Mast Cell-Mediated Orchestration of Airway Epithelial Responses in Chronic Respiratory Diseases

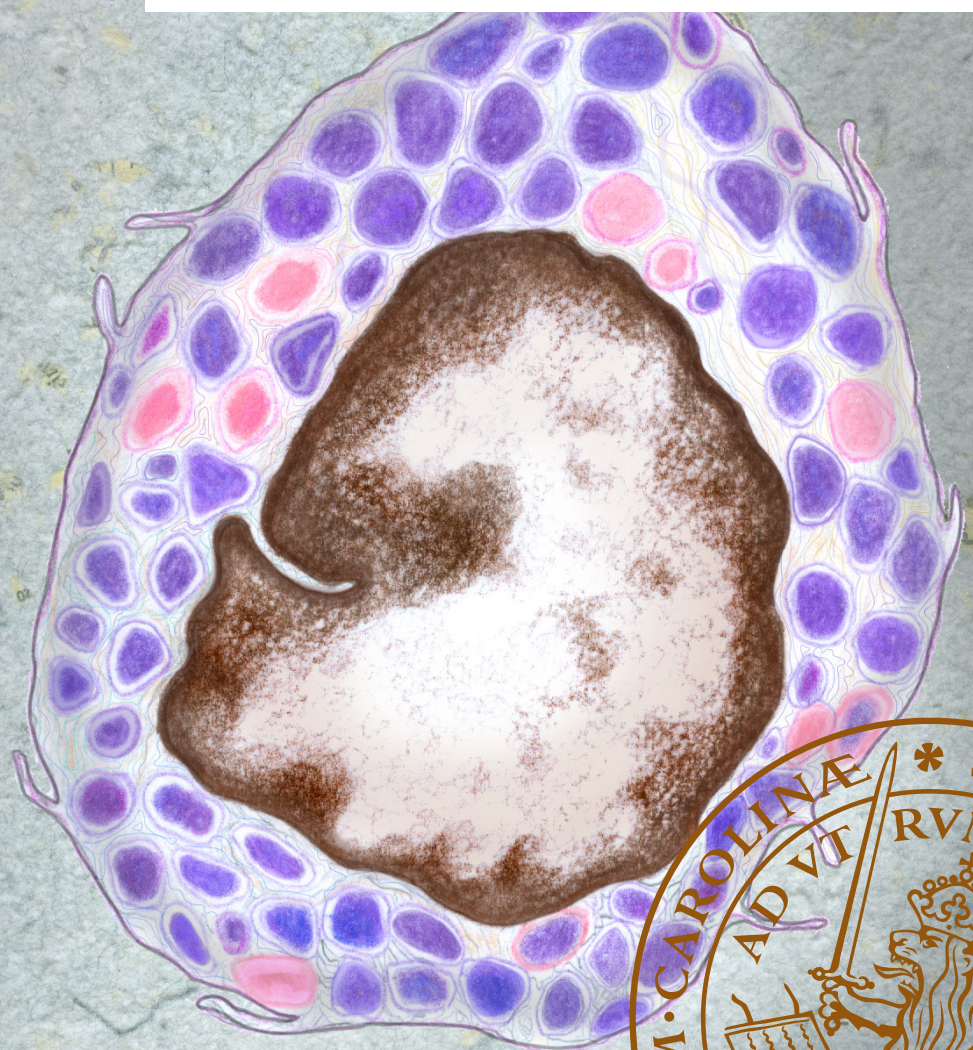
Berlin, Frida

2023

Document Version:
Publisher's PDF, also known as Version of record

Mast Cell-Mediated Orchestration of Airway Epithelial Responses in Chronic Respiratory Diseases

FRIDA BERLIN
EXPERIMENTAL MEDICAL SCIENCE | FACULTY OF MEDICINE | LUND UNIVERSITY



Printed by Media-Tryck, Lund 2023 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Experimental Medical Science

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2023:101
ISBN 978-91-8021-441-4
ISSN 1652-8220

Mast Cell-Mediated Orchestration of Airway Epithelial Responses in Chronic Respiratory Diseases

Mast Cell-Mediated Orchestration of Airway Epithelial Responses in Chronic Respiratory Diseases

Frida Berlin

LUNDS UNIVERSITET

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Experimental Medical Science at Lund University to be publicly defended on the 7th of September at 09.15 in Belfragesalen, BMC D15, Department of Medicine, Klinikgatan 32, Lund

Faculty opponent
Dr. Sara Wernersson,
Associate Professor of Molecular Inflammation Research
Swedish University of Agricultural Science, Sweden

Organization: LUND UNIVERSITY **Document name:** Doctoral Dissertation

Date of issue 2023-09-07

Author(s): Frida Berlin **Sponsoring organization:**

Title and subtitle: Mast Cell-Mediated Orchestration of Airway Epithelial Responses in Chronic Respiratory Diseases

Abstract:

Chronic respiratory diseases, such as asthma, are an increaseing health issue worldwide and cause about 3.9 million deaths annually. Despite this, little is know about the molecular mechanisms underpinning disease pathogenesis. Bronchial and alveolar remodeling and impaired epithelial function are typical characteristics of chronic respiratory diseases. In these patients, an increased number of mast cells, positive for the serine proteases; tryptase and chymase, infiltrate the epithelium and the alveolar parenchyma. While it is likely that the epithelial cells are exposed to various amounts of released tryptase and chymase, the interaction between mast cells and epithelial cells remains unknown. This thesis aimed to investigate the impact of mast cell proteases on bronchial and alveolar remodelling. Human bronchial and alveolar epithelial cells were treated with tryptase and chymase. Holographic live cell imaging, fluorescent microscopy, and gene and protein assays were used to analyze various parameters such as proliferation patterns, protein expressions and distributions. The results showed that both tryptase and chymase promoted epithelial remodelling in several ways. Tryptase induced cell growth, cell survival, and wound healing, whereas chymase reduced cell growth, altered cell morphology and impaired epithelial barrier proprties. In conclusion, our results suggest that intraepithelial and alveolar mast cell release of proteases plays a crucial role in epithelial homeostasis, and that an inbalance of the protease release may be involved in respiratory disease progression and in disruption of critical epithelial functions.

Key words: Mast cells, proteases, tryptase, chymase, chronic respiratory diseases, asthma

Classification system and/or index terms (if any)

Supplementary bibliographical information **Language:** English

ISSN and key title: 1652-8220, Lund University, Faculty of Medicine Doctoral Dissertation Series 2023:101

ISBN: 978-91-8021-441-4

Recipient's notes **Number of pages**: 64

Price Security classification

Signature Date 2023-07-26

Mast Cell-Mediated Orchestration of Airway Epithelial Responses in Chronic Respiratory Diseases

Frida Berlin

LUNDS
UNIVERSITET

Cover photo: Mast cell with intracellular granula, illustrated by Gunnel Berlin

Faculty of Medicine
Department of Experimental Medical Science

ISSN 1652-8220
ISBN 978-91-8021-441-4
Lund University, Faculty of Medicine Doctoral Dissertation Series 2023:101

Printed in Sweden by Media-Tryck, Lund University
Lund 2023

“If we knew what it was we were doing,
it would not be called research, would it?

- Albert Einstein

Table of Content

List of Papers **10**
Additional peer-reviewed papers, not included in the thesis **11**
Abbreviations **12**
Introduction **13**
The Mast Cell **13**
Origin of Mast Cells 13
Role of Mast Cells in Healthy Conditions 13
Activation 14
Phenotypes 16
Respiratory Diseases **17**
Asthma 18
Mast Cells in Diseases **19**
Tryptase 20
Chymase 22
Experimental Research Models 23
The Airway Epithelium **24**
The Lung Epithelium and the Role of Epithelial Cells 24
Epithelial Alterations in Asthma 25
Aims of the Thesis **28**
Methodology **29**
Cell Culture Models **29**
Bronchial Epithelial Cell Line: Beas-2B 29
Bronchial Epithelial Cell Line: BCi-NS1.1 29
Alveolar Epithelial Cell Line: A549 30
Primary Bronchial Epithelial Cells 30
ALI Cultures 30
In Vitro Cell Stimulation **31**
Tryptase and Chymase 31
PAR-2 inhibitor 32
Pro-inflammatory and Viral Stimulations 32
Experimental Set-Up **32**
Holographic Live-Cell Imaging **33**

Immunocytochemsitry **34**
Submerged cultures 34
Differentiated ALI cultures 34
Results **35**
Tryptase **35**
Tryptase-mediated Effects on Cell Morphology, Migration and Wound Healing Capacity in BECs 35
Tryptase Promotes Cell Growth in Alveolar and Bronchial Epithelial Cells 36
Increased Epithelial Release of GFs and Expression of Pro-Survival Proteins in Tryptase-Stimulated Cells 37
Chymase **38**
Chymase Altered Cell Morphology, Enhanced Cell Migration, and Decreased Cell Growth 38
Chymase-Mediated Disruption of Barrier Integrity 38
Summary of Results **39**
Conclusions **41**
Discussion **42**
Morphology and Migration **42**
Dual Roles in Epithelial Homeostasis **43**
Chymase Disrupts Bronchial Epithelial Barrier Properties **44**
Advantages and Limitations with the Experimental Design **45**
The Significance of Our Findings **46**
Future Perspectives **48**
Identify the relevant molecular mechanisms 48
The role of MCs in epithelial barrier disruption 48
Explore other MC-associated proteins 49
Inhibition of specific targets? 49
Populärvetenskaplig Sammanfattning **50**
Acknowledgement **52**
References **55**

List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I. **Berlin F,** Mogren S, Tutzauer J, and Andersson C.K. Mast Cell Proteases Tryptase and Chymase Induce Migratory and Morphological Alterations in Bronchial Epithelial Cells. *International Journal of Molecular Science* 2021, 22(10):5250.

II. Mogren S, **Berlin F**, Ramu S, Sverrild A, Porsbjerg C, Uller L and Andersson CK. Mast cell tryptase enhances wound healing by promoting migration in human bronchial epithelial cells. *Cell Adhesion and Migration* 2021, 15(1): 202-214.

III. **Berlin F,** Mogren S, Ly C, Ramu S, Hvidtfeldt M, Uller L, Porsbjerg C, and Andersson CK. Mast cell tryptase promotes airway remodeling by inducing anti-apoptotic and cell growth properties in human alveolar and bronchial epithelial cells. *Cells* 2023, 12(10):1439.

IV. **Berlin F,** Eminton A, Horton KL, Ridley R, Blume C, Naftel J, Rupani H, Swindle EJ* and Andersson CK*. Mast Cell Chymase Disrupts Airway Barrier Function in Healthy and Severe Asthma patients. (Manuscript).

* These authors contributed equally to this work.

Additional peer-reviewed papers, not included in the thesis

- Kadefors M, **Berlin F**, Wildt M, Dellgren G, Rolandsson Enes S, Aspberg A, Westergren-Thorsson G. Dipeptidyl peptidase 4 expression is not associated with an activated fibroblast phenotype in idiopathic pulmonary fibrosis. *Frontiers in Pharmacology* 2022, 31;13:953771.

- Mogren S, **Berlin F**, Eskilsson L, Van Der Burg N, Tufvesson E, Andersson CK. Mast Cell Proteases Promote Diverse Effects on the Plasminogen Activation System and Wound Healing in A549 Alveolar Epithelial Cells. *Cells* 2022, 18;11(18):2916.

- Callesen KT, Mogren S, **Berlin F**, Andersson C, Schmidt S, Klitfod L, Esteban V, Poulsen LK, Jensen BM. Characterization of Mast Cells from Healthy and Varicose Human Saphenous Vein. *Biomedicines* 2022; 10(5):1062.

- Ramu S, Akbarshahi H, Mogren S, **Berlin F**, Cerps S, Menzel M, Hvidtfeldt M, Porsbjerg C, Uller L, Andersson CK. Direct effects of mast cell proteases, tryptase and chymase, on bronchial epithelial integrity proteins and anti-viral responses. *BMC Immunology* 2021, 2;22(1):35.

Abbreviations

ALI	Air-liquid interface
BAL	Bronchoalveolar lavage
BEC	Bronchial epithelial cell
bFGF	Basic fibroblast growth factor
CPA3	Carboxypeptidase A
ECM	Extracellular matrix
FeNO	Fractional exhaled nitric oxide
GF	Growth factor
Ig	Immunoglobulin
ILC2	Type 2 innate lymphoid cells
MC	Mast cell
mMCP	mouse MC protease
MMP	Matrix metalloproteinase
PAR-2	Protease-activator receptor-2
PBEC	Primary bronchial epithelial cell
PDGF-AA	Platelet-derived growth factor-AA
TGF	Transforming growth factor
Th2	T helper 2
TLR	Toll-Like receptor
VEGF	Vascular endothelial growth factor

Introduction

The Mast Cell

Origin of Mast Cells

Mast cells (MCs) were first discovered by the German scientist Paul Erlich in 1878 and were later recognized for their multifunctional roles in processes such as homeostasis and pathogen defence (1, 2). MCs originate from pluripotent $CD34^+$ hematopoietic precursors in the bone marrow and circulate in the bloodstream as immature precursors. When the precursors home into the target tissue, they undergo terminal differentiation. This cell differentiation, or maturation, is highly regulated by the local environment, and is particularly dependent on tissue-specific growth factors (GFs) and cytokines. An essential GF for MC survival is the stem cell factor, which can be expressed and released by various structural cells, such as epithelial cells (3-6). MCs are widely distributed in our bodies and are typically located at the interface between the host and the external world, such as the gut, skin, and lungs (7, 8).

MCs have played a crucial function in the innate immunity of animals throughout evolutionary history. These immune cells appeared early in evolution and have since evolved into multifunctional cells in vertebrates. The development of MCs can be traced back to an ancestor approximately 450-500 million years ago, which includes hagfish, lampreys, and sharks. In invertebrates, granular haemocytes resembling primitive versions of MCs have been identified in species such as Arthropoda. This evolutionary conservation highlights the critical role of MCs in immune responses, pathogen defence, and homeostatic maintenance (1, 9).

Role of Mast Cells in Healthy Conditions

MCs are strategically located in the connective tissue and mucosal surfaces in close proximity to the outside world, which indicates their vital role in recognizing and responding to environmental insults and tissue injury (1, 6): due to their location and fast response, MCs are among the first cells to react to harmful compounds and initiate an immune response. MCs have a great number of receptors situated on their plasma membranes and in the cytosol, and these allow them to detect many different

stimuli as part of both innate and acquired immunity. MCs express pattern recognition receptors (PRRs), including toll-like receptors (TLRs) and NOD-like receptors, that are receptors that recognize different kinds of microbial products, viruses and bacteria and thus play a role in innate immunity. The antibody receptor for immunoglobulin E (IgE), FcεRI, is located on the MC membrane surface and plays a role in acquired immunity (4, 10). Additionally, MCs can respond to various endogenous stimuli, such as IL-33 and ATP that are released upon tissue injury. MCs contribute to all stages of wound healing, including acute inflammation, proliferation, and remodelling, which are required to close and heal the wound gaps (11).

Unlike many other innate immune cells, MCs are long-lived and can survive in the body for months or even years. Upon recognition and activation, MCs respond by releasing cytosolic granules that contain a wide range of powerful proteins. After this so-called degranulation, they can recover and replenish their granules within just a few days (12, 13). Consequently, MCs play significant roles in host defence and tissue repair, and, interestingly, congenital MC deficiency has never been reported, which indicates their essential role in maintaining healthy tissues (1, 14).

In the human lung, MCs are found in the whole respiratory system, including the upper airways, bronchi, bronchioles, and the alveolar parenchyma in the lower airways (15) with an increased density from the central airways to the peripheral compartments (15, 16). The preliminary role of lung MCs is to actively maintain the healthy lung by protecting against infection by respiratory pathogens.

Activation

Because MCs express a vast number of receptors, their activation can be mediated through various kinds of stimuli. When activated, MCs release their secretory cytoplasmic granulates. These granules can be released either by anaphylactic degranulation, with complete granule release, or piecemeal degranulation, where small amounts of mediators are released without complete degranulation. Both IgE-mediated and non-IgE-mediated activation can cause the release of various immunologically active substances which will be explained below (2, 17).

