
78. Hata AN, Zent R, Breyer MD, Breyer RM. Expression and molecular pharmacology of the mouse CRTH2 receptor. J Pharmacol Exp Ther. 2003;306:463-70.
79. Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med. 1999;340:1888-99.
80. Vane JR, Botting RM. Anti-inflammatory drugs and their mechanism of action. Inflamm Res. 1998;47 Suppl 2:S78-87.
81. Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, et al. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. JAMA. 2000;284:1247-55.

82. Crofford LJ, Lipsky PE, Brooks P, Abramson SB, Simon LS, van de Putte LB. Basic biology and clinical application of specific cyclooxygenase-2 inhibitors. Arthritis Rheum. 2000;43:4-13.
83. Verburg KM, Maziasz TJ, Weiner E, Loose L, Geis GS, Isakson PC. Cox-2-specific inhibitors: definition of a new therapeutic concept. Am J Ther. 2001;8:49-64.
84. Weir MR, Sperling RS, Reicin A, Gertz BJ. Selective COX-2 inhibition and cardiovascular effects: a review of the rofecoxib development program. Am Heart J. 2003;146:591-604.
85. Iyer JP, Srivastava PK, Dev R, Dastidar SG, Ray A. Prostaglandin E(2) synthase inhibition as a therapeutic target. Expert Opin Ther Targets. 2009;13:849-65.
86. Gyllfors P, Bochenek G, Overholt J, Drupka D, Kumlin M, Sheller J, et al. Biochemical and clinical evidence that aspirin-intolerant asthmatic subjects tolerate the cyclooxygenase 2-selective analgetic drug celecoxib. J Allergy Clin Immunol. 2003;111:1116-21.
87. Catella-Lawson F, McAdam B, Morrison BW, Kapoor S, Kujubu D, Antes L, et al. Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. J Pharmacol Exp Ther. 1999;289:735-41.
88. McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, FitzGerald GA. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. Proc Natl Acad Sci U S A. 1999;96:272-7.
89. Ayoub SS, Botting RM, Goorha S, Colville-Nash PR, Willoughby DA, Ballou LR. Acetaminophen-induced hypothermia in mice is mediated by a prostaglandin endoperoxide synthase 1 gene-derived protein. Proc Natl Acad Sci U S A. 2004;101:11165-9.
90. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci U S A. 1993;90:11693-7.
91. Esser R, Berry C, Du Z, Dawson J, Fox A, Fujimoto RA, et al. Preclinical pharmacology of lumiracoxib: a novel selective inhibitor of cyclooxygenase-2. Br J Pharmacol. 2005;144:538-50.
92. Ochi T, Motoyama Y, Goto T. The analgesic effect profile of FR122047, a selective cyclooxygenase-1 inhibitor, in chemical nociceptive models. Eur J Pharmacol. 2000;391:49-54.
93. Thien FC, Walters EH. Eicosanoids and asthma: an update. Prostaglandins Leukot Essent Fatty Acids. 1995;52:271-88.
94. Peters-Golden M, Brock TG. Intracellular compartmentalization of leukotriene synthesis: unexpected nuclear secrets. FEBS Lett. 2001;487:323-6.
95. Peters-Golden M, Henderson WR, Jr. Leukotrienes. N Engl J Med. 2007;357:1841-54.
96. Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. J Allergy Clin Immunol. 2003;111:450-63; quiz 64.
97. Smit JJ, Lukacs NW. A closer look at chemokines and their role in asthmatic responses. Eur J Pharmacol. 2006;533:277-88.
98. Wahl SM, Wong H, McCartney-Francis N. Role of growth factors in inflammation and repair. J Cell Biochem. 1989;40:193-9.
99. Bosse Y, Rola-Pleszczynski M. FGF2 in asthmatic airway-smooth-muscle-cell hyperplasia. Trends Mol Med. 2008;14:3-11.
100. Voelkel NF, Vandivier RW, Tuder RM. Vascular endothelial growth factor in the lung. Am J Physiol Lung Cell Mol Physiol. 2006;290:L209-21.