FcεRI

MCs express large numbers of FcεRIs on their cell surface, estimated to amount to about 500,000 receptors per cell, but may vary depending on the circulating concentration of IgE (18). When one multivalent allergen binds to two IgE antibodies, which in turn are bound to a high-affinity FcεRI each, the allergen crosslinks the IgE on the MC surface (Figure 1). Crosslinking of IgE receptors induce an intracellular signal transduction pathway, leading to activation and degranulation by initiate fusion and the subsequent exocytosis of granules. This

degranulation includes release of granules containing pre-formed mediators, such as proteases (tryptase and chymase), histamine, TNF-α, as well as *de novo* production of lipid mediators, such as prostaglandins and leukotrienes and various cytokines and chemokines. This type of activation usually results in bronchoconstriction, plasma extravasation, recruitment of inflammatory cells and inflammation and is typically associated with immediate hypersensitivity reactions and allergic responses (19-21).

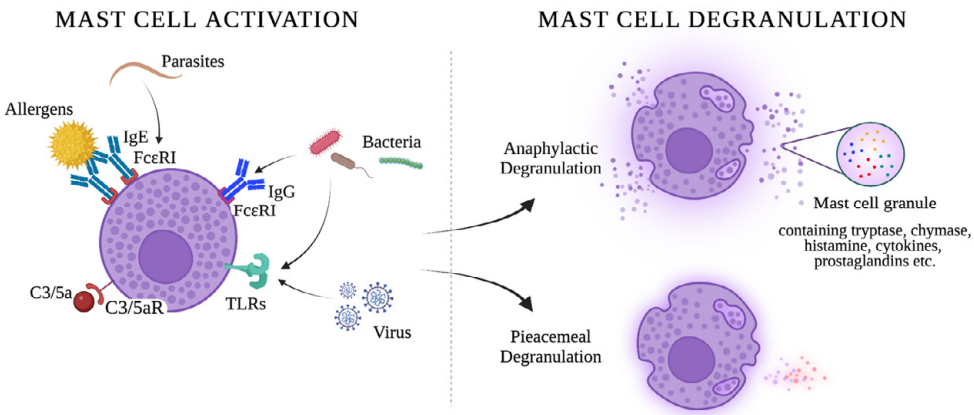


Figure 1. Illustration of different stimuli and surface receptors that can activate mast cells (left), which induces either anaphylactic degranulation or piecemeal degranulation (right).

Previous investigations have been conducted to gain insights into other biological roles of FcεRI activation. One study has for example shown the involvement of IgE-mediated response in parasite defence. IgE-deficient mice exhibited a disturbed defence against intestinal parasites and increased numbers of parasite larvae in the skeletal muscles, in comparison to their wild type controls (22). This implies that IgE-mediated activation is important for parasite elimination and that MCs are vital for regulating homeostasis *in vivo*, although they are well recognized for their role in allergic responses.

Non-FcεRI activation

Besides FcεRI activation, MCs can be activated by other immunoglobulins as well. IgG, for example, can bind to the FcγRs on the MC surface (Figure 1), a process involved in allergic reactions as well as in the recognition of bacteria and parasite expulsion (12, 23, 24).

As part of the innate immune response, MCs can also recognize, and be directly activated by, invasive pathogens through PRR-dependent and complement-

dependent pathways. TLRs recognize pathogen-associated molecular patterns (PAMPs) such as different types of bacteria and viruses (25). Generally, for human MCs, TLR1, TLR2, TLR4, TLR5 and TLR6 are situated on the cell surface and recognize bacteria and fungi, whereas TLR3, TLR7, and TLR9 are located in the cytosol and are involved in virus recognition (Figure 1). Upon TLR activation, distinct profiles of MC-derived mediators and cytokines are released, usually without degranulation, depending on the specific TLR subtype. Some central cytokines included in TLR activation are TNF-α, IL-6, IL-13, and IL-1β. Importantly, the individual expression patterns of TLRs may differ depending on the localisation of the MC (26, 27).

C3a and C5a belong to the complement system and can initiate degranulation and chemotaxis of MCs via the C3aR and C5aR receptors. These compounds induce degranulation particularly of MC_{TC} (27-29). Moreover, MCs can respond to other host endogenous compounds, including neurotensin, substance P, and endothelin 1 (30-32).

Phenotypes

MCs are known for their heterogeneity, even within the same organ. Therefore, their intracellular granules, which contain a wide range of proteins, are highly influenced by the local environment, and the MC content may vary a lot depending on their localization. Along with the granules, the ultrastructure, immunological and non-immunological activation, and receptor expression, exhibit marked heterogeneity; up to 50% of the MC granules consist of proteases, which therefore makes them the majority fraction of the MC protein content (33). Among these proteases, β-tryptase is the predominant protease in human MCs (34). Chymase may also be present in large quantities in some MC populations. Due to these different expression profiles, human MCs have been defined into two major phenotypes based on their granule content, namely MC_T, which is tryptase positive, and MC_{TC}, which is tryptase, chymase, and carboxypeptidase A (CPA3) positive (4, 35, 36). In healthy human lungs, MC_T is the predominant phenotype, and is found in structures including the bronchial mucosa and alveolar parenchyma (37, 38). In contrast, the MC_{TC} phenotype is typically found in the connective tissue, such as the skin.

The different levels of protease expression in MCs and their anatomical locations implies that they have distinct functions, but these particular roles remain poorly defined (38). In the pathological conditions of respiratory diseases, a disturbed MC balance has been observed; the numbers of MCs are vastly elevated, they are found in locations that usually have very few MCs (e.g., the bronchial epithelium), and they seem to undergo a phenotypic switch from MC_T to MC_{TC}. However, the underpinning mechanisms and implications of these events are less understood.

Differences between Human and Other Animals

Rodents demonstrate a different MC distribution in the lungs compared to humans; their MCs are present in the bronchial airway wall, but, in contrast to humans, they lack MCs in the small airways and alveolar parenchyma. (15, 16). However, a recent study has identified parenchymal MCs in wild mice, suggesting that environmental factors may impact the tissue distribution of MCs in mice (39). Hence, rodents kept in animal facilities may not display a natural distribution of MCs when compared to wild animals. Additionally, the granule content may have a different profile than humans, i.e., mouse MCs express various numbers of serine proteases. For example, humans have only one identified chymase gene, belonging to the α-family, whereas rodents have several chymase genes, mouse MC protease 1 (mMCP-1), mMCP-2 and mMCP-4 which belongs to the β-chymases as well as one α-chymase, mMCP-5. Even though human chymase and mMCP-5 both are α-chymase, mMCP-4 is suggested to be the functional homologue to human chymase. The different chymases differ significantly in expression patterns depending on their tissue distribution. Mouse MCs also express four types of tryptases, i.e., mMCP-6 and mMCP-7 which are the main tryptases studied in mice, but also mouse transmembrane tryptase and mMcp-11 (40-43). In humans, four different groups of tryptase have been defined (α, β, γ, and σ), which have been further categorized in several subtypes. β-tryptase is the main active form in humans (3). However, it is not fully understood which mouse proteases are functionally translational to human proteases.

Respiratory Diseases

Chronic respiratory diseases cause significant health problems worldwide and include a diverse range of conditions affecting the lungs and respiratory system. Among the most prevalent respiratory diseases are asthma, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF), which impose a substantial burden on individuals, healthcare systems and society (44, 45). In 2017, more than 500 million people suffered from chronic respiratory diseases, which is almost a 40% increase since 1990, and together these diseases account for 3.9 million deaths per year. COPD is the most prevalent disease with 3.9% global prevalence (46). COPD is primarily caused by exposure to harmful particles or gases, e.g., tobacco smoke, and is a progressive lung disease characterized by irreversible airflow limitation, chronic bronchitis and parenchymal destruction (emphysema) (47). Asthma is the second prevalent disease with 3.6% global prevalence (46)

The pathologies behind chronic respiratory diseases are highly diverse. However, common features include chronic inflammation, structural changes of the airways

and alveolar parenchyma and impaired lung function. Their symptoms are associated with coughing, shortness of breath and reduced exercise tolerance, which has a significant impact on the life quality of the patients. Today there are no available cures for any of these diseases, only various forms of treatment to control the symptoms and improve the daily life of these patients (45, 46, 48).

The understanding of pathogenesis of chronic respiratory diseases is limited, while being crucial to develop effective prevention, diagnosis, and treatment strategies. Despite vast complexities, research has stressed the roles of immune dysregulation, oxidative stress, genetic susceptibility, environmental factors and tissue remodelling processes, as drivers of the development and progression of chronic respiratory diseases.

Asthma

Asthma affects approximately 300 million people worldwide: 1 in 7 children and 1 in 12 adults (49, 50). It is a chronic inflammatory lung disease which leads to airflow obstruction, structural alterations, and bronchial hyperresponsiveness (51, 52). The airflow limitations are due to bronchoconstriction, airway wall thickening and increased mucus.

Asthma is a heterogenous disease, and has further been divided into subphenotypes with differing pathology, clinical expression, and responses (53), such as (1) clinical and physiological phenotypes, including severity, exacerbation frequency, treatment resistance, and age onset, (2) phenotype related triggers, such as drugs, environmental allergens, and exercise, and (3) inflammatory phenotypes, such as eosinophilic, and neutrophilic (53).

The two most common way of treating asthma symptoms are with the inhaled glucocorticosteroids (ICS), which has anti-inflammatory properties, and/or the short- and long-acting β2 adrenergic agonists which induce smooth muscle relaxation (54, 55). However, not all patients receive adequate relief through these medications, which is likely to be a consequence of asthma heterogeneity and unknown underpinning mechanisms. Approximately 10% of asthma patients have severe disease and require high dose of ICS with additional steroids administration. These patients run high risk of developing severe exacerbations which require hospitalization (55). Therefore, more studies are needed in order to deepen the insight on asthma heterogeneity and develop new treatment approaches.

Most asthmatics display T2-high asthma, including early-onset allergic eosinophilic asthma as well as late-onset non-allergic eosinophilic asthma. T2-high asthma patients usually have high levels of fractional exhaled nitric oxide (FeNO) (56, 57), sometimes referred to as a FeNO-high phenotype. The T2-high cytokine profile are generally associated with increased levels of inflammatory alarmins (IL-25, IL-33 and TLSP) (58) and cytokines (IL-3, IL-4, IL-5, IL-9, IL-13 and GM-CSF) and other

mediators (55), which is produced by the epithelium and inflammatory cells, such as T helper 2 (Th2) cells and type 2 innate lymphoid cells (ILC2s). These cytokines promote characteristic features such as eosinophilia, mucus hypersecretion, bronchial hyperresponsiveness and IgE production (59). Some recent clinical trials have yielded successful results in targeting the T2-signalling pathways in severe asthma, including anti-IgE (omalizumab), anti-IL-4 and IL-13 (dupilumab) and anti-IL-5 (mepolizumab, benralizumab and relizumab).

Contrasting T2-high asthma, T2-low asthma is less studied, and has no clear endotype definition or salient biomarkers (60). Therefore, the lack of T2-high biomarkers can be used for characterizing T2-low asthma (61). However, Th1 and Th17 cells are suggested to be involved in this endotype and some studies have reported that several T2 cytokines may also be implicated in T2-low asthma by activation of Th17 cells (59, 62, 63). In terms of treatment, this endotype display resistance to ICS and have presence of either neutrophilic or paucigranulocytic inflammation, and yet, no biologicals are approved.

Mast Cells in Diseases

MCs have also been recognized for their role in various pathological conditions across different organs. They were first recognized for their role in IgE-dependent allergic diseases, as they express high quantities of the IgE receptor, but are now also known for their involvement in chronic respiratory diseases as well as inflammatory autoimmune diseases as well. Additionally, MCs demonstrates opposing roles in tumour progressions, where they may facilitate or diminish tumour growth, depending on the type of tumour (10).

In asthmatic patients, MC are found in high densities in the bronchial epithelium, mucosa and airway smooth muscle and their activation can cause release of pro-inflammatory mediators which are believed to contribute to airway inflammation and bronchoconstriction. In a study comparing MC phenotypes and distribution in different asthma severities, Balzar et al. (64) found that the total number of submucosal MC were highest in mild asthma, whereas MC_{TC} were the predominant MC phenotype in severe asthma. When looking at intraepithelial MCs, patients with mild asthma and severe asthma had similar numbers of MCs, but the proportion of MC_T and MC_{TC} was shifted in the severe asthmatic epithelium, where MC_{TC} was the dominating subtype. Hence, they conclude that severe asthma is associated with a predominance of MC_{TC} in airway submucosa and epithelium.

Furthermore, MCs have, in several studies, demonstrated a phenotypic switch, re-localisation, and correlations with different clinical parameters. For example, the airway smooth muscle bundles have an increased infiltration of MCs in asthma patients, which was further correlated with hyperresponsiveness (65). A more recent

study found that the density of MC_{TC}s infiltrating the airway epithelium correlated with airway hyperresponsiveness in patients with FeNO-high asthma (66). Also in the distal lung compartments, i.e., alveolar parenchyma, the proportion and density of MC_{TC} was significantly increased in uncontrolled atopic asthma patients, both compared to healthy as well as controlled asthma subjects, and was associated with alveolar collagen VI (67).

Similar observations have also been confirmed in the pathophysiology of other chronic respiratory diseases, including chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), and cystic fibrosis (CF). Commonly, they demonstrate an increased density of the MC_{TC} population, which home into structures such as the alveolar parenchyma and bronchial epithelium, where they are associated with pro-inflammatory responses and tissue remodelling, similarly to the pathology of asthma (64, 68-73). In COPD, studies have shown that both the C5a expression and the C5aR (CD88) expression in MC_T and MC_{TC} cells was increased, which may be a source for the chronic MC activation in COPD (69, 74). The alveolar parenchyma in lungs from IPF patients, had a higher proportion of MC_{TC} with an increased transforming growth factor β (TGF-β) expression. Furthermore, both the density and percentage of MC_{TC} in IPF was positively correlated with the degree of fibrosis (70); in CF patients, the numbers of MC_{TC} were increased in the inflammatory and fibrotic areas and an elevated IL-6 expression was found in the lungs of CF patients (70). Various respiratory diseases at severe stages are characterized by epithelial proliferation, remodelling and loss of function, however, the knowledge about these tissue alterations is limited and needs further investigation.

Exacerbations in asthma and COPD are often associated with viral infections. Some respiratory viral infections elevate the population and change the function of MCs (75, 76), where the activated MCs secrete cytokines and mediators that can activate the endothelium to recruit immune cells to the infection area (26). However, little is known about MC-epithelial crosstalk in pro-inflammatory and viral settings.

In summary, the histopathology of several respiratory diseases indicates that MCs, in particular MC_{TC}, are associated with disease severity, but despite MC pathological presence and phenotypic switch from MC_T to MC_{TC}, little is actually known regarding their functional role in disease pathogenesis. As both tryptase and chymase are predominantly synthesized in MCs, it is important to study the effects of these proteases on airway cells in order to understand the impact of the infiltrating MCs and how they may prevent or promote disease development.

Tryptase

Within the cytosolic granules, tryptase is stored as an inactive precursor but can rapidly convert to active tryptase upon release. The active tryptase is a composition

of four monomeric substructures which are stabilized by heparin complexes, creating the tetrameric active protease. Tryptase, as well as chymase, belongs to serine proteases family and act by cleaving a variety of different substrates (77).

In homeostasis, tryptase is acknowledged for its multifunctional roles, such as activation of cytokines, stimulation of cytokine release in cells such as epithelial cells, endothelial cells and fibroblast, cleavage of cell surface receptors, regulation of extracellular matrix (ECM) deposition by activating fibroblast and matrix metalloproteinases (MMPs) and promoting wound healing. Tryptase can also activate pro-inflammatory pathways, which may result in airway inflammation and mucus secretion, and induce collagen synthesis in fibroblasts (1, 78-81). One particular receptor that tryptase is known to cleave and activate, is the protease-activated receptor-2 (PAR-2), which belongs to the G protein-couples receptor family. PAR-2 is widely expressed on various cell types such as epithelial cells and fibroblasts, and is involved in inflammation, coagulation and cell proliferation (78). However, tryptase may activate other receptors as well, as PAR-2 inhibition may not reduce tryptase induction of all biological events, but these underpinning mechanisms and receptors remain to be identified.

In the context of lung diseases, tryptase exhibits various activities that are believed to be involved in several disease progressions, including asthma and COPD. Respiratory diseases are characterized by tissue remodelling and thickening of different lung structures, such as the smooth muscle cell layer, fibrotic tissue, and epithelial layers. As the number of MCs is significantly increased in lung diseases, tryptase is believed to be involved in the thickening of lung tissues as it acts as a mitogen (e.g., induce cell division and proliferation) for several cell types. For example, tryptase exhibits mitogenic effects in human airway smooth muscle (ASM) cells (82, 83), fibroblasts and epithelial cells (84). In fibroblasts, PAR-2 inhibition reduced tryptase mitogenic and migratory effects (85, 86).

In asthmatic patients, the number of MCs in the bronchoalveolar lavage (BAL) fluid is increased, as well as the levels of tryptase, indicating an on-going degranulation of tryptase in asthmatic conditions (87). Tryptase has also been reported to be involved in airway hyperresponsiveness, airway remodelling, and bronchoconstriction, and to directly induce smooth muscle contraction (88-91), which is associated with asthma. In addition, tryptase has been shown to induce the production and release of TGF-β in human ASM cells and MCs (92, 93), and to be involved in pathogenesis of emphysema and small airway remodelling, in COPD (94).

Taken together, tryptase appears to promote several events on lung structures and cells, which suggests that tryptase may be an important factor in airway remodelling and pathogenesis of respiratory diseases. However, while it is of great interest to develop therapeutic strategies that targets tryptase, functional and mechanistic

knowledge is still lacking, and more studies are thus warranted to identify potential therapeutic targets.

Chymase

Chymase is a monomeric protease with chymotrypsin-like cleavage specificity which can cleave large number of proteins and peptides in different sizes, to regulate a variety of physiological functions (91). For example, chymase can cleave ECM proteins (e.g., pro-MMP2 and 9), cytokines (e.g., IL-1β, IL-6, IL-13, IL-33, TNFs), latent TGF-β, fibronectin and tight junction proteins. It is also well known for its role in regulating blood pressure by cleaving angiotensin (Ang) I to generate Ang II (95-102). One study reported that mice deficient for Mcpt1 demonstrated a delayed removal of the nematode *Trichinella spiralis* but not in *Nippostrongylus brasiliensis*, suggesting that chymase is important in the defence of selected parasite worms (103).

Whether chymases are detrimental or beneficial in respiratory diseases is debated, and different studies reports opposing findings, which further highlights chymase complexity in pathogenesis (102). As an example, by cleaving and degrading pro-inflammatory cytokines such as IL-6, IL-13 and IL-33, chymase may dampen the pro-inflammatory response, which would have a protective effect in asthma, whereas activation of IL-1β could promote airway inflammation (91). In addition, chymase can also regulate many substrates that are associated with tissue remodelling, who can also serve dual roles in pathogenesis. Cleavage and activation of e.g., MMPs and fibronectin may prevent excessive ECM deposition in lung tissue, which could potentially alleviate airway remodelling. On the other hand, activation of TGF-β can promote ECM deposition in allergic airways (96, 104) and, in the context of IPF, TGF-β activation contribute to fibrosis, since it is a cytokine involved in the fibrotic response (96, 104).

In addition to ECM remodelling, chymase has been shown to provoke profound morphological alterations in explanted primary bronchial tubes with fragmentation of ECM and dissociation of epithelial cells from the basement membrane in lung tissue (105). Additionally, chymase disrupts vital barrier protein junctions in cell line cultures (105, 106). These studies imply a strong detrimental role of chymase on epithelial barrier properties, which can enhance the risk of translocation of inhaled invaders into the subepithelial space and lead to immunological responses and airway and mucosal inflammation.

Taken together, several of these events induced by chymase may contribute to host protection but also in detrimental regulations of ECM remodelling and thus provoke severe complications in inflammatory lung diseases.

It may seem contradictive that MCs, including tryptase and chymase, show both detrimental and beneficial properties in host defence and survival. It is important to note that the MC activation needs to be in balance to maintain a proper biological beneficial function, and that, in the pathological conditions, the MC activation is believed to be dysregulated, which in turn may lead to harmful responses. However, more studies are however needed to better understand MCs role in the pathogenesis of chronic respiratory diseases.

Experimental Research Models

Studies and correlations using histopathology is critical to understand how the cellular architecture and phenotypes are organized in disease pathologies. These studies mirror the actual consequences and developments in tissues which have already happened and enable us to understand many underlying events causing clinical symptoms. For example, such studies have helped researchers not only to find the presence of high numbers of MCs in respiratory diseases and phenotyping them, but also to further find distinct pathological disease phenotypes, which is highly important for therapeutical aspects. At this stage, it is difficult to draw any conclusions regarding the impact of MCs on disease development, as they might be beneficial and/or detrimental. From here, it is therefore important to complement histopathological studies by performing additional research, investigating for example functional implications and biochemical properties of these cells. This is typically done using different *in vivo* and *in vitro* models.

Typical *in vitro* models used to study implication of MCs in respiratory disease, make use of either purified or recombinant proteins related to MC expression (e.g., tryptase or chymase) to stimulate the cell type of interest. Those cells can originate from a cell line or, for example, directly from human samples (i.e., primary human cells). As *in vitro* studies are performed in a closed and highly regulated environment, they can never fully reflect the physiological events which may happened in an *in vivo* context, but they can be designed in different ways which are more or less comparable to the physiological lung.

Furthermore, some common ways of studying the functional role of a certain cells, proteins, or pathways *in vivo* are to genetically modify an animal model or use specific inhibitors targeting the protein of interest. A vast number of animal models have been developed and used over the years and have had a major impact on research by giving us deeper insights into molecular mechanisms, correlations, and biological physiology (102). For example, a chymase-deficient mouse model (Mcpt4-/-) for asthma showed enhanced airway reactivity and increased eosinophil infiltration, and was suggested to be a result of impairment of degrading IL-33 (107). Also, another study used a chymase inhibitor, showing that inhibition of chymase dampened the airway inflammation (108). These kinds of studies are

important for studying the effects of specific proteins, cells, or mechanistic pathways in the complex *in vivo* setting.

While these translational models are indeed important and have helped us to understand the physiological effects of, for example, chymase in certain contexts, but it is also important to underscore that human MC and protease expression profiles and distribution are different than that of rodents, and that these *in vivo* studies cannot be directly transferable to human physiology – although, they serve as important platforms to evaluate fundamental physiological functions.

As these are examples of experimental models, they will never fully replicate the human disease development, but at least, they will provide valuable understanding of chemical, cellular, functional, and pathological implications of the disease or protein of interest.

The Airway Epithelium

The Lung Epithelium and the Role of Epithelial Cells

The human lung has an estimated surface of 70m^2, which is directly in contact with the external environment, and every day the inner surface of the lungs is exposed to more than 10,000L of air. The main function of the lung is to maintain gas exchange in order to oxygenate the host body (109).

The lung comprises distinct compartments, which have different functions and structures, stretching from the mucosal tissue in the upper and lower respiratory tract to the alveoli in the peripheral lung parenchyma, where the gas exchange is taking place (Figure 2). Each lung compartment has developed its specific population of immune cells which actively participates in inflammatory responses.

The inhaled air contains many particles and pathogens, which can cause harm for the host. This is largely overcome by efficient host defence systems and mechanisms, wherein the differentiated respiratory epithelium plays a central role. The inner surface of the lung is covered by the epithelium, providing a physical barrier, immune defence and mucociliary clearance of inhaled particles, to maintain a healthy state of the host. (50, 109).

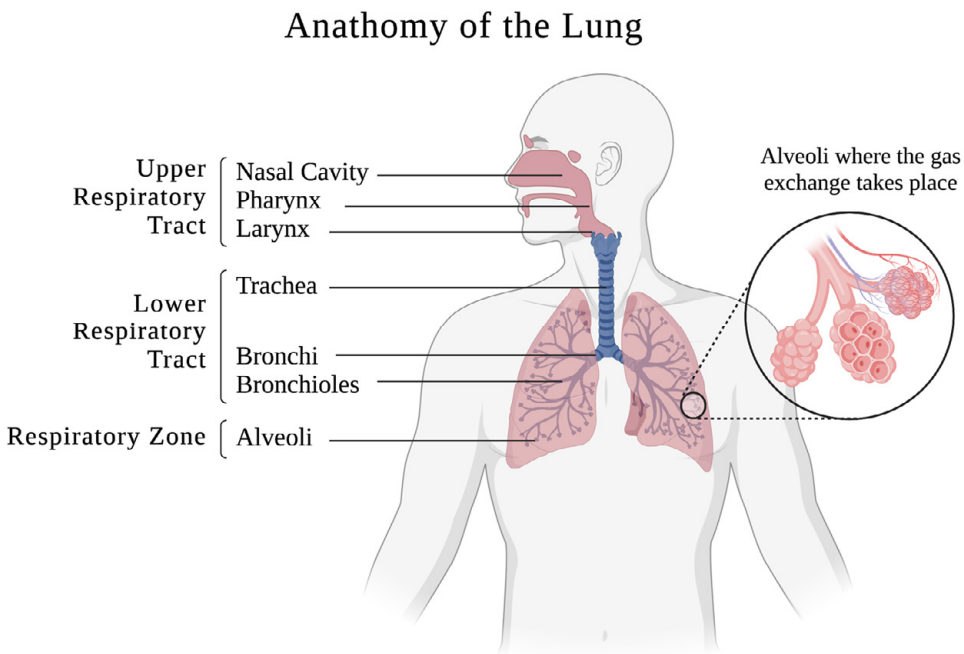


Figure 2. Illustration of lung anathomy, streching from the upper respiratory tract, to the lower respiratory tract and the alveoli in the respiratory zone.

An important element in the epithelial barrier is the formation of protein junctions between the lining cells, to allow strong cell-cell contact and paracellular passages of ions, water, and macromolecules. The epithelial barrier consists of linked complexes of tight junctions (TJ) or adherens junctions (AJ) and intracellular cytoskeleton proteins. Occludin is an integral TJ protein regulating paracellular permeability and zonula occludens-1 (ZO-1) is an intracellular protein that links TJ to the actin cytoskeleton on the intracellular site. These protein complexes, accompanied by a range of other barrier-associated proteins, constitute the apical cell polarity and barrier properties of the epithelium, which are involved in signal transductions and cell homeostasis (110, 111).

Epithelial Alterations in Asthma

Altered epithelial structure and function are typical hallmarks of lung diseases (Figure 3) (110, 112). Considering barrier function, asthma patients demonstrate a decreased physical barrier compared to healthy subjects. This is apparent by increased ionic and macromolecular permeability as well as impaired TJ and AJ protein organization and expression (113, 114). Cell cultures from asthmatic patients are also more sensitive to disruption by cigarette smoke extract (113).

However, the underpinning mechanisms of compromised epithelium are not fully understood.

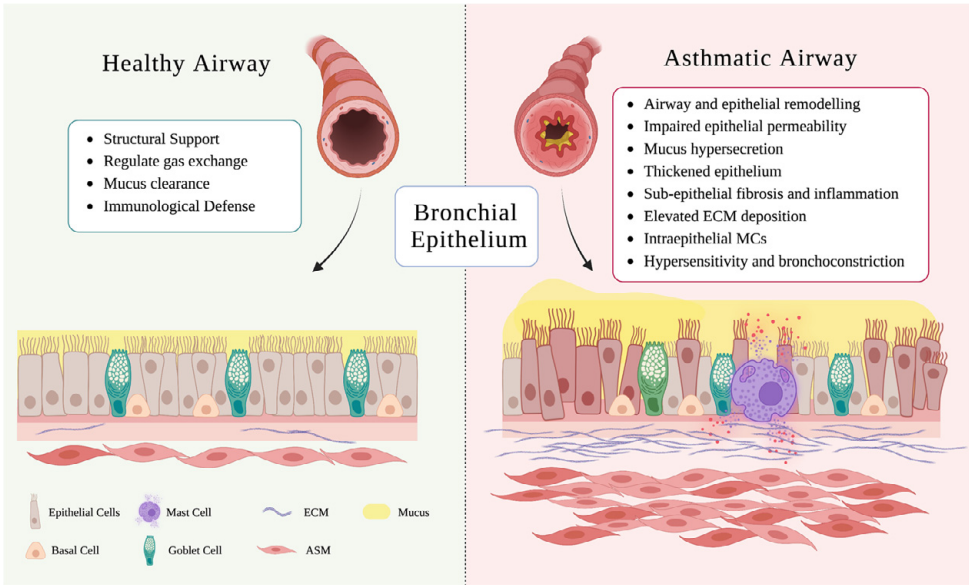


Figure 3: The airways in asthma patients are altered, with various structural and functional impairments including epithelial remodelling, MC infiltration and elevated ECM deposition. ECM: extracellular matrix, MC: mast cell, ASM: airway smooth muscle.

In the asthmatic epithelium, structural abnormalities are typically referred to airway and epithelial remodelling. Importantly, physiologic remodelling is critical for maintaining a healthy epithelium and includes regeneration of injured tissue by parenchymal cells. In asthma, however, all compartments of the airway wall – including inner, outer, and total – are significantly thickened due to airway remodelling, resulting in airway narrowing. In cases of fatal asthma, the airway wall is increased by 50-300%, and 10-100% in non-fatal asthma, compared to non-asthmatic controls (115, 116). This is partly explained by abnormal cell growth and replacement of injured tissue by ECM, leading to impaired airway structure (116). In particular, pathological remodelling as seen in asthma includes subepithelial fibrosis, goblet cell hyperplasia, and smooth muscle hypertrophy. Additionally, a study by Cohen et al. (117) demonstrates that the airway epithelium in severe asthma has a much higher rate of cell proliferation and a dysregulated proliferative response than in mild asthma and in healthy subjects. The authors propose that this may drive the processes leading to the epithelial hyperplasia and thickening seen in severe asthma, despite treatment with high doses of corticosteroids.

In addition to increased proliferation rate, the enhanced levels of various growth factors, including basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF), are also proposed to be implicated in the tissue remodelling, thickening of airway structures, and asthma pathology (118, 119). Furthermore, elevated expression of the pro-survival protein BIRC3 has been linked to asthma pathogenesis (120), which also suggests a dysregulation of cell survival-death balance. Although this proliferative response may be initiated by persistent airway inflammation, viral infection or injury, it is still not clear what drives the proliferative epithelial remodelling process in asthma.

Aims of the Thesis

MCs appear in high numbers within the airway epithelium in patients with respiratory diseases. The general aim of this thesis was to study how MC specific proteases released from intraepithelial MCs impacts the airway epithelium and their potential role in disease progression.

The specific objectives of the four studies were as follows:

- In paper I, the aim was to explore the effects of tryptase and chymase on fundamental epithelial cell appearance and properties, in particular cell morphology, cell growth, and migratory capacity.

- In paper II, the objective was to study the impact of tryptase in the context of wound healing, with a focus on direct gap closure effects as well as in regulating the release of wound healing-associated proteins and gene expression patterns in epithelial cells.

- In paper III, the aim was to further investigate the role of tryptase in epithelial cell growth properties and to characterize its ability to regulate homeostasis in alveolar and bronchial epithelial cells. We additionally aimed to study these parameters in a pro-inflammatory and viral environment.

- In paper IV, the aim was to study if chymase affects the barrier properties of fully differentiated bronchial epithelial cells and to test whether the potential effects differed between cells from healthy and asthmatic donors.

Methodology

To understand the molecular and cellular role of MCs in the pathophysiology of respiratory diseases, we used different *in vitro* experimental approaches, including submerged cell cultures (Figure 4A) and air-liquid interface (ALI) cultures (Figure 4B). This section will provide an overall introduction to the methods utilized in our research. Further information about specific methods or materials are provided in individual papers I-IV.

Cell Culture Models

Bronchial Epithelial Cell Line: Beas-2B

The bronchial epithelial cell line Beas-2B was originally established as a human immortalized, non-cancerous bronchial epithelial cell line in 1988 (121) and was used in papers I, II and III. Cells were maintained in RPMI-medium 1640 supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin and cultured in a humidified incubator at 37°C in 5% CO_2. Experiments were performed in starvation medium (RPMI supplemented with 1% foetal bovine serum and 1% penicillin-streptomycin). For experiments, cells were used in submerged cultures.

Bronchial Epithelial Cell Line: BCi-NS1.1

The immortalized BCi-NS1-1 (BCi) cell line was used in paper IV for the barrier study because BCi cells have, in contrast to Beas-2B, a multipotent differentiation capacity, which allows them to form TJs and cilia and to produce mucus (122). Cells were maintained in collagen-coated flasks in complete airway epithelial medium, i.e., airway epithelial cell basal medium supplemented with an airway epithelial growth medium supplement pack (Promocell) in a humidified incubator at 37°C in 5% CO_2. For experiments, these BECs were differentiated in ALI cultures.