101. Zosky GR, Sly PD. Animal models of asthma. Clin Exp Allergy. 2007;37:973-88.
102. Kips JC, Anderson GP, Fredberg JJ, Herz U, Inman MD, Jordana M, et al. Murine models of asthma. Eur Respir J. 2003;22:374-82.
103. Chang YS, Kim YK, Bahn JW, Kim SH, Park HW, Kim TB, et al. Comparison of asthma phenotypes using different sensitizing protocols in mice. Korean J Intern Med. 2005;20:152-8.
104. Nelde A, Teufel M, Hahn C, Duschl A, Sebald W, Brocker EB, et al. The impact of the route and frequency of antigen exposure on the IgE response in allergy. Int Arch Allergy Immunol. 2001;124:461-9.
105. Nials AT, Uddin S. Mouse models of allergic asthma: acute and chronic allergen challenge. Dis Model Mech. 2008;1:213-20.
106. Epstein MM. Do mouse models of allergic asthma mimic clinical disease? Int Arch Allergy Immunol. 2004;133:84-100.
107. Gueders MM, Paulissen G, Crahay C, Quesada-Calvo F, Hacha J, Van Hove C, et al. Mouse models of asthma: a comparison between C57BL/6 and BALB/c strains regarding bronchial responsiveness, inflammation, and cytokine production. Inflamm Res. 2009.

108. Irvin CG, Bates JH. Measuring the lung function in the mouse: the challenge of size. Respir Res. 2003;4:4.
109. Bates JH, Lutchen KR. The interface between measurement and modeling of peripheral lung mechanics. Respir Physiol Neurobiol. 2005;148:153-64.
110. Bates JH, Irvin CG. Measuring lung function in mice: the phenotyping uncertainty principle. J Appl Physiol. 2003;94:1297-306.
111. Gomes RF, Shen X, Ramchandani R, Tepper RS, Bates JH. Comparative respiratory system mechanics in rodents. J Appl Physiol. 2000;89:908-16.
112. Hantos Z, Daroczy B, Suki B, Nagy S, Fredberg JJ. Input impedance and peripheral inhomogeneity of dog lungs. J Appl Physiol. 1992;72:168-78.
113. Tomioka S, Bates JH, Irvin CG. Airway and tissue mechanics in a murine model of asthma: alveolar capsule vs. forced oscillations. J Appl Physiol. 2002;93:263-70.
114. Barnas GM, Ho G, Green MD, Harinath P, Smalley AJ, Campbell DN, et al. Effects of analysis method and forcing waveform on measurement of respiratory mechanics. Respir Physiol. 1992;89:273-85.
115. Neimert-Andersson T, Thunberg S, Swedin L, Wiedermann U, Jacobsson-Ekman G, Dahlen SE, et al. Carbohydrate-based particles reduce allergic inflammation in a mouse model for cat allergy. Allergy. 2008;63:518-26.
116. Ellis R, Leigh R, Southam D, O'Byrne PM, Inman MD. Morphometric analysis of mouse airways after chronic allergen challenge. Lab Invest. 2003;83:1285-91.
117. Leigh R, Ellis R, Wattie J, Southam DS, De Hoogh M, Gauldie J, et al. Dysfunction and remodeling of the mouse airway persist after resolution of acute allergen-induced airway inflammation. Am J Respir Cell Mol Biol. 2002;27:526-35.
118. Leigh R, Ellis R, Wattie JN, Hirota JA, Matthaei KI, Foster PS, et al. Type 2 cytokines in the pathogenesis of sustained airway dysfunction and airway remodeling in mice. Am J Respir Crit Care Med. 2004;169:860-7.
119. Leigh R, Southam DS, Ellis R, Wattie JN, Sehmi R, Wan Y, et al. T-cell-mediated inflammation does not contribute to the maintenance of airway dysfunction in mice. J Appl Physiol. 2004;97:2258-65.
120. Southam DS, Ellis R, Wattie J, Inman MD. Components of airway hyperresponsiveness and their associations with inflammation and remodeling in mice. J Allergy Clin Immunol. 2007;119:848-54.