Alveolar Epithelial Cell Line: A549

Because increased MC density also is found in the alveolar parenchyma in diseased conditions, we also wanted to investigate the role of MC protease in alveolar epithelial cells. Hence, both Beas-2B and the alveolar epithelial cell line A549 were studied in paper III. A549 was originally an adenocarcinomic human alveolar epithelial cell line. In our experiments, the cells were maintained in Ham's F12-medium supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin. Cells were cultured in the same manner as the Beas-2B cells, except for the starvation medium, which was based on Ham's F12-medium. For experiments, the cells were used in submerged cultures.

Primary Bronchial Epithelial Cells

Two different patient cohorts were used in this thesis. In paper III, primary human BECs were collected from patients with mild to moderate asthma by bronchoscopy. These patients were not treated with inhaled corticosteroids (for at least 3 months) and had airway hyperresponsiveness to mannitol. The patients were originally a part of the Reconstruct study at Bispebjerg Hospital, Denmark (66), and the samples (n=5) used in paper III were previously collected by Ramu et al. (106). Briefly, primary cells were collected through epithelial brushings using a sterile fibre-optic bronchoscope, vortexed, centrifuged, washed and then cultured in collagen-coated cell culture flasks in bronchial epithelial growth medium (BEGM) from Lonza. Cells were cultured in a humidified incubator at 37°C in 5% CO_2. For the experiments, these primary cells were used in submerged cultures.

In paper IV, primary BECs were obtained from non-asthmatic (n = 6) and asthmatic donors (n = 4) by epithelial brushings using fibre-optic bronchoscopy, followed by isolation and expansion. Small airway cells were maintained in small airway epithelial medium and large airway cells were maintained in large airway epithelial medium (Promocell). The experiments comparing cell responses between non-asthmatic and asthmatic volunteers were run in pairs and were sex and age matched. For the experiments, these primary BECs were differentiated in ALI cultures.

ALI Cultures

Cells from the BCi cell line, non-asthmatic donors, and asthmatic donors (both small and large airways) were used to study the impact of chymase on barrier properties in paper IV. To be able to study the barrier function, one first needs to establish a proper epithelial barrier layer. By culturing cells at the ALI, cells will differentiate into ciliated and mucus-producing cells and will become polarised with the apical expression of tight junction proteins, e.g., ZO-1 and occludin (Figure 4B). This experimental design is also referred to as three-dimensional (3D) *in vitro* modelling.

For differentiation, cells were first seeded onto collagen-coated transwell inserts (0.4 µm pore size, Corning) with 200 µl apical and 500 µl basolateral media. Then 2-3 days later the cultures were changed to ALI by replacing the old media with 300 µl ALI-culture media (1:1 complete airway epithelial medium and complete DMEM) in the basolateral compartment only. After this, cells were cultured for 3 weeks at the ALI with media replacement 3 times/week, and the transepithelial electrical resistance (TER) was measured once a week to track the formation of the physical barrier. TER measurements were performed using chopstick electrodes after a 15-minute incubation with 100 µl pre-heated HBSS in the apical compartment.

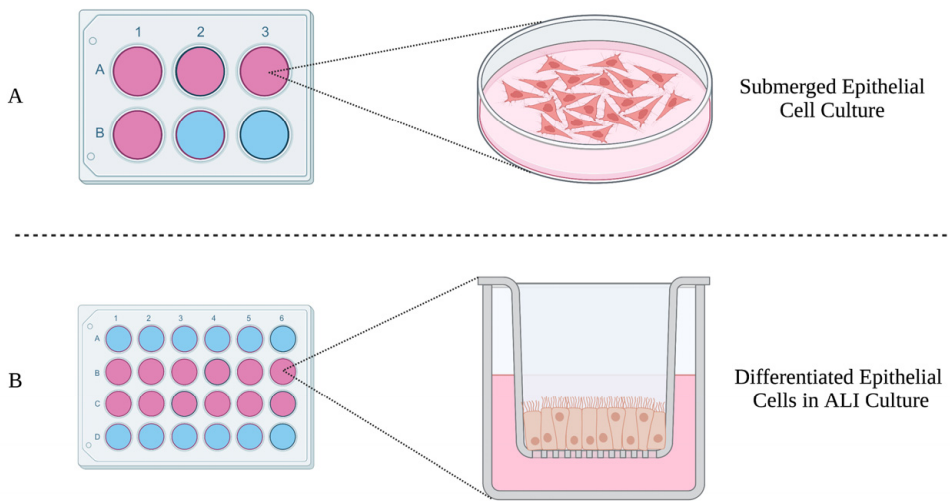


Figure 4. Illustration of the submerged epithelial cell culture system (A) and the pseudostratified epithelium with differentiated epithelial cells in ALI cultures (B). The image was created in BioRender by Frida Berlin.

In Vitro Cell Stimulation

Tryptase and Chymase

In this thesis, the MC proteases tryptase and chymase were used to study MCs' roles in airway remodelling, barrier integrity, and homeostasis. Highly purified human lung tryptase (Merck Millipore) with a molecular wight of ~135kDa were used in this thesis. The tetramer was non-covalently linked with two sets of dissimilar subunits of α and β isoforms sequences. The undiluted tryptase had an enzymatic activity of 62.5 units/mg protein according to the manufacturer's description. The

chymase was a monomeric recombinant human chymase (Sigma-Aldrich) with an enzymatic activity of 90 units/mg protein.

To establish specific concentrations for tryptase and chymase in submerged cultures (paper I, II, III), epithelial cells were stimulated with different concentrations of the proteases. Supernatants were collected and validated at different timepoints using a lactate dehydrogenase (LDH) cytotoxicity assay and cytokine measurements. Dose response experiments were also performed using a holographic live-cell imaging system to validate protease effects, cell survival, and potential toxicity. For submerged epithelial cell cultures, 0.5 µg/mL was carefully chosen as the appropriate concentration for both tryptase and chymase. For the ALI cultures (project IV), high (5 µg/mL) and low (0.5 µg/mL) concentrations of chymase were used, and no visible signs of toxicity was observed.

PAR-2 inhibitor

To explore tryptase's mechanism of action, a synthetic inhibitor was used to block the protease-activated receptor-2 (PAR-2). Cells were pre-treated with the antagonist I-191 (MedChemExpress) for 30 minutes at 37°C in 5% CO_2, followed by one wash in PBS and then stimulation with tryptase.

Pro-inflammatory and Viral Stimulations

The TLR3 agonist polyinosinic:polycytidylic acid (PolyI:C) is a synthetic analogue of dsRNA which was used as a viral mimic (InvivoGen, France). The TLR4 agonist lipopolysaccharide (LPS, InvivoGen, France) and in-house made cigarette smoke extract were used to initiate pro-inflammatory responses in BECs. Cells were pre-treated with tryptase for 3h and then co-stimulated with the indicated viral or pro-inflammatory stimuli.

Experimental Set-Up

The general experimental set-up for submerged alveolar and bronchial epithelial cell cultures will be explained in this section (Figure 5). Cells were seeded onto cell culture plates 3 days prior to start for RNA, supernatant and protein collection as well as for immunocytochemistry and 24 h prior to start for the Holomonitor M4 experiments. On the experimental day, cells were stimulated with tryptase or chymase along with non-stimulated control cells. In paper III, cells were co-stimulated with pro-inflammatory or viral stimuli, which were added into the supernatant 3 h after protease stimulation. RNA, cell culture supernatants, and cell lysates were collected at 6 h and 24 h for further protein and gene analysis. Cells

used for immunocytochemistry were seeded onto 4-well chamber slides and were fixated in 2% paraformaldehyde at 24h after protease exposure.

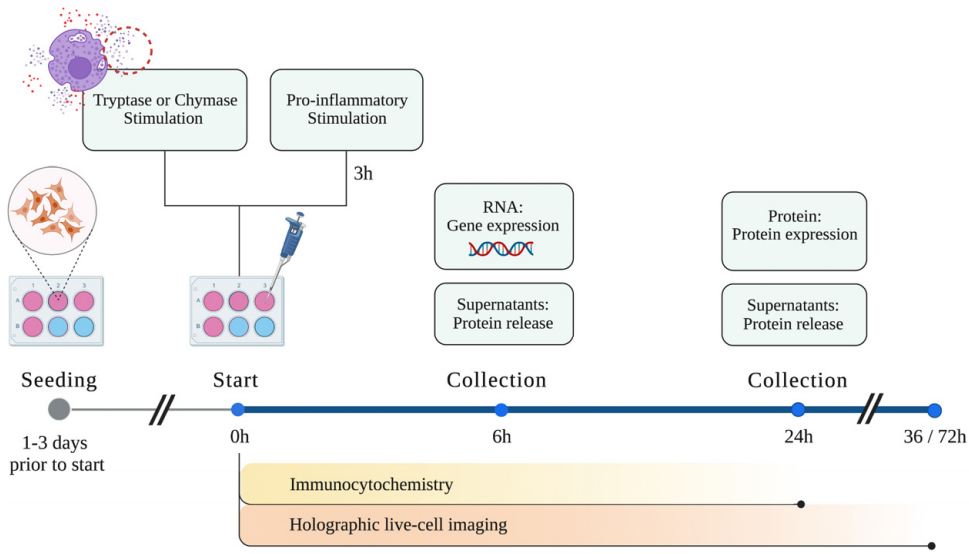


Figure 5. Illustration of the experimental set-up for submerged cell cultures. The image was created in BioRender by Frida Berlin.

Cells cultured in ALI, had a similar experimental set-up but with some minor changes. First, TER measurements were performed in the "old" media, as described above, for baseline comparison. Then 10µl of 30× of the indicated chymase concentration was spiked and mixed into fresh basolateral cell culture media when starting the experiments. TER measurements were repeated at the different read out time points, i.e., 1, 2, 6, and 24h after chymase exposure. The transwells were placed on different plates, based on their read-out time, un order to avoid external disturbances. After the TER measurements, the cells were fixed in 4% paraformaldehyde for 15 minutes, followed by washings in PBS. Apical and basolateral supernatants were collected for further analysis.

Holographic Live-Cell Imaging

To assess the functional properties of tryptase and chymase on epithelial migration, morphology, proliferation and wound healing capacity, a novel live-cell imaging system, the Holomonitor M4 from Phase Holographic Imaging (Lund, Sweden), was used (paper I, II, III). The Holomonitor uses digital holography to record cells

in real time and provides reconstructed 3D images and videos and quantitative data on a single-cell level. The method is label free, and all experiments are performed inside the humidified incubator at 37°C in 5% CO_2. The epithelial cells were split and seeded onto a Sarstedt TC 6-well plate 24 h prior to the start of the monitoring. In the wound healing model, cells were seeded into 2-well culture-inserts (Ibidi, Germany) at high densities. For all experiments, fresh media (with or without proteases) was added 30 minutes prior to start, and a random capture pattern of at least 5 different positions per well was chosen. Images were captured every 15 minutes over 24, 36, or 72 h, giving >1000 images in total per repeat. All data were analysed using HStudio.

Immunocytochemsitry

Submerged cultures

Cells were permeabilized in 0.1% Tween-20 and immunostained with the primary antibody BIRC3 (Thermo Fisher Scientific, USA) at a 1:50 dilution or Ki67 (Dako, Denmark) at a 1:300 dilution and thereafter incubated with the secondary antibody Alexa Fluor 488 (Invitrogen) at a 1:200 dilution. For cytoskeletal immunostaining, FITC-conjugated phalloidin 488 (Sigma-Aldrich, USA) was used at a 1:1000 dilution. Nuclei counterstaining was performed using the mounting medium ProLong Gold antifade reagent with DAPI (Invitrogen, USA).

Differentiated ALI cultures

Fixed cells were permeabilized with 0.1% Triton X-100, blocked in 1% BSA and then immunostained with the occludin-conjugated monoclonal antibody 488 (Invitrogen, USA) at a 1:100 dilution and with the F-actin 555 phalloidin (Acti-stain, Cytoskeleton) at a 1:200 dilution. DAPI (Roche, Switzerland) at a 1:500 dilution as used for nuclei stainings. Cells were finally mounted with Mowiol (Sigma-Aldrich, USA).

Results

In the following section, the main results from the studies included in this thesis are presented. A more detailed description of the findings can be found in the result section of each paper.

Tryptase

Respiratory diseases such as asthma, are characterized by increased number of MCs in specific lung compartments, including the bronchial epithelium and alveolar parenchyma. MCs are known to express high levels of tryptase, suggesting that neighbouring cells and the local environment are exposed to this protease at higher concentrations in diseases conditions than in non-diseased. However, the MC-epithelial interactions in the lung remain poorly understood. Therefore, we aimed to functionally study how MC tryptase may affect bronchial and alveolar epithelial cells, with a particular focus on airway remodelling and wound healing capacity.

Tryptase-mediated Effects on Cell Morphology, Migration and Wound Healing Capacity in BECs

First, the tryptase effect on fundamental cell shape was evaluated. BECs stimulated with tryptase exhibited a significantly elongated cell morphology and a reorganization of the cytoskeleton (Figure 6A and B) in comparison to non-stimulated cells (Paper I). Because these features are an indication of migratory cells, we then used the Holomonitor M4 to analyse tryptase's effect on cell motility. Single-cell tracking analysis revealed that tryptase significantly increased cell migration, motility, and speed in BECs in submerged cultures (Paper I, Figure 6C and D). When comparing different time intervals, we found that tryptase induced a significant increase in cell motility already within 3 h post stimulation, which remained throughout the entire experiment (36h).

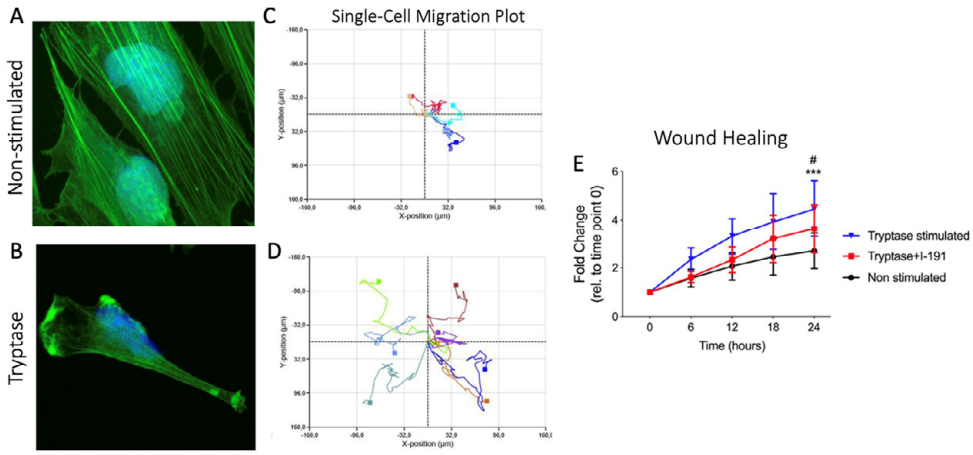


Figure 6. Tryptase induced morphological alterations and reorganization of the cytoskeleton (A) and enhanced migration (B) and wound healing capacity (C) in BECs. Immunofluorescent images (A,B) were taken by confocal microscopy. XY-plots (B) obtained from the Holomonitor M4 represents the exact migratory pattern of non-stimulated (above) and tryptase stimulated (below) BECs over 12 h.

When further analysing motility properties in a wound healing model, we found that tryptase's enhancement of migratory capacity remained in the wound gap, and, accordingly, tryptase enhanced the wound healing capacity in BECs (Figure 6E). To study the mechanistic pathway, a PAR-2 inhibitor (I-191) was used and showed that tryptase induction of wound gap closure was reduced in cells pre-treated with the inhibitor compared to controls (Paper II).

Tryptase Promotes Cell Growth in Alveolar and Bronchial Epithelial Cells

The Holomonitor M4 was used to functionally study the proliferative effect of tryptase on BECs and AECs. The analysis revealed that tryptase significantly shortened the cell division intervals, indicating an acceleration of cell divisions initiated by tryptase. Moreover, tryptase promoted epithelial cell growth in both cell types when compared to individual control cells (Figure 7B). To further understand the underlying mechanisms of cell growth, we investigated the involvement of the PAR-2 receptor. When AECs were pre-stimulated with the PAR-2 inhibitor (I-191), the induction of cell growth by tryptase was diminished, although the reduction was not statistically significant (Figure 7C). The study in paper III also examined primary BECs from asthma patients to assess the impact of tryptase on cell growth in a disease-specific context. In contrast to the cell lines, no difference in cell growth was observed in the PBECs. However, a significant upregulation of the proliferation marker Ki67 was found in PBECs stimulated with tryptase (Paper III), which was also observed in the tryptase stimulated Beas2-b (Paper I). This implies that the

effects of tryptase on cell growth may vary between cell lines and primary cells. Notably, the cell line experiments were performed at 72 h while the PBECs experiments were performed at 36 h, which also may explain the difference.

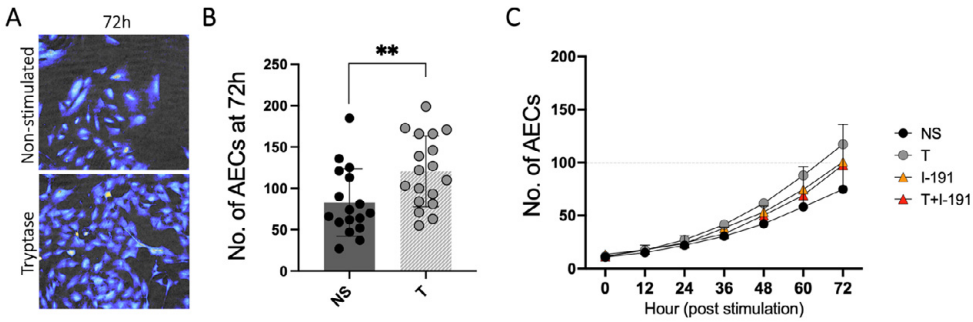


Figure 7. Tryptase increased the cell growth in AECs and BECs. Representative images of AECs obtained with Holomonitor M4 demonstrating the number of cells for non-stimulated (above) and tryptase-stimulated cells (below) at 72h (A). Tryptase significantly elevated the cell growth in AECs at 72h (B). A cell growth curve of AECs showed diminished proliferative effect by tryprase when pre-stimulated with a PAR-2 inhibitor (C). Non-parametric Mann–Whitney tests were used for the comparison of the non-stimulated (NS) and tryptase-treated groups.

Because patients with asthma displays airway inflammation, we additionally studied BEC growth properties in pro-inflammatory and viral settings (Paper III). Interestingly, tryptase induction of cell growth remained in the pro-inflammatory settings (LPS and cigarette smoke extract), but not in the viral setting (poly I:C).

Increased Epithelial Release of GFs and Expression of Pro-Survival Proteins in Tryptase-Stimulated Cells

To further investigate whether tryptase can alter the epithelial responses and homeostasis, cell culture supernatants, and protein expressions were analysed (Paper III). Tryptase stimulation resulted in a significant upregulation of the anti-apoptotic protein BIRC3 in PBECs, BECs, and AECs, indicating enhanced cell survival in tryptase-stimulated cells. Additionally, tryptase altered the release of various cytokines and GFs by bronchial and alveolar cell lines as well as in PBECs. In the PBECs, tryptase induced the release of GFs such as Granzyme B, G-CSF, MIP-1a, and PDGF-AA and there was a significant elevation in bFGF release (500-fold relative to controls). On the other hand, tryptase stimulation also led to a significant reduction in proteins such as GRO-β, TGF-α, and VEGF.

Taken together, these results suggest that tryptase promotes epithelial cell growth and survival and improves wound healing capacity.

Chymase

Because there is a phenotypically shifted MC population in the pathological conditions of respiratory diseases, with an increased proportion of MCs expressing both tryptase and chymase, we also sought to understand the role of chymase on bronchial epithelial remodelling and barrier function.

Chymase Altered Cell Morphology, Enhanced Cell Migration, and Decreased Cell Growth

BECs stimulated with chymase demonstrated a statistically significant difference in cell morphology when compared to non-stimulated cells. Chymase-treated BECs had reduced cell area and optical volume, increased cell thickness, and were more elongated. Similar to tryptase-treated cells, chymase induced the reorganization of the cytoskeleton and induce strong migratory effects, with increased cell motility, migration, and speed (Paper I).

Opposing the proliferative effect induced by tryptase, chymase stimulated BECs showed a reduction in cell growth compared to non-stimulated cells (Paper I). This was functionally assessed by Holomonitor M4 analysis, demonstrating a significant decrease in cell growth and in the percentage of dividing cells, accompanied by decreased Ki67 protein expression and reduced metabolic activity. Importantly, toxicity assays did not indicate cellular toxicity at the concentration used in the experiments.

Chymase-Mediated Disruption of Barrier Integrity

Chymase effect on ionic epithelial permeability was evaluated by performing TER measurements at different time points in fully differentiated primary BECs from healthy donors and from patients with severe asthma (Paper IV). The ALI cultures were stimulated with either low (0.5 µg/mL) or high (5 µg/mL) concentrations of chymase (Figure 8A). The obtained results indicated an early drop in TER, already at 1 h, followed by a quick recovery at 2 h for both concentrations and in both groups. The high concentrations of chymase showed a partial, but not full recovery, as was seen for the low concentrations of chymase. Furthermore, all groups had a second drop in TER (approximately 10-15%) at 6 h, and from here only the BECs from healthy donors treated with low concentration of chymase fully recovered at 24 h. Whereas the BECs from severe asthma patient treated with low concentration of chymase recovered only slightly and the other groups remained at similar permeability levels as after the second drop. These findings indicate that chymase caused an increase in ionic leakage in BECs from both healthy individuals and those with and severe asthma, where BECs from healthy donors were the only group

exhibited a full barrier recovery after the impairment induced by chymase earlier in the experiment.

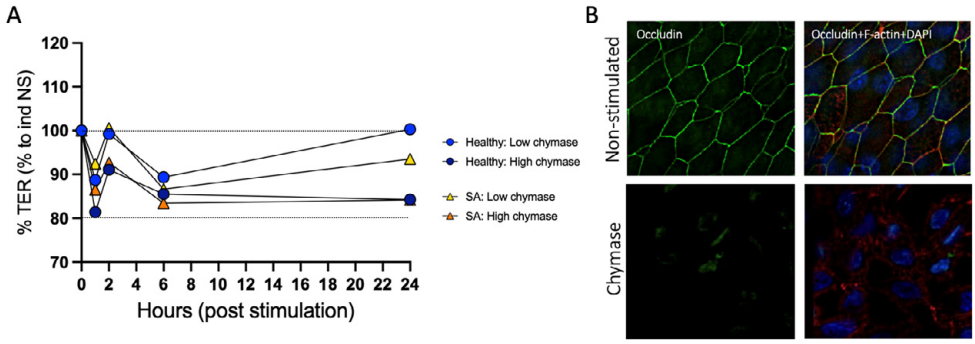


Figure 8. Chymase impaired barrier integrity in ALI cultures from healthy donors and those with severe asthma. TER values taken over time (A) revealed the recovery of healthy cells treated with low concentrations of chymase, but not in severily asthmatic cells. Immunofluorescent stainings of non-stimulated and chymase-stimulated (high concentration) cells from a non-asthmatic donor (B). Green: occludin, red: F-actin (cytoskeleton), and blue: nuclei.

Immunostainings indicated the decreased expression of the tight junction protein occludin (Figure 8B, green) and the reorganisation of the cytoskeleton (red) in cells from a non-asthmatic patient treated with a high concentration of chymase for 24h, compared to non-stimulated cells. While non-stimulated cells displayed a uniform and even pericellular expression, chymase-treated cells demonstrated a patchy expression pattern of occludin throughout the sample.

Summary of Results

The main findings from the present thesis are summarised in a graphical figure (Figure 9). Collectively, the results from the included papers show that intraepithelial MC release of the tryptase and chymase proteases enhance cell migration, induce morphological alterations, and had opposing effects on cell division patterns and cell growth. Tryptase increased pro-survival gene and protein expression, altered epithelial release of various GFs and cytokines, and promoted wound gap closure, while chymase impaired the epithelial barrier function.

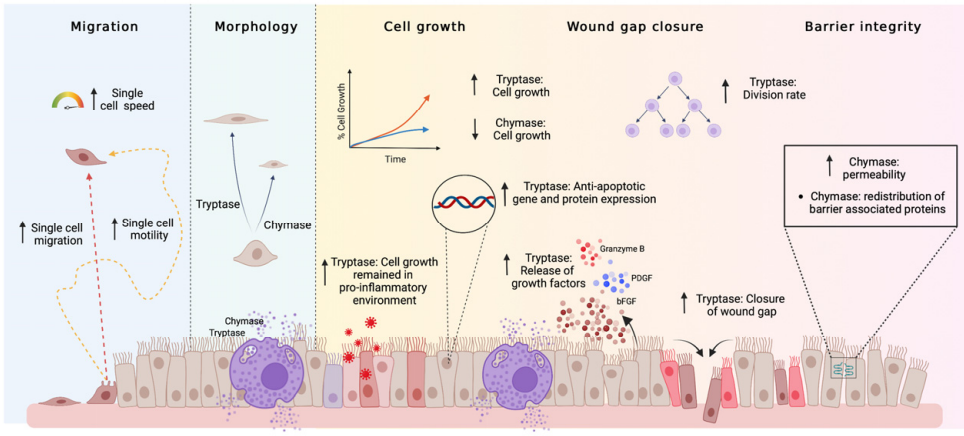


Figure 9. Illustration summarising the results obtained in the present thesis. The image was created in BioRender by Frida Berlin.

Taken together, these results indicate vast and powerful effects of tryptase and chymase on epithelial cell function and thus, are likely to play critical roles in airway remodelling and in the disruption of epithelial homeostasis and function under pathological conditions.

Conclusions

The findings presented in this thesis shed light on the role of tryptase and chymase on airway remodelling and epithelial dysfunction.

Tryptase exerts significant influence on various remodelling events, particularly those related to cell growth, cell survival, and wound healing. Hence, tryptase may be involved in the cell growth of different lung structures commonly observed in patients with respiratory diseases. Pro-migratory effects induced by tryptase were PAR-2 dependent, suggesting an important role for tryptase activation of PAR-2 in rapid gap closure following epithelial damage.

Chymase has a profound impact on bronchial remodelling and barrier integrity, and this is primarily associated with loss of cell-cell contact, increased cell migration, and disruption of barrier integrity.

Collectively, the release of tryptase and chymase by intraepithelial MCs appears to induce critical activities that are involved in respiratory disease progression and in disruption of crucial epithelial functions.

Discussion

Morphology and Migration

The role of MCs and their mediators on epithelial function has been poorly studied. Using the holographic live-cell imaging technology Holomonitor M4, we quantified different morphological parameters, performed single-cell tracking analysis, and evaluated wound healing capacity in submerged cultures stimulated with tryptase or chymase. The results indicate that both proteases can actively induce changes in the shape and migratory effects of the bronchial epithelium, which may have implication for cell-cell contact and tissue structure and functions in the lungs. However, the molecular mechanisms involved in these features remain to be explored, although such mechanisms might be limited to epithelial cells, since no pro-migratory effects have been seen in fibroblasts stimulated with chymase (123). Notably, the migratory and morphological effects are not identical, and this suggests that the proteases might partly mediate responses through different signalling mechanisms or might activate distinct cellular pathways.

Epithelial proliferation and migration are required in the wound healing process and can be induced by various GFs, cytokines and other triggers after epithelial injury or viral infection (117, 124, 125). Because cell migration is associated with important wound healing processes, the wound healing capacity of tryptase was investigated. The results revealed that tryptase promotes wound gap closure via PAR-2 by inducing migratory properties in BECs. These results are in line with previous results showing that tryptase is involved in tissue repairment of several cell types (126). However, in pathological contexts tryptase's involvement in proliferation, migration, and wound healing may contribute to critical epithelial expansion, which may lead to chronic inflammation and subsequently remodelling (80). Therefore, tryptase and PAR-2 are potential targets for therapeutic strategies aimed at promoting efficient gap closure while minimizing the detrimental effects associated with chronic inflammation and remodelling.

Dual Roles in Epithelial Homeostasis

A hallmark of airway remodelling in asthma is thickening of the airway wall, including the epithelium, submucosa, and smooth muscle layer, and these effects are suggested to be a result of chronic inflammation (127). Previous *in vitro* studies have reported that tryptase can induce proliferative properties in various cell types based on the altered regulation of genes and proteins that are involved in proliferation (82, 84, 128). However, as far as we are aware, this has never been investigated functionally in either bronchial or alveolar epithelial cells, and thus the underpinning mechanisms of airway thickening remain poorly understood. Therefore, the results from this thesis provide valuable insights into the functional effects of tryptase on epithelial cells in the context of lung diseases. Because tryptase exhibited mitogenic properties on BECs and AECs by promoting cell growth, increasing cell survival expression, and the release of cytokines, these findings contribute to a better understanding of the role of tryptase in airway remodelling and epithelial dysfunction. We also show that the effect on cell growth may at least partially be induced by PAR-2 receptor, however; this needs to be further functionally investigated.

Interestingly, this work showed that tryptase significantly shortened the cell division intervals, which indicates an acceleration in the cell division rate, both in the early and the late phase of the experiment. This suggests that the mitogenic effects of tryptase remained even after more than 24 h post-stimulation, which is when the enzymatic activity had been lost. This may be explained by unknown intracellular effects and/or by the fact that tryptase significantly induced the release of potent GFs in the epithelial cells. These GFs are likely to influence the cells within the cell culture, and importantly, seen from an *in vivo* perspective, these GFs may have implications on the local microenvironment as well. For example, bFGF and PDGF-AA are strongly associated with airway remodelling in asthma (118, 119), and since tryptase has the ability to induce the release of these GFs, our data suggests that tryptase has a substantial impact on the cell growth not only of epithelial cells, but also on other cell types (1).

In contrast, functional and molecular assays demonstrated that chymase-stimulated BECs had a reduction in cell growth, compared to non-stimulated BECs (Paper I). Further analyses revealed that the decreased cell growth was not due to cell death or cytotoxicity but rather to a decreased division rate, reduced metabolic activity, and reduced Ki67 expression. Interestingly, chymase has been reported to induce some proliferative effects in other cell types, including fibroblasts and small airway epithelial cells (129, 130). These opposing results imply that chymase may induce different biological activities depending on cell type. Additionally, the small airway epithelial cells were primary cells, which may also explain the opposing results. However, the results from this study suggest that chymase has a diminishing effect

on bronchial epithelial cell growth, which may cause impaired maintenance of bronchial epithelium and weakened repair capacity upon injury.

Chymase Disrupts Bronchial Epithelial Barrier Properties

Because there is an increased number of MC_{TC}s in alveolar and bronchial epithelium under pathological conditions (64, 67, 69, 70), it is likely that these structures are exposed to various concentrations of chymase. Zhou et al. has shown that chymase acts detrimentally on barrier integrity in epithelial layers (105), but the impact of chymase on barrier function has not been investigated in PBECs previously. As cells from patients with severe asthma show greater epithelial permeability at baseline compared to healthy donors (113), we aimed to compare healthy and asthmatic patients' responses to chymase exposure. Therefore, we used a translational *in vitro* model, i.e., fully differentiated BCis as well as PBECs from patients with severe asthma and healthy donors in order to study how the epithelial integrity is affected by chymase.

Both healthy and asthmatic PBECs stimulated with chymase showed a fluctuating ionic permeability pattern over time. This was demonstrated by an initial drop at 1 h, a quick recovery at 2 h and a second drop at 6 h. At the first drop and recovery, the high concentrations of chymase had a slightly bigger drop and poorer recovery than the cells treated with the low concentration, which showed full recovery. However, after the second drop at 6 h, only the healthy cells stimulated with a low concentration of chymase were able to fully recover. These observations may be explained by the different ability for cells to initiate epithelial restorative responses and/or by the proteolytic properties of chymase. The drop at 1 h may be caused by the direct enzymatic activity of chymase, followed by an initial recovery response induced by the epithelial cells. The second drop observed at 6 h, may be due to a second (delayed) epithelial defence mechanisms, which might cause the increased permeability. Until now, the differences in permeability were possibly associated with the concentrations of chymase, where no clear differences between healthy and severely asthmatic ALI cultures were observed. A further, an explanation for why the severely asthmatic, but not the healthy ALI cultures stimulated with low concentration of chymase failed to recover may be due to impaired epithelial healing mechanisms in these subjects. Importantly, these hypotheses are not yet confirmed, and we aim to further investigate these preliminary results to broaden our insights into the role of chymase on epithelial barrier integrity and to compare the responses between PBECs from healthy and asthmatic patients. It has previously been reported that inhibition of TNF-α neutralized barrier permeability (131), and therefore, it

would be of importance to further analyse the cellular response in order to understand these differences.