121. Inman MD, Ellis R, Wattie J, Denburg JA, O'Byrne PM. Allergen-induced increase in airway responsiveness, airway eosinophilia, and bone-marrow eosinophil progenitors in mice. Am J Respir Cell Mol Biol. 1999;21:473-9.
122. Kumar RK, Herbert C, Foster PS. The "classical" ovalbumin challenge model of asthma in mice. Curr Drug Targets. 2008;9:485-94.
123. Boost KA, Flondor M, Hofstetter C, Platacis I, Stegewerth K, Hoegl S, et al. The beta-adrenoceptor antagonist propranolol counteracts anti-inflammatory effects of isoflurane in rat endotoxemia. Acta Anaesthesiol Scand. 2007;51:900-8.
124. Reutershan J, Chang D, Hayes JK, Ley K. Protective effects of isoflurane pretreatment in endotoxin-induced lung injury. Anesthesiology. 2006;104:511-7.
125. Wagers SS, Haverkamp HC, Bates JH, Norton RJ, Thompson-Figueroa JA, Sullivan MJ, et al. Intrinsic and antigen-induced airway hyperresponsiveness are the result of diverse physiological mechanisms. J Appl Physiol. 2007;102:221-30.
126. Whitehead GS, Walker JK, Berman KG, Foster WM, Schwartz DA. Allergen-induced airway disease is mouse strain dependent. Am J Physiol Lung Cell Mol Physiol. 2003;285:L32-42.
127. Zhang Y, Lamm WJ, Albert RK, Chi EY, Henderson WR, Jr., Lewis DB. Influence of the route of allergen administration and genetic background on the murine allergic pulmonary response. Am J Respir Crit Care Med. 1997;155:661-9.
128. Jonasson S, Hedenstierna G, Hedenstrom H, Hjoberg J. Comparisons of effects of intravenous and inhaled methacholine on airway physiology in a murine asthma model. Respir Physiol Neurobiol. 2009;165:229-36.
129. Zhu W, Gilmour MI. Comparison of allergic lung disease in three mouse strains after systemic or mucosal sensitization with ovalbumin antigen. Immunogenetics. 2009;61:199-207.
130. Zosky GR, Larcombe AN, White OJ, Burchell JT, von Garnier C, Holt PG, et al. Airway hyperresponsiveness is associated with activated CD4+ T cells in the airways. Am J Physiol Lung Cell Mol Physiol. 2009;297:L373-9.

131. Lundequist A, Nallamshetty SN, Xing W, Feng C, Laidlaw TM, Uematsu S, et al. Prostaglandin E(2) exerts homeostatic regulation of pulmonary vascular remodeling in allergic airway inflammation. J Immunol.184:433-41.
132. Hartert TV, Dworski RT, Mellen BG, Oates JA, Murray JJ, Sheller JR. Prostaglandin E(2) decreases allergen-stimulated release of prostaglandin D(2) in airways of subjects with asthma. Am J Respir Crit Care Med. 2000;162:637-40.
133. Schulman ES, Newball HH, Demers LM, Fitzpatrick FA, Adkinson NF, Jr. Anaphylactic release of thromboxane A2, prostaglandin D2, and prostacyclin from human lung parenchyma. Am Rev Respir Dis. 1981;124:402-6.
134. Dahlen SE, Hansson G, Hedqvist P, Bjorck T, Granstrom E, Dahlen B. Allergen challenge of lung tissue from asthmatics elicits bronchial contraction that correlates with the release of leukotrienes C4, D4, and E4. Proc Natl Acad Sci U S A. 1983;80:1712-6.
135. Palmqvist C, Wardlaw AJ, Bradding P. Chemokines and their receptors as potential targets for the treatment of asthma. Br J Pharmacol. 2007;151:725-36.
136. Erjefalt JS, Uller L, Malm-Erjefalt M, Persson CG. Rapid and efficient clearance of airway tissue granulocytes through transepithelial migration. Thorax. 2004;59:136-43.
137. Uller L, Lloyd CM, Rydell-Tormanen K, Persson CG, Erjefalt JS. Effects of steroid treatment on lung CC chemokines, apoptosis and transepithelial cell clearance during development and resolution of allergic airway inflammation. Clin Exp Allergy. 2006;36:111-21.