An important element for providing a proper barrier is the formation of junctions between the epithelial cells lining the lungs. Previous studies have reported that the physical bronchial epithelium is decreased in asthma pathology, with impaired tight junction and adheres junction proteins (113, 114). Differentiated PBECs stimulated with a high concentration of chymase had low expression of the tight junction protein occludin and showed a rearrangement of the cytoskeleton at 24 h post stimulation, when compared to non-stimulated controls. This suggests that chymase induces damage to barrier-associated proteins which may have severe consequences for epithelial barrier function. Thus, in the context of the epithelial barrier, the presence of intraepithelial MC_{TC}s along with their release of chymase, may play a critical role in the susceptibility to external pathogens and chronic inflammation.

Advantages and Limitations with the Experimental Design

The majority of experiments conducted in this thesis were based on cell lines. Using cell lines and primary cells each has its advantages and disadvantages. Immortalized cell lines are readily available and can be maintained in culture for long periods, they are often homogenous, thus providing a consistent and standardized cell population for experiments, and they are cost-effective (122). Additionally, with regards to ethical considerations and respect to the donors, I believe that in explorative studies like the ones conducted in the thesis, the preliminary experiments and optimisation of experimental protocols would best be performed in cell lines, and then confirmed in primary cells in order to minimize waste of valuable human material. However, cell lines may not fully recapitulate the complexity and heterogeneity of primary cells, thus potentially limiting their relevance to *in vivo* conditions (132). Primary cells (and some exceptional cell lines, such as BCi) also allow a more translational model, as was used in paper IV, because they exhibit multipotent differentiation capacity and thus enable culturing in ALI and 3D experimental set ups (122). The 3D models have several advantages over the 2D models (submerged cultures) by better resembling the cellular phenotypes found *in vivo* and exhibiting epithelial barrier formation (132).

While 3D models are considered more translational and physiologically relevant compared to submerged cultures, they do also have limitations, particularly when using a single cell type. To better mimic the complex human lung environment *in vitro*, additional experimental methods, such as co-cultures, can offer advantages for future research. The use of co-cultures involves culturing multiple cell types together, allowing for interactions and crosstalk between different cell populations.

In the context of the aim of this thesis, incorporated co-cultures of epithelial cells and MCs may provide a more comprehensive understanding of the cellular responses and their interactions.

In order to investigate the effects of tryptase and chymase on epithelial cells, it is important to consider the *in vivo* conditions and complexities associated with their concentrations and the type of MC degranulation. The exact concentrations of the mature proteases *in vivo* remain uncertain and variable (133-135). Furthermore, the degradation and regulation of proteases may differ *in vivo*, where additional factors and cells are present, compared to our *in vitro* set-up where only one cell type was used.

The assessment of ideal protease concentrations for *in vitro* studies was challenging due to the complexity of replicating the *in vivo* milieu. Although these concentrations may not fully reproduce the *in vivo* scenario, our objective was to use concentrations that elicit epithelial responses based on various parameters. The findings from this thesis serve as a basis for further investigation to better understand the interaction between MC proteases and epithelial cells in respiratory diseases.

The Significance of Our Findings

Previous MC research has predominantly focused on IgE-mediated activation and mechanisms in allergic responses, and this thesis expands our understanding of MC function in the light of tissue remodelling.

It is increasingly recognized that MCs play fundamental roles beyond IgE-mediated pathways. The results obtained in this thesis highlight the functional effects of tryptase and chymase on bronchial and alveolar epithelial cells, their contribution to the maintenance of cellular homeostasis as well as their potential involvement in respiratory disease pathogenesis. The different effects of tryptase and chymase on epithelial function indicate the involvement of different mode of actions, further emphasizing the complexity of MC-mediated processes in the lungs. In line with previous hypotheses, our results suggest that MCs are important for regulating cell growth and homeostasis, but whether tryptase and chymase are beneficial or detrimental are still not evident.

If intraepithelial MCs release both tryptase and chymase, it is likely that both proteases induce different functional events on the microenvironment. Our studies suggest that tryptase stimulate cell growth and increase wound healing which are important for protecting the epithelial barrier and close wound gaps. On the other hand, if large amounts of tryptase are released continuously by MCs, it may lead to excessive cell growth and thus promote detrimental tissue remodelling, particularly

towards a thickening of airway structures. In contrast, chymase may play an important role in dampening these events by reducing cell growth, cleaving cytokines and GFs, degrading junction proteins, etc. However, as chymase exhibits relatively strong proteolytic effects on barrier structures, released chymase may lead to barrier impairments and cause epithelial injuries, which in turn may result in inflammation and tissue remodelling.

Since our results from this thesis show contradicting effects not only of the proteases in epithelial homeostasis, but also of the proteases role in the respiratory disease pathogenesis, the impact of MCs, and in particularly MC_{TC}s, in disease prevention/development is still not fully understood. Importantly, it is not known how much of tryptase and/or chymase that are released *in vivo*, although both proteases are expressed and stored in the cytosol – but overactivated MCs or excessive release of tryptase and/or chymase may play a central role in the pathogenesis of respiratory diseases.

Further research is warranted to uncover the precise underlying molecular mechanisms that are involved in respiratory conditions and to explore the potential therapeutic targeting of MC proteases. Developing clinical interventions are needed to alleviate airway remodelling and restore epithelial homeostasis.

Future Perspectives

The findings presented in this thesis have shed light on the functional role of tryptase and chymase, both potent MC proteases, on lung epithelial cells and highlight the importance of investigating these proteases in human disease. However, there is still much to explore in order to fully comprehend the impact of MCs on disease pathogenesis *in vivo*.

Identify the relevant molecular mechanisms

Little is known regarding the mechanism of action of both tryptase and chymase. While the present study, along with other research, has demonstrated significant effects of these proteases on epithelial and other structural cells, the underlying mechanisms are still poorly characterized. In addition, our understanding of the signalling pathways and cellular events induced by tryptase and chymase are limited. Although PAR-2 appears to play a role in certain cellular activations and activities (136), numerous events remain uncovered and require additional investigation to fully understand the comprehensive actions of tryptase and chymase on cellular responses and their remodelling effects. This would provide vital insights for the development of therapeutic strategies. To address these knowledge gaps, future research should focus on downstream signalling cascades and identifying specific receptors or extracellular targets.

The role of MCs in epithelial barrier disruption

As observed in paper IV, chymase may have a direct proteolytic effect but may also be able to cause changes in permeability due to its initiation of the epithelial response. Thus, it is crucial to analyse the epithelial release of different cytokines, alarmins, and other proteins in order to understand the underpinning molecular mechanisms causing epithelial impairments. In addition, gene and protein profiling and characterization are needed. Finally, to get a bigger picture of the role of intraepithelial MC_{TC}s in maintaining barrier function, it would be essential to perform additional experiments using tryptase and perhaps MC supernatants because they also might affect barrier properties.

Explore other MC-associated proteins

Since MCs are highly plastic cells, and because the composition of their granules can be greatly influenced by the local microenvironment, it would be of great interest to further characterize MC heterogeneity in different lung compartments under different disease conditions. It is important to note that MCs express various other proteins that may also have significant implications for disease development. One such protein is CPA3, which warrants further investigation. Performing similar experiments to those conducted in this study could provide valuable insights into the roles of MCs in disease pathogenesis and may involve MC-associated proteins related to cell growth, migration, inflammation, and tissue remodelling in lung epithelial cells or relevant models. In line with this, it will be highly relevant to advance the *in vitro* models by, for example, using ALI cultures, co-cultures, or organoids.

Inhibition of specific targets?

By incorporating inhibitors into the experimental design, we can gain insights into the specific contributions of tryptase and chymase to cellular processes and can evaluate the potential of inhibitors to alleviate or modulate these effects. As an example, it would be interesting to inhibit certain potential proteins that may be involved in the second drop in epithelial permeability, which was observed in paper IV. These kinds of studies would contribute to our understanding of MC-derived secondary mechanisms and help identifying potential therapeutic strategies that can disrupt MC-mediated pathogenesis in lung diseases.

Populärvetenskaplig Sammanfattning

Kroniska luftvägssjukdomar är ett samlingsnamn för bestående sjukdomar som påverkar luftvägarna och andra delar av lungan, här inkluderas bland annat astma och KOL. Omkring 450 miljoner människor världen över är drabbade av någon form av kronisk respiratorisk sjukdom och sjukdomarna utgör den tredje vanligaste dödsorsaken i världen med över 3 miljoner dödsfall årligen. Den ursprungliga orsaken till varför luftvägssjukdomarna utvecklas är fortfarande okänt, däremot har exempelvis tobaksrök, luftföroreningar, damm och frekventa respiratoriska infektioner i barndomen identifierats som riskfaktorer till sjukdomsutvecklingen. Idag finns det ingen bot mot dessa sjukdomar, men det finns olika typer av symptomlindrande mediciner tillgängliga. Trots detta är det inte alla som blir hjälpta.

Varje dag andas vi in 10 000 liter luft. I och med det är det en stor risk att lungorna kommer i kontakt med externa, potentiellt skadliga partiklar, såsom virus, bakterier och mikrober. Den delen av lungans yta som vetter mot den inandade luften består av miljontals, hårt sammanlänkade epitelceller, som bildar lungepitelet. Lungepitelet är en ytterst viktig komponent i immunförsvaret genom att det skapar en fysisk barriär som skyddar oss mot utomstående partiklar. Dessutom kan lungepitelet reagera på potentiella faror och påkalla förstärkning av immunceller med syfte att eliminera dessa faror, på så vis kan en frisk vävnad upprätthållas. En av de första immuncellerna att exponeras för externa partiklar är mastcellen. Mastceller är en del av det medfödda immunförsvaret och finns i alla kroppens organ, bland annat i lungorna. När mastceller stöter på externa partiklar blir de aktiverade och utsöndrar en stor mängd olika kraftfulla proteiner som finns lagrade inne i cellen, häribland tryptas och kymas, som har till uppgift att skydda oss mot en potentiell skada och läka eventuella sår. I denna avhandling belyses två faktorer i utvecklingen av luftvägssjukdomar: proteinerna tryptas och kymas.

De senaste årens forskning har visat att mastceller är involverade i sjukdomsutvecklingen av olika typer av lungsjukdomar, som exempelvis astma. Astma är en komplex lungsjukdom, som leder till nedsatt andningsförmåga och hosta samt kan ge bröstsmärtor. En astmatikers tillstånd förvärras i regel när de exponeras för partiklar som triggar inflammationen, som till exempel virus. Trots att forskning genomförts i decennier är det fortfarande inte klarlagt vilka faktorer det är som driver sjukdomsutvecklingen.

Studier har visat att mastceller finns i mycket högt antal i astmatikers lungor, dock är effekten av mastcellers, eller dess utsöndrade proteiners, verkan på sjukdomen är inte känd. Då tryptas och kymas är de mest frekvent förekommande proteinerna i mastceller är en hypotes att astmatikers lungvävnad exponeras för högre nivåer av tryptas och kymas än hos friska. Det är därför av intresse att studera dessa proteiners inverkan på sjukdomsutvecklingen, vare sig den är positiv eller negativ, för att på så vis eftersträva djupare förståelse för mastcellernas roll i luftvägssjukdomar. Därför är forskningen i den här doktorsavhandlingen ägnad åt att fördjupa kunskapen om mastcellers roll i sjukdomsutveckling av astma, med fokus på tryptas och kymas.

Genom att i laboratoriet stimulera epitelceller med tryptas och kymas har olika typer av parametrar kunnat läsas av. Resultaten visar att dessa två proteiner har olika effekter på epitelceller, som i sin tur skulle kunna spela centrala roller i sjukdomsutvecklingen av kroniska lungsjukdomar, såsom astma. Resultaten visar också att proteinerna delvis har olika egenskaper.

Tryptas uppvisade egenskaper som bidrog till att epitelcellerna ökade kraftigt i tillväxt och överlevnad samt inducerade ytterligare frisläppning av potenta tillväxt-proteiner från epitelcellerna – som i sin tur kan påverka tillväxten av olika typer av lungceller, inklusive epitelcellerna själva. Detta skulle kunna leda till förtjockning av olika typer av lungvävnader, vilket är karakteristiskt för astma.

Kymas å andra sidan, minskade tillväxten av epitelceller. Utöver det så ändrade kymas också cellernas form och inducerade förhöjda rörelsemönster. Dessutom observerades att kymas hade negativa effekter på epitelbarriären och skadade de andra proteinerna som länkar samman epitelcellerna och ökade genomsläppligheten av små molekyler. När astmatiker jämfördes med friska framkom att astmatiker hade nedsatt återhämtning efter kymas-inducerad skada, vilket tyder på att kymas bryter upp den fysiska barriären i både astmatiker och friska, men att astmatiker har fördröjd återhämtning jämfört med friska.

Sammantaget visar studierna i avhandlingen att tryptas och kymas har olika funktioner i celltillväxt där de båda proteinerna stör den normala celltillväxtbalansen och inducerar ytterligare strukturförändringar i epitelet, vilka är vanligt förekommande kännetecken i astma. Kymas orsakade också skada på lungbarriären, vilket tyder på att kymas kan öka genomsläppligheten av skadliga ämnen och att astmatiker har försämrad återhämtning jämfört med friska. Sammanfattningsvis belyser resultaten att mastceller, genom utsöndrandet av framför allt tryptas och kymas, verkar ha en central roll i utvecklingen av luftvägssjukdomar. Genom en ökad förståelse för samspelet mellan mastceller och lungvävnad kan nya terapeutiska strategier för att återställa epitelfunktionen tas fram och leda till förbättring den allmänna hälsan hos drabbade patienter.

Acknowledgement

I wish to express my sincere gratitude to all who have contributed and supported me during my PhD studies. This would never have been possible without your hard work and encouragement.

Först av allt, vill jag tacka min otroliga handledare **Cecilia Andersson** för precis allt. En komplett person som jag har haft äran att ha som handledare under dessa år och inspirerat mig sen dag ett. Du har fått mig att utvecklas och växa som ingen annan skulle kunna ha gjort. Tack för att du alltid har trott på mig, stöttat mig, utmanat mig, delat med dig av ditt lugn, ditt tålamod och givetvis stort tack för alla gapskratt!

Sofia Mogren, jag vore ingenting utan dig. Tack för all support, alla skratt, fenomenala samtal och inte minst alla ägg som vi har delat under åren. Du är min livs levande joke-box, jag vet aldrig riktigt vad som kommer hoppa ut ur din mun, och det är precis det som gör det så fantastiskt att få vara din närmsta kollega. Jag är fylld med tacksamhet och jag skulle aldrig kunna önska mig en bättre grupp att arbeta i. Tack Cecilia, Sofia och Lilla-Tuss för en helt fantastisk tid tillsammans!

Mina duktiga bi-handledare **Jonas Erjefält, Ellen Tufvesson** och **Lena Uller** för er support genom åren som har betytt mycket för mig och för min forskning.

Emily Swindle, at University of Southampton, for hosting me during the ERS fellowship and for your invaluable support and encouragement. Our collaboration has improved the scientific impact of my thesis greatly and it has been a pleasure to get to know you and your team.

Present members of the entire **Brooke-Lab** for your support and making my time in Southampton fantastic and so much fun. Special thanks to **Cornelia Blume, Alice Eminton, Katie Horton** and **Rob Wiley** for your important contribution to the laboratory work and training me. **Anna Willis** for the jazz!

Celeste Porsbjerg and **Morten Hvidtfeldt** and the rest of the group at Bispebjerg Hospital in Denmark, for your scientific support, discussions, and clinical material. This collaboration has also been vital for the thesis, and it has been a pleasure to work together with you and develop new projects together.

Samuel Cerps, Sofia Tillgren Malm och **Julia Tutzauer** för fina samarbeten, alla goa stunder på kontoret och jag är så glad att ni har hjälpt mig att tömma alla kakförråd som Sofia fyller upp. Ni har förgyllt mina dagar.

Jonas Erjefält and the whole Airway Inflammation and Immunology group, **Prem, Julia, Daisy, Renan, Manar, Hodan**, and Medetect AB, **Monika, Johan, Caroline L, Caroline S, Jimmie** och **Karin** for great meetings, AWs and parties!