138. Williams CM, Galli SJ. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. J Exp Med. 2000;192:455-62.
139. Martin TR, Takeishi T, Katz HR, Austen KF, Drazen JM, Galli SJ. Mast cell activation enhances airway responsiveness to methacholine in the mouse. J Clin Invest. 1993;91:1176-82.
140. Hakonarson H, Halapi E, Whelan R, Gulcher J, Stefansson K, Grunstein MM. Association between IL-1beta/TNF-alpha-induced glucocorticoid-sensitive changes in multiple gene expression and altered responsiveness in airway smooth muscle. Am J Respir Cell Mol Biol. 2001;25:761-71.
141. Slats AM, Janssen K, van Schadewijk A, van der Plas DT, Schot R, van den Aardweg JG, et al. Expression of smooth muscle and extracellular matrix proteins in relation to airway function in asthma. J Allergy Clin Immunol. 2008;121:1196-202.
142. Wardlaw AJ, Brightling CE, Green R, Woltmann G, Bradding P, Pavord ID. New insights into the relationship between airway inflammation and asthma. Clin Sci (Lond). 2002;103:201-11.
143. Knight DA, Asokananthan N, Watkins DN, Misso NL, Thompson PJ, Stewart GA. Oncostatin M synergises with house dust mite proteases to induce the production of PGE(2) from cultured lung epithelial cells. Br J Pharmacol. 2000;131:465-72.
144. Knight DA, Stewart GA, Thompson PJ. The respiratory epithelium and airway smooth muscle homeostasis: its relevance to asthma. Clin Exp Allergy. 1994;24:698-706.
145. Guerrero MD, Aquino M, Bruno I, Riccio R, Terencio MC, Paya M. Anti-inflammatory and analgesic activity of a novel inhibitor of microsomal prostaglandin E synthase-1 expression. Eur J Pharmacol. 2009;620:112-9.
146. Trebino CE, Stock JL, Gibbons CP, Naiman BM, Wachtmann TS, Umland JP, et al. Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. Proc Natl Acad Sci U S A. 2003;100:9044-9.
147. Kojima F, Kapoor M, Yang L, Fleishaker EL, Ward MR, Monrad SU, et al. Defective generation of a humoral immune response is associated with a reduced incidence and severity of collagen-induced arthritis in microsomal prostaglandin E synthase-1 null mice. J Immunol. 2008;180:8361-8.
148. Jakobsson PJ, Thoren S, Morgenstern R, Samuelsson B. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc Natl Acad Sci U S A. 1999;96:7220-5.
149. Samuelsson B, Morgenstern R, Jakobsson PJ. Membrane prostaglandin E synthase-1: a novel therapeutic target. Pharmacol Rev. 2007;59:207-24.
150. Krug N, Rabe KF. Animal models for human asthma: the perspective of a clinician. Curr Drug Targets. 2008;9:438-42.
151. Melillo E, Woolley KL, Manning PJ, Watson RM, O'Byrne PM. Effect of inhaled PGE2 on exercise-induced bronchoconstriction in asthmatic subjects. Am J Respir Crit Care Med. 1994;149:1138-41.

152. Pavord ID, Wong CS, Williams J, Tattersfield AE. Effect of inhaled prostaglandin E2 on allergen-induced asthma. Am Rev Respir Dis. 1993;148:87-90.
153. Sestini P, Armetti L, Gambaro G, Pieroni MG, Refini RM, Sala A, et al. Inhaled PGE2 prevents aspirin-induced bronchoconstriction and urinary LTE4 excretion in aspirin-sensitive asthma. Am J Respir Crit Care Med. 1996;153:572-5.
154. Wenzel S, Holgate ST. The mouse trap: It still yields few answers in asthma. Am J Respir Crit Care Med. 2006;174:1173-6; discussion 6-8.
155. Persson CG. Con: mice are not a good model of human airway disease. Am J Respir Crit Care Med. 2002;166:6-7; discussion 8.