Phase Holographic Imaging AB, especially **Kersti Alm** for invaluable support, service, and collaboration. It has had a great impact on my work!

Gunilla Westergren-Thorsson and the rest of **C12** for great collaboration, your welcoming and support. Special thanks to **Måns Kadefors** and **Zackarias Söderlund** for all funny chats, support and always being so nice.

Lisa Karlsson and **Maria Weitoft** for dejlige coffee breaks, book clubs, and your amazing spirits. You always make me smile!

The people at my research floor **Elisabeth Blankell, Bengt-Olof Nilsson, Sangeetha Ramu, Mandy Menzel, Juan José Nieto Fontarigo, Sara Bodahl, Catarina Rippe, Katarzyna Krawczyk, Katarzyna Kawka, Li Liu, Lotte Vanherle, Nicole van der Burg, Carin Larsson** and many, many more, for making D12 a wonderful place to work with all Friday breakfasts, the running club and spontaneous discussions.

My bachelor students **Camilla Ly, Lykke Eskilsson** and **Emanuel Behi Åkesson** for great contribution and for letting me supervise you.

I also want to send gratitude to a few people outside of my research network:

Min mamma **Gunnel Berlin** för att ha lett mig in på cellbiologi från första början, för din stöttning genom alla år och för att låta Bub-trollen leva vidare.

Min nära vän **Julia Dahlqvist** för alla korrekturläsningar som du har hjälpt mig med och för att följt med mig i både uppförsbackar och nedförsbackar under min doktorandtid. Det har betytt otroligt mycket och du har varit en riktig pärla, tack!

Rasmus Vesterlund för att du alltid har funnits där för mig och för att du har stöttat och uppmuntrat mig varje gång cellerna har dött, när jag närmar mig en deadline eller bara tålmodigt tittat på mina vackra filmer på cellerna. Tack älskade du!

Till sist vill jag skicka frustrationer och förtvivlan åt cancer som har gjort den här tiden extra utmanande för mig. Med det sagt, vill jag understryka tyngden av vetenskaplig forskning och tacka alla som på olika sätt ägnar sig åt och bidrar till att fördjupa kunskapen om människokroppen och sjukdomar. Vi vill åt samma håll, vi har samma mål och vi behöver varandra!

Tack min pappa **Torsten Bengtsson** för din humor som jag ofta tänker på och som får mig att se lättare på problem och livet! Din bortgång är det hårdaste jag har upplevt, men samtidigt fått mig mer motiverad än någonsin till att lära mig, forska och utvecklas!

References

1. Douaiher J, Succar J, Lancerotto L, Gurish MF, Orgill DP, Hamilton MJ, Krilis SA, Stevens RL. Development of mast cells and importance of their tryptase and chymase serine proteases in inflammation and wound healing. *Adv Immunol* 2014; 122: 211-252.
2. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev* 1997; 77: 1033-1079.
3. Hallgren J, Pejler G. Biology of mast cell tryptase. An inflammatory mediator. *Febs j* 2006; 273: 1871-1895.
4. Gilfillan AM, Austin SJ, Metcalfe DD. Mast cell biology: introduction and overview. *Adv Exp Med Biol* 2011; 716: 2-12.
5. Dahlin JS, Hallgren J. Mast cell progenitors: origin, development and migration to tissues. *Mol Immunol* 2015; 63: 9-17.
6. Virk H, Arthur G, Bradding P. Mast cells and their activation in lung disease. *Transl Res* 2016; 174: 60-76.
7. Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* 2006; 117: 1277-1284.
8. da Silva EZ, Jamur MC, Oliver C. Mast cell function: a new vision of an old cell. *J Histochem Cytochem* 2014; 62: 698-738.
9. Crivellato E, Ribatti D. The mast cell: an evolutionary perspective. *Biol Rev Camb Philos Soc* 2010; 85: 347-360.
10. Moon TC, St Laurent CD, Morris KE, Marcet C, Yoshimura T, Sekar Y, Befus AD. Advances in mast cell biology: new understanding of heterogeneity and function. *Mucosal Immunol* 2010; 3: 111-128.
11. Komi DEA, Khomtchouk K, Santa Maria PL. A Review of the Contribution of Mast Cells in Wound Healing: Involved Molecular and Cellular Mechanisms. *Clin Rev Allergy Immunol* 2020; 58: 298-312.
12. Abraham SN, St John AL. Mast cell-orchestrated immunity to pathogens. *Nat Rev Immunol* 2010; 10: 440-452.
13. Dvorak AM, Schleimer RP, Schulman ES, Lichtenstein LM. Human mast cells use conservation and condensation mechanisms during recovery from degranulation. In vitro studies with mast cells purified from human lungs. *Lab Invest* 1986; 54: 663-678.
14. Brockow K, Metcalfe DD. Mastocytosis. *Chem Immunol Allergy* 2010; 95: 110-124.
15. Carroll NG, Mutavdzic S, James AL. Distribution and degranulation of airway mast cells in normal and asthmatic subjects. *Eur Respir J* 2002; 19: 879-885.

16. Battaglia S, Mauad T, van Schadewijk AM, Vignola AM, Rabe KF, Bellia V, Sterk PJ, Hiemstra PS. Differential distribution of inflammatory cells in large and small airways in smokers. *J Clin Pathol* 2007; 60: 907-911.

17. Méndez-Enríquez E, Hallgren J. Mast Cells and Their Progenitors in Allergic Asthma. *Front Immunol* 2019; 10: 821.

18. Kraft S, Kinet JP. New developments in FcepsilonRI regulation, function and inhibition. *Nat Rev Immunol* 2007; 7: 365-378.

19. Bischoff SC, Dahinden CA. c-kit ligand: a unique potentiator of mediator release by human lung mast cells. *J Exp Med* 1992; 175: 237-244.

20. Brightling CE, Bradding P, Pavord ID, Wardlaw AJ. New insights into the role of the mast cell in asthma. *Clin Exp Allergy* 2003; 33: 550-556.

21. Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol* 2008; 8: 205-217.

22. Gurish MF, Bryce PJ, Tao H, Kisselgof AB, Thornton EM, Miller HR, Friend DS, Oettgen HC. IgE enhances parasite clearance and regulates mast cell responses in mice infected with Trichinella spiralis. *J Immunol* 2004; 172: 1139-1145.

23. Woolhiser MR, Okayama Y, Gilfillan AM, Metcalfe DD. IgG-dependent activation of human mast cells following up-regulation of FcgammaRI by IFN-gamma. *Eur J Immunol* 2001; 31: 3298-3307.

24. Finkelman FD, Khodoun MV, Strait R. Human IgE-independent systemic anaphylaxis. *J Allergy Clin Immunol* 2016; 137: 1674-1680.

25. Elieh Ali Komi D, Wöhrl S, Bielory L. Mast Cell Biology at Molecular Level: a Comprehensive Review. *Clin Rev Allergy Immunol* 2020; 58: 342-365.

26. Marshall JS, Portales-Cervantes L, Leong E. Mast Cell Responses to Viruses and Pathogen Products. *Int J Mol Sci* 2019; 20.

27. Yu Y, Blokhuis BR, Garssen J, Redegeld FA. Non-IgE mediated mast cell activation. *Eur J Pharmacol* 2016; 778: 33-43.

28. el-Lati SG, Dahinden CA, Church MK. Complement peptides C3a- and C5a-induced mediator release from dissociated human skin mast cells. *J Invest Dermatol* 1994; 102: 803-806.

29. Nilsson G, Johnell M, Hammer CH, Tiffany HL, Nilsson K, Metcalfe DD, Siegbahn A, Murphy PM. C3a and C5a are chemotaxins for human mast cells and act through distinct receptors via a pertussis toxin-sensitive signal transduction pathway. *J Immunol* 1996; 157: 1693-1698.

30. Salahi M, Parsa S, Nourmohammadi D, Razmkhah Z, Salimi O, Rahmani M, Zivary S, Askarzadeh M, Tapak MA, Vaezi A, Sadeghsalehi H, Yaghoobpoor S, Mottahedi M, Garousi S, Deravi N. Immunologic aspects of migraine: A review of literature. *Front Neurol* 2022; 13: 944791.

31. Lauritano D, Mastrangelo F, D'Ovidio C, Ronconi G, Caraffa A, Gallenga CE, Frydas I, Kritas SK, Trimarchi M, Carinci F, Conti P. Activation of Mast Cells by Neuropeptides: The Role of Pro-Inflammatory and Anti-Inflammatory Cytokines. *Int J Mol Sci* 2023; 24.

32. Maurer M, Wedemeyer J, Metz M, Piliponsky AM, Weller K, Chatterjea D, Clouthier DE, Yanagisawa MM, Tsai M, Galli SJ. Mast cells promote homeostasis by limiting endothelin-1-induced toxicity. *Nature* 2004; 432: 512-516.

33. Stevens RL, Adachi R. Protease-proteoglycan complexes of mouse and human mast cells and importance of their beta-tryptase-heparin complexes in inflammation and innate immunity. *Immunol Rev* 2007; 217: 155-167.

34. Schwartz LB. Tryptase: a clinical indicator of mast cell-dependent events. *Allergy Proc* 1994; 15: 119-123.

35. Irani AM, Schwartz LB. Human mast cell heterogeneity. *Allergy Proc* 1994; 15: 303-308.

36. 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: 4464-4468.

37. 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.

38. Bradding P. Human lung mast cell heterogeneity. *Thorax* 2009; 64: 278-280.

39. Yeh YW, Chaudhuri AS, Zhou L, Fang Y, Boysen P, Xiang Z. Mast Cells Are Identified in the Lung Parenchyma of Wild Mice, Which Can Be Recapitulated in Naturalized Laboratory Mice. *Front Immunol* 2021; 12: 736692.

40. Reynolds DS, Stevens RL, Lane WS, Carr MH, Austen KF, Serafin WE. Different mouse mast cell populations express various combinations of at least six distinct mast cell serine proteases. *Proc Natl Acad Sci U S A* 1990; 87: 3230-3234.

41. Stevens RL. Gene expression in different populations of mouse mast cells. *Ann N Y Acad Sci* 1991; 629: 31-37.

42. Stevens RL, Friend DS, McNeil HP, Schiller V, Ghildyal N, Austen KF. Strain-specific and tissue-specific expression of mouse mast cell secretory granule proteases. *Proc Natl Acad Sci U S A* 1994; 91: 128-132.

43. Xing W, Austen KF, Gurish MF, Jones TG. Protease phenotype of constitutive connective tissue and of induced mucosal mast cells in mice is regulated by the tissue. *Proc Natl Acad Sci U S A* 2011; 108: 14210-14215.

44. The L. GBD 2017: a fragile world. *Lancet* 2018; 392: 1683.

45. Prevalence and attributable health burden of chronic respiratory diseases, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Respir Med* 2020; 8: 585-596.

46. Labaki WW, Han MK. Chronic respiratory diseases: a global view. *Lancet Respir Med* 2020; 8: 531-533.

47. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. *Am J Respir Crit Care Med* 2001; 163: 1256-1276.

48. Mehta M, Dhanjal DS, Paudel KR, Singh B, Gupta G, Rajeshkumar S, Thangavelu L, Tambuwala MM, Bakshi HA, Chellappan DK, Pandey P, Dureja H, Charbe NB, Singh SK, Shukla SD, Nammi S, Aljabali AA, Wich PR, Hansbro PM, Satija S, Dua K. Cellular signalling pathways mediating the pathogenesis of chronic inflammatory respiratory diseases: an update. *Inflammopharmacology* 2020; 28: 795-817.

49. Levy ML, Bacharier LB, Bateman E, Boulet LP, Brightling C, Buhl R, Brusselle G, Cruz AA, Drazen JM, Duijts L, Fleming L, Inoue H, Ko FWS, Krishnan JA, Mortimer K, Pitrez PM, Sheikh A, Yorgancıoğlu A, Reddel HK. Key recommendations for primary care from the 2022 Global Initiative for Asthma (GINA) update. *NPJ Prim Care Respir Med* 2023; 33: 7.

50. Swindle EJ, Collins JE, Davies DE. Breakdown in epithelial barrier function in patients with asthma: identification of novel therapeutic approaches. *J Allergy Clin Immunol* 2009; 124: 23-34; quiz 35-26.

51. Holgate ST, Wenzel S, Postma DS, Weiss ST, Renz H, Sly PD. Asthma. *Nat Rev Dis Primers* 2015; 1: 15025.

52. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012; 18: 716-725.

53. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet* 2008; 372: 1107-1119.

54. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, Adcock IM, Bateman ED, Bel EH, Bleecker ER, Boulet LP, Brightling C, Chanez P, Dahlen SE, Djukanovic R, Frey U, Gaga M, Gibson P, Hamid Q, Jajour NN, Mauad T, Sorkness RL, Teague WG. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014; 43: 343-373.

55. Lambrecht BN, Hammad H. The immunology of asthma. *Nat Immunol* 2015; 16: 45-56.

56. Fahy JV. Type 2 inflammation in asthma--present in most, absent in many. *Nat Rev Immunol* 2015; 15: 57-65.

57. Jackson DJ, Busby J, Pfeffer PE, Menzies-Gow A, Brown T, Gore R, Doherty M, Mansur AH, Message S, Niven R, Patel M, Heaney LG. Characterisation of patients with severe asthma in the UK Severe Asthma Registry in the biologic era. *Thorax* 2021; 76: 220-227.

58. Byers DE. Defining the roles of IL-33, thymic stromal lymphopoietin, and IL-25 in human asthma. *Am J Respir Crit Care Med* 2014; 190: 715-716.

59. Hammad H, Lambrecht BN. The basic immunology of asthma. *Cell* 2021; 184: 1469-1485.

60. Kyriakopoulos C, Gogali A, Bartziokas K, Kostikas K. Identification and treatment of T2-low asthma in the era of biologics. *ERJ Open Res* 2021; 7.

61. Hong H, Liao S, Chen F, Yang Q, Wang DY. Role of IL-25, IL-33, and TSLP in triggering united airway diseases toward type 2 inflammation. *Allergy* 2020; 75: 2794-2804.

62. Gauvreau GM, Sehmi R, Ambrose CS, Griffiths JM. Thymic stromal lymphopoietin: its role and potential as a therapeutic target in asthma. *Expert Opin Ther Targets* 2020; 24: 777-792.

63. Adatia A, Wahab M, Satia I. Is tezepelumab more than just an anti-eosinophil drug? *Eur Respir J* 2022; 59.

64. Balzar S, Fajt ML, Comhair SA, Erzurum SC, Bleecker E, Busse WW, Castro M, Gaston B, Israel E, Schwartz LB, Curran-Everett D, Moore CG, Wenzel SE. Mast cell phenotype, location, and activation in severe asthma. Data from the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2011; 183: 299-309.

65. 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: 1699-1705.

66. Hvidtfeldt M, Sverrild A, Pulga A, Frøssing L, Silberbrandt A, Hostrup M, Thomassen M, Sanden C, Clausson CM, Siddhuraj P, Bornesund D, Nieto-Fontarigo JJ, Uller L, Erjefält J, Porsbjerg C. Airway hyperresponsiveness reflects corticosteroid-sensitive mast cell involvement across asthma phenotypes. *J Allergy Clin Immunol* 2023.

67. Andersson CK, Weitoft M, Rydell-Törmänen K, Bjermer L, Westergren-Thorsson G, Erjefält JS. Uncontrolled asthmatics have increased FceRI(+) and TGF-β-positive MC(TC) mast cells and collagen VI in the alveolar parenchyma. *Clin Exp Allergy* 2018; 48: 266-277.

68. Balzar S, Chu HW, Strand M, Wenzel S. Relationship of small airway chymase-positive mast cells and lung function in severe asthma. *Am J Respir Crit Care Med* 2005; 171: 431-439.

69. Andersson CK, Mori M, Bjermer L, Lofdahl CG, Erjefalt JS. Alterations in lung mast cell populations in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010; 181: 206-217.

70. Andersson CK, Andersson-Sjoland A, Mori M, Hallgren O, Pardo A, Eriksson L, Bjermer L, Lofdahl CG, Selman M, Westergren-Thorsson G, Erjefalt JS. Activated MCTC mast cells infiltrate diseased lung areas in cystic fibrosis and idiopathic pulmonary fibrosis. *Respir Res* 2011; 12: 139.

71. Andersson CK, Tufvesson E, Aronsson D, Bergqvist A, Mori M, Bjermer L, Erjefalt JS. Alveolar mast cells shift to an FcepsilonRI-expressing phenotype in mild atopic asthma: a novel feature in allergic asthma pathology. *Allergy* 2011; 66: 1590-1597.

72. Lai Y, Altemeier WA, Vandree J, Piliponsky AM, Johnson B, Appel CL, Frevert CW, Hyde DM, Ziegler SF, Smith DE, Henderson WR, Jr., Gelb MH, Hallstrand TS. Increased density of intraepithelial mast cells in patients with exercise-induced bronchoconstriction regulated through epithelially derived thymic stromal lymphopoietin and IL-33. *J Allergy Clin Immunol* 2014; 133: 1448-1455.

73. Paivandy A, Pejler G. Novel Strategies to Target Mast Cells in Disease. *J Innate Immun* 2021; 13: 131-147.

74. Marc MM, Korosec P, Kosnik M, Kern I, Flezar M, Suskovic S, Sorli J. Complement factors c3a, c4a, and c5a in chronic obstructive pulmonary disease and asthma. *Am J Respir Cell Mol Biol* 2004; 31: 216-219.

75. Castleman WL, Sorkness RL, Lemanske RF, Jr., McAllister PK. Viral bronchiolitis during early life induces increased numbers of bronchiolar mast cells and airway hyperresponsiveness. *Am J Pathol* 1990; 137: 821-831.

76. van Schaik SM, Tristram DA, Nagpal IS, Hintz KM, Welliver RC, 2nd, Welliver RC. Increased production of IFN-gamma and cysteinyl leukotrienes in virus-induced wheezing. *J Allergy Clin Immunol* 1999; 103: 630-636.

77. Atiakshin D, Buchwalow I, Samoilova V, Tiemann M. Tryptase as a polyfunctional component of mast cells. *Histochem Cell Biol* 2018; 149: 461-477.

78. Wernersson S, Pejler G. Mast cell secretory granules: armed for battle. *Nat Rev Immunol* 2014; 14: 478-494.

79. Compton SJ, Cairns JA, Holgate ST, Walls AF. The role of mast cell tryptase in regulating endothelial cell proliferation, cytokine release, and adhesion molecule expression: tryptase induces expression of mRNA for IL-1 beta and IL-8 and stimulates the selective release of IL-8 from human umbilical vein endothelial cells. *J Immunol* 1998; 161: 1939-1946.

80. Cairns JA. Mast cell tryptase and its role in tissue remodelling. *Clin Exp Allergy* 1998; 28: 1460-1463.

81. Mogren S, Berlin F, Eskilsson L, Van Der Burg N, Tufvesson E, Andersson CK. Mast Cell Proteases Promote Diverse Effects on the Plasminogen Activation System and Wound Healing in A549 Alveolar Epithelial Cells. *Cells* 2022; 11.

82. Brown JK, Jones CA, Rooney LA, Caughey GH. Mast cell tryptase activates extracellular-regulated kinases (p44/p42) in airway smooth-muscle cells: importance of proteolytic events, time course, and role in mediating mitogenesis. *Am J Respir Cell Mol Biol* 2001; 24: 146-154.

83. Brown JK, Tyler CL, Jones CA, Ruoss SJ, Hartmann T, Caughey GH. Tryptase, the dominant secretory granular protein in human mast cells, is a potent mitogen for cultured dog tracheal smooth muscle cells. *Am J Respir Cell Mol Biol* 1995; 13: 227-236.

84. Cairns JA, Walls AF. Mast cell tryptase is a mitogen for epithelial cells. Stimulation of IL-8 production and intercellular adhesion molecule-1 expression. *J Immunol* 1996; 156: 275-283.

85. Bagher M, Larsson-Callerfelt AK, Rosmark O, Hallgren O, Bjermer L, Westergren-Thorsson G. Mast cells and mast cell tryptase enhance migration of human lung fibroblasts through protease-activated receptor 2. *Cell Commun Signal* 2018; 16: 59.

86. Akers IA, Parsons M, Hill MR, Hollenberg MD, Sanjar S, Laurent GJ, McAnulty RJ. Mast cell tryptase stimulates human lung fibroblast proliferation via protease-activated receptor-2. *Am J Physiol Lung Cell Mol Physiol* 2000; 278: L193-201.

87. Cruse G, Bradding P. Mast cells in airway diseases and interstitial lung disease. *Eur J Pharmacol* 2016; 778: 125-138.

88. Bühling F, Groneberg D, Welte T. Proteases and their role in chronic inflammatory lung diseases. *Curr Drug Targets* 2006; 7: 751-759.

89. Barrios VE, Middleton SC, Kashem MA, Havill AM, Toombs CF, Wright CD. Tryptase mediates hyperresponsiveness in isolated guinea pig bronchi. *Life Sci* 1998; 63: 2295-2303.

90. Abraham WM. Tryptase: potential role in airway inflammation and remodeling. *Am J Physiol Lung Cell Mol Physiol* 2002; 282: L193-196.

91. Pejler G. The emerging role of mast cell proteases in asthma. *Eur Respir J* 2019; 54.

92. Woodman L, Siddiqui S, Cruse G, Sutcliffe A, Saunders R, Kaur D, Bradding P, Brightling C. Mast cells promote airway smooth muscle cell differentiation via autocrine up-regulation of TGF-beta 1. *J Immunol* 2008; 181: 5001-5007.

93. Mortaz E, Givi ME, Da Silva CA, Folkerts G, Redegeld FA. A relation between TGF-β and mast cell tryptase in experimental emphysema models. *Biochim Biophys Acta* 2012; 1822: 1154-1160.

94. Mortaz E, Folkerts G, Redegeld F. Mast cells and COPD. *Pulm Pharmacol Ther* 2011; 24: 367-372.

95. Urata H, Kinoshita A, Misono KS, Bumpus FM, Husain A. Identification of a highly specific chymase as the major angiotensin II-forming enzyme in the human heart. *J Biol Chem* 1990; 265: 22348-22357.

96. Taipale J, Lohi J, Saarinen J, Kovanen PT, Keski-Oja J. Human mast cell chymase and leukocyte elastase release latent transforming growth factor-beta 1 from the extracellular matrix of cultured human epithelial and endothelial cells. *J Biol Chem* 1995; 270: 4689-4696.

97. Tchougounova E, Lundequist A, Fajardo I, Winberg JO, Abrink M, Pejler G. A key role for mast cell chymase in the activation of pro-matrix metalloprotease-9 and pro-matrix metalloprotease-2. *J Biol Chem* 2005; 280: 9291-9296.

98. Groschwitz KR, Wu D, Osterfeld H, Ahrens R, Hogan SP. Chymase-mediated intestinal epithelial permeability is regulated by a protease-activating receptor/matrix metalloproteinase-2-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol* 2013; 304: G479-489.

99. Vartio T, Seppä H, Vaheri A. Susceptibility of soluble and matrix fibronectins to degradation by tissue proteinases, mast cell chymase and cathepsin G. *J Biol Chem* 1981; 256: 471-477.

100. Mizutani H, Schechter N, Lazarus G, Black RA, Kupper TS. Rapid and specific conversion of precursor interleukin 1 beta (IL-1 beta) to an active IL-1 species by human mast cell chymase. *J Exp Med* 1991; 174: 821-825.

101. Fu Z, Thorpe M, Alemayehu R, Roy A, Kervinen J, de Garavilla L, Åbrink M, Hellman L. Highly Selective Cleavage of Cytokines and Chemokines by the Human Mast Cell Chymase and Neutrophil Cathepsin G. *J Immunol* 2017; 198: 1474-1483.

102. Pejler G. Novel Insight into the in vivo Function of Mast Cell Chymase: Lessons from Knockouts and Inhibitors. *J Innate Immun* 2020; 12: 357-372.

103. Knight PA, Wright SH, Lawrence CE, Paterson YY, Miller HR. Delayed expulsion of the nematode Trichinella spiralis in mice lacking the mucosal mast cell-specific granule chymase, mouse mast cell protease-1. *J Exp Med* 2000; 192: 1849-1856.

104. Cho SH, Lee SH, Kato A, Takabayashi T, Kulka M, Shin SC, Schleimer RP. Cross-talk between human mast cells and bronchial epithelial cells in plasminogen activator inhibitor-1 production via transforming growth factor-β1. *Am J Respir Cell Mol Biol* 2015; 52: 88-95.

105. Zhou X, Wei T, Cox CW, Jiang Y, Roche WR, Walls AF. Mast cell chymase impairs bronchial epithelium integrity by degrading cell junction molecules of epithelial cells. *Allergy* 2019; 74: 1266-1276.

106. Ramu S, Akbarshahi H, Mogren S, Berlin F, Cerps S, Menzel M, Hvidtfeldt M, Porsbjerg C, Uller L, Andersson CK. Direct effects of mast cell proteases, tryptase and chymase, on bronchial epithelial integrity proteins and anti-viral responses. *BMC Immunol* 2021; 22: 35.

107. Waern I, Lundequist A, Pejler G, Wernersson S. Mast cell chymase modulates IL-33 levels and controls allergic sensitization in dust-mite induced airway inflammation. *Mucosal Immunol* 2013; 6: 911-920.

108. de Garavilla L, Greco MN, Sukumar N, Chen ZW, Pineda AO, Mathews FS, Di Cera E, Giardino EC, Wells GI, Haertlein BJ, Kauffman JA, Corcoran TW, Derian CK, Eckardt AJ, Damiano BP, Andrade-Gordon P, Maryanoff BE. A novel, potent dual inhibitor of the leukocyte proteases cathepsin G and chymase: molecular mechanisms and anti-inflammatory activity in vivo. *J Biol Chem* 2005; 280: 18001-18007.

109. Davies DE. Epithelial barrier function and immunity in asthma. *Ann Am Thorac Soc* 2014; 11 Suppl 5: S244-251.

110. Hellings PW, Steelant B. Epithelial barriers in allergy and asthma. *J Allergy Clin Immunol* 2020; 145: 1499-1509.

111. Matter K, Balda MS. Signalling to and from tight junctions. *Nat Rev Mol Cell Biol* 2003; 4: 225-236.

112. Rock JR, Randell SH, Hogan BL. Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis Model Mech* 2010; 3: 545-556.

113. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, Haitchi HM, Vernon-Wilson E, Sammut D, Bedke N, Cremin C, Sones J, Djukanović R, Howarth PH, Collins JE, Holgate ST, Monk P, Davies DE. Defective epithelial barrier function in asthma. *J Allergy Clin Immunol* 2011; 128: 549-556.e541-512.

114. de Boer WI, Sharma HS, Baelemans SM, Hoogsteden HC, Lambrecht BN, Braunstahl GJ. Altered expression of epithelial junctional proteins in atopic asthma: possible role in inflammation. *Can J Physiol Pharmacol* 2008; 86: 105-112.

115. Kuwano K, Bosken CH, Paré PD, Bai TR, Wiggs BR, Hogg JC. Small airways dimensions in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1993; 148: 1220-1225.

116. Elias JA, Zhu Z, Chupp G, Homer RJ. Airway remodeling in asthma. *J Clin Invest* 1999; 104: 1001-1006.

117. Cohen L, E X, Tarsi J, Ramkumar T, Horiuchi TK, Cochran R, DeMartino S, Schechtman KB, Hussain I, Holtzman MJ, Castro M. Epithelial cell proliferation contributes to airway remodeling in severe asthma. *Am J Respir Crit Care Med* 2007; 176: 138-145.

118. Kardas G, Daszyńska-Kardas A, Marynowski M, Brząkalska O, Kuna P, Panek M. Role of Platelet-Derived Growth Factor (PDGF) in Asthma as an Immunoregulatory Factor Mediating Airway Remodeling and Possible Pharmacological Target. *Front Pharmacol* 2020; 11: 47.

119. Doherty T, Broide D. Cytokines and growth factors in airway remodeling in asthma. *Curr Opin Immunol* 2007; 19: 676-680.

120. Du L, Xu C, Zeng Z, Chen F, Tang K, Liang Y, Guo Y. Exploration of induced sputum BIRC3 levels and clinical implications in asthma. *BMC Pulm Med* 2022; 22: 86.

121. Reddel RR, Ke Y, Gerwin BI, McMenamin MG, Lechner JF, Su RT, Brash DE, Park JB, Rhim JS, Harris CC. Transformation of human bronchial epithelial cells by infection with SV40 or adenovirus-12 SV40 hybrid virus, or transfection via strontium phosphate coprecipitation with a plasmid containing SV40 early region genes. *Cancer Res* 1988; 48: 1904-1909.

122. Walters MS, Gomi K, Ashbridge B, Moore MA, Arbelaez V, Heldrich J, Ding BS, Rafii S, Staudt MR, Crystal RG. Generation of a human airway epithelium derived basal cell line with multipotent differentiation capacity. *Respir Res* 2013; 14: 135.

123. Bagher M, Rosmark O, Elowsson Rendin L, Nybom A, Wasserstrom S, Müller C, Zhou XH, Dellgren G, Hallgren O, Bjermer L, Larsson-Callerfelt AK, Westergren-Thorsson G. Crosstalk between Mast Cells and Lung Fibroblasts Is Modified by Alveolar Extracellular Matrix and Influences Epithelial Migration. *Int J Mol Sci* 2021; 22.

124. Semlali A, Jacques E, Koussih L, Gounni AS, Chakir J. Thymic stromal lymphopoietin-induced human asthmatic airway epithelial cell proliferation through an IL-13-dependent pathway. *J Allergy Clin Immunol* 2010; 125: 844-850.

125. Barrow RE, Wang CZ, Evans MJ, Herndon DN. Growth factors accelerate epithelial repair in sheep trachea. *Lung* 1993; 171: 335-344.

126. Trautmann A, Toksoy A, Engelhardt E, Bröcker EB, Gillitzer R. Mast cell involvement in normal human skin wound healing: expression of monocyte chemoattractant protein-1 is correlated with recruitment of mast cells which synthesize interleukin-4 in vivo. *J Pathol* 2000; 190: 100-106.

127. James AL, Paré PD, Hogg JC. The mechanics of airway narrowing in asthma. *Am Rev Respir Dis* 1989; 139: 242-246.

128. Hartmann T, Ruoss SJ, Raymond WW, Seuwen K, Caughey GH. Human tryptase as a potent, cell-specific mitogen: role of signaling pathways in synergistic responses. *Am J Physiol* 1992; 262: L528-534.

129. Dong X, Chen J, Zhang Y, Cen Y. Mast cell chymase promotes cell proliferation and expression of certain cytokines in a dose-dependent manner. *Mol Med Rep* 2012; 5: 1487-1490.

130. Zhao XO, Sommerhoff CP, Paivandy A, Pejler G. Mast cell chymase regulates extracellular matrix remodeling-related events in primary human small airway epithelial cells. *J Allergy Clin Immunol* 2022; 150: 1534-1544.

131. Hardyman MA, Wilkinson E, Martin E, Jayasekera NP, Blume C, Swindle EJ, Gozzard N, Holgate ST, Howarth PH, Davies DE, Collins JE. TNF-α-mediated bronchial barrier disruption and regulation by src-family kinase activation. *J Allergy Clin Immunol* 2013; 132: 665-675.e668.

132. Aydin M, Naumova EA, Bellm A, Behrendt AK, Giachero F, Bahlmann N, Zhang W, Wirth S, Paulsen F, Arnold WH, Ehrhardt A. From Submerged Cultures to 3D Cell Culture Models: Evolution of Nasal Epithelial Cells in Asthma Research and Virus Infection. *Viruses* 2021; 13.

133. Moon TC, Befus AD, Kulka M. Mast cell mediators: their differential release and the secretory pathways involved. *Front Immunol* 2014; 5: 569.

134. Schwartz LB, Min HK, Ren S, Xia HZ, Hu J, Zhao W, Moxley G, Fukuoka Y. Tryptase precursors are preferentially and spontaneously released, whereas mature tryptase is retained by HMC-1 cells, Mono-Mac-6 cells, and human skin-derived mast cells. *J Immunol* 2003; 170: 5667-5673.

135. Blank U, Madera-Salcedo IK, Danelli L, Claver J, Tiwari N, Sánchez-Miranda E, Vázquez-Victorio G, Ramírez-Valadez KA, Macias-Silva M, González-Espinosa C. Vesicular trafficking and signaling for cytokine and chemokine secretion in mast cells. *Front Immunol* 2014; 5: 453.

136. Caughey GH. Mast cell tryptases and chymases in inflammation and host defense. *Immunol Rev* 2007; 217: 141-154